Ther Adv Reprod Health
2021, Vol. 15: 1–16
DOI: 10.1177/26334941211023544

Mitochondrial enrichment in infertile patients: a review of different mitochondrial replacement therapies

Cristina Rodríguez-Varela, Sonia Herraiz and Elena Labarta

***Abstract*:** Poor ovarian responders exhibit a quantitative reduction in their follicular pool, and most cases are also associated with poor oocyte quality due to patient's age, which leads to impaired *in vitro* fertilisation outcomes. In particular, poor oocyte quality has been related to mitochondrial dysfunction and/or low mitochondrial count as these organelles are crucial in many essential oocyte processes. Therefore, mitochondrial enrichment has been proposed as a potential therapy option in infertile patients to improve oocyte quality and subsequent *in vitro* fertilisation outcomes. Nowadays, different options are available for mitochondrial enrichment treatments that are encompassed in two main approaches: heterologous and autologous. In the heterologous approach, mitochondria come from an external source, which is an oocyte donor. These techniques include transferring either a portion of the donor's oocyte cytoplasm to the recipient oocyte or nuclear material from the patient to the donor's oocyte. In any case, this approach entails many ethical and safety concerns that mainly arise from the uncertain degree of mitochondrial heteroplasmy deriving from it. Thus the autologous approach is considered a suitable potential tool to improve oocyte quality by overcoming the heteroplasmy issue. Autologous mitochondrial transfer, however, has not yielded as many beneficial outcomes as initially expected. Proposed mitochondrial autologous sources include immature oocytes, granulosa cells, germline stem cells, and adipose-derived stem cells. Presently, it would seem that these autologous techniques do not improve clinical outcomes in human infertile patients. However, further trials still need to be performed to confirm these results. Besides these two main categories, new strategies have arisen for oocyte rejuvenation by improving patient's own mitochondrial function and avoiding the unknown consequences of third-party genetic material. This is the case of antioxidants, which may enhance mitochondrial activity by counteracting and/or preventing oxidative stress damage. Among others, coenzyme-Q10 and melatonin have shown promising results in low-prognosis infertile patients, although further randomised clinical trials are still necessary.

Keywords: mitochondria, mitochondrial enrichment, oocyte rejuvenation, poor oocyte quality

Received: 16 December 2020; revised manuscript accepted: 7 May 2021.

Correspondence to:
Cristina Rodríguez-Varela
IVI Foundation – IIS La Fe, 46026 Valencia, Spain.
cristina.rodriguez@ivirma.com

Sonia Herraiz
IVI Foundation – IIS La Fe, Valencia, Spain

Elena Labarta
IVI Foundation – IIS La Fe, Valencia, Spain; IVIRMA Valencia, Valencia, Spain

Introduction

Ovarian ageing is one of the main causes of infertility in poor ovarian responders (POR), along with genetic or physiological factors (such as obesity and genetic polymorphisms affecting gonadotropin receptors), which can alter women's responsiveness to ovarian stimulation.[1] Ovarian ageing, regardless of it being premature or physiological, reduces the ovarian reserve, characterised by a quantitative and qualitative alteration of oocytes.[2] Hence this condition results in not only poor response to ovarian stimulation, but also in poor oocyte quality, especially in those POR of advanced maternal age. This in turn cuts

pregnancy chances by *in vitro* fertilisation (IVF) techniques.[3]

In particular, poor oocyte quality is characterised by impaired nuclear and/or cytoplasmic maturity. It has been recently related to either mitochondrial dysfunction or low mitochondrial count as these organelles are crucial for acquiring oocyte competence. These two conditions are also associated with the ageing process. On one hand, mtDNA mutations accumulate over time, insofar as a higher mtDNA mutation rate present in aged oocytes may cause their mitochondrial dysfunction.[2] On the other hand, as a measure of mitochondrial content, low mitochondrial DNA (mtDNA) levels have been found in aged oocytes from not only advanced maternal age but also from diminished ovarian reserve patients compared to oocytes from women with normal ovarian reserves.[4]

Mitochondria are the powerhouse organelles of cells, as they are the main source of energy that comes in the form of adenosine triphosphate (ATP). This molecule is essential in oocytes for successful meiotic spindle assembly, proper chromosome segregation, maturation, fertilisation, and subsequent pre-implantation embryogenesis. Mitochondria also play a role in $Ca^{2}+$ homeostasis, apoptosis regulation, and management of the oxidative stress, which are crucial processes for proper fertilisation and embryo development.[5] Consequently, an alteration of these mechanisms could compromise oocyte quality and further embryo development. Indeed, low ATP content has been related to increased fertilisation failure, arrested division, and abnormal embryo development in infertile patients.[6]

Hence an optimal number of functional mitochondria are crucial to acquire oocyte competence but also for proper blastomere survival in the developing embryo.[7] However, mitochondrial replication is inhibited during embryo development.[8] One plausible explanation may be to keep mtDNA mutations at a minimum, given its high exposure to free radicals, along with its poor genomic repair and protective mechanisms compared to nuclear genetic material.[9] Therefore, both the number and quality of mitochondria present in mature oocytes determine the total number and quality of the mitochondria present in the peri-implantational blastocyst and are randomly distributed among all blastomeres during embryo development until the hatched blastocyst stage.[10] Indeed, a minimum mtDNA copy number threshold in the mature oocyte has been proposed to enable embryo development.[11]

By taking into account the close relation between mitochondrial function/quantity and oocyte/embryo quality, mtDNA has been proposed as a potential biomarker for IVF outcome. Regarding the metaphase II (MII) oocyte, a higher content of mtDNA relates to better oocyte quality and development potential,[12] but mtDNA content in blastocyst stage cells is low compared to oocyte cells as a result of the dilution of mitochondria between embryonic cells throughout development.[10] Thus unlike mature oocytes, a higher mtDNA content in the blastocyst stage has been related to aneuploidy,[12,13] implantation failure[12,14] and poor embryo morphology.[14] It has been hypothesised that mtDNA in blastocyst stage cells replicates as a response to any stresses or threats that could compromise their viability.[15]

Mitochondrial replacement techniques for oocyte rejuvenation

The clinical management of POR women in ART includes modified ovarian stimulation protocols or using exogenous adjuvants in an attempt to increase the number and quality of retrieved oocytes,[16] although no specific approach has been demonstrated to significantly increase the ovarian response in all POR patients. Innovative procedures using stem cell ovarian infusion are already being investigated based on the assumption that the factors secreted from these cells may improve the follicular niche and, hence, promote follicular growth.[17] In contrast, strategies enhancing the quality of already-available oocytes constitute another possibility to improve IVF outcomes in these patients.

Poor oocyte quality in POR patients can be improved by oocyte rejuvenation. In past decades, several cytoplasmic replacement techniques have emerged to accomplish this, given the crucial role of mitochondria and many other cellular components in achieving oocyte competence. These emerging therapeutic strategies are based on the assumption that several RNAs, proteins, energy-producing components, mitochondria, and many other yet undetected factors,[18] exist in

the cytoplasm of young and healthy oocytes that contribute to the correct function of these cells. Therefore, the transfer of these components could promote oocyte rejuvenation by enhancing its subsequent maturation, fertilisation and embryo development.

Of these cytoplasmic components, mitochondria are the main treatment target given their proven crucial role in oocyte acquisition of competence and subsequent embryo development. Currently, heterologous and autologous approaches for mitochondrial replacement are employed for oocyte rejuvenation with varying success rates. Initially, the first therapeutic strategies focus on replacing the cytoplasm,[19–23] while later approaches centre on the direct isolation and replacement of purified mitochondria.[24–26]

In the sections below, we describe the main therapeutic strategies using young cytoplasm donations or purified functional mitochondria of either heterologous or autologous sources to restore oocyte quality and, therefore, improve clinical reproductive outcomes in infertile patients.

Heterologous approach

The source of mitochondria in the heterologous approach is healthy oocytes from donors. Mitochondrial enrichment can be performed in this context by relocating a healthy cytoplasm into the patient's oocyte (partial cytoplasm transfer) or replacing the compromised cytoplasm with a competent one by means of nuclear transfer technology (total cytoplasm transfer) (Figure 1).

Partial cytoplasm transfer. The first technique designed to overcome impaired oocyte quality was ooplasmic transfer (OT), also called cytotransfer, and has laid the foundations of oocyte rejuvenation treatments. Since the first attempt of OT in mice,[27] many other studies have applied different techniques to enhance oocyte quality in both animal and human models.

OT involves transferring a cytoplasm portion from a donor's oocyte to the patient's oocyte to introduce potentially beneficial components that might restore oocyte viability.[28] OT can be synchronous or asynchronous for the relation between the donor and the recipient oocytes. In the synchronous transfer, the donor and recipient share the same developmental stage, and it is usually performed from an MII to an MII oocyte.[29] In the asynchronous transfer, donor and patient oocytes are in different development stages, such as from a tripronucleated zygote to an MII oocyte.[30] This technique can also be performed by either the electrofusion of the donor's enucleated cytoplasm with the recipient oocyte or injecting the donor enucleated cytoplasm at the same time as the spermatozoa during the intracytoplasmic sperm injection (ICSI) procedure.[19,28]

In 1997, Cohen and colleagues[19] announced the first human pregnancy after OT. Following this achievement, this method has been successfully used in low-prognosis patients.[28,30,31] Despite these good results, in 2001, the Food and Drug Administration (FDA) suspended its use owing to ethical and technical concerns,[32] as the introduction of foreign cytoplasm leads to mitochondrial heteroplasmy in the patient's oocyte. It has been suggested that the presence of the third genetic material (mtDNA from the donor) could interfere with the close communication between the nuclear and mitochondrial DNA from the recipient and may lead to unpredictable consequences for not only the developing embryo but also for the offspring's subsequent long-term health.[33,34] In any case, these potential consequences are still under debate, and its safety remains to be clarified, as there are opposite points of view in this respect.[35]

Total cytoplasm transfer. Mitochondrial heteroplasmy in the offspring and its unknown consequences have led to alternative strategies being proposed to improve oocyte quality. Germinal vesicle (GV), spindle, pronuclear, polar body and blastomere transfer constitute different ways of relocating the genetic material from a patient's compromised oocyte or zygote to a healthy cytoplasm. Unlike OT, this approach allows the possibility of reducing the amount of the patient's mitochondria transferred along with the genetic material into the reconstructed oocyte.

GV transfer: The GV is the nuclear structure of an immature oocyte in prophase I of the first meiosis, a stage in which human oocytes are arrested for varied time periods until final maturation.[36] Indeed, this technique was initially proposed to treat advanced maternal age patients because it can prevent the age-related increase in

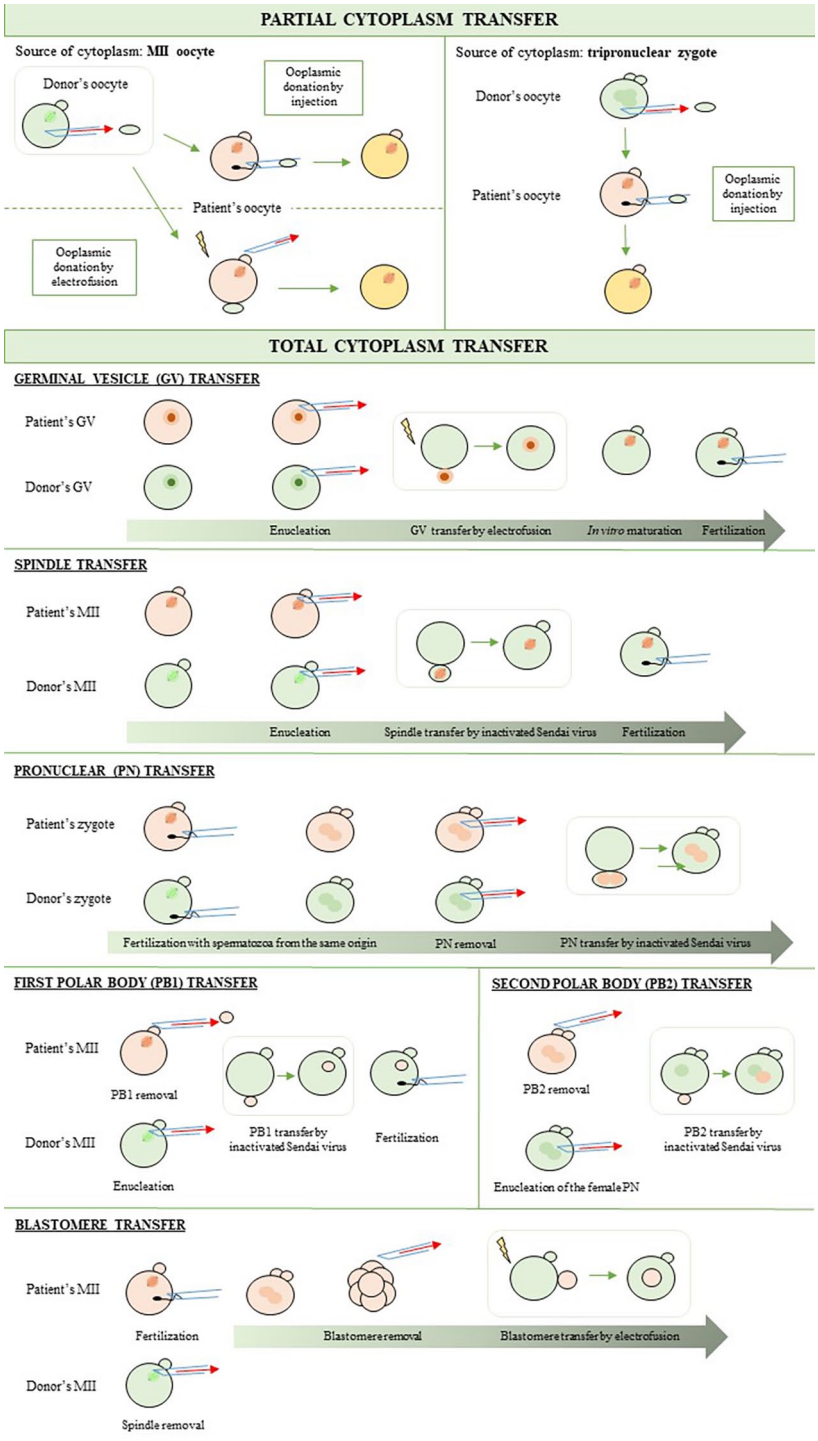

Figure 1. Schematic representation of the different heterologous mitochondrial replacement techniques.

chromosomal abnormalities deriving from the first meiosis.

GV transfer consists of relocating the GV from the compromised oocyte to an enucleated healthy oocyte in the same developmental stage by electrofusion, subsequently matured *in vitro* to the MII stage.[37] Enucleation must be performed in oocytes previously treated with cytochalasin B (CB), a cytoskeleton disrupting agent that avoids

the manipulation-induced extrusion of the first polar body (PB1).[38] After fertilisation, a zygote is generated with healthy mitochondria from the donor, but with nuclear DNA from its original parents.

The first GV transfer in humans was performed in 1999 by Zhang's group. In this study, up to 80% of treated oocytes successfully reached the MII stage, but no indications were provided on the reconstructed oocytes' developmental capacity.[21] In 2004, Takeuchi and colleagues[39] demonstrated that human oocytes reconstituted with GV nuclei were able to undergo maturation (with similar maturation rates to the study of Zhang and colleagues), fertilisation and early embryo development, while maintaining normal ploidy. No live birth and healthy offspring have been described in humans yet, contrary to what has been found in animal studies,[40,41] probably due to the current poor efficiency of the *in vitro* maturation technique in our species.

One of the main advantages of GV transfer is its defined structure, which eases its visualisation and manipulation. However, the main limitations of this technique include: high mitochondrial aggregation around the GV, which could lead to mitochondrial heteroplasmy after transfer;[42] and the maturation process needed from the GV to the MII stage, which requires further improvement.[21] On one hand, the *in vitro* maturation technique itself needs to be optimized, and on the other hand, the use of the cytoskeleton inhibitor CB hampers meiotic progression. Moreover, data on the amount of mtDNA carryover following this technique on human oocytes are lacking,[37] although no detectable mtDNA was found in any of the reconstructed oocytes after GV transfer in mice.[43]

Spindle transfer: Nuclear genetic materials assemble in a spindle structure in the metaphase of the second meiosis. In spindle transfer, this structure is transferred from the patient's oocyte to an enucleated healthy oocyte of the same developmental stage, by electrofusion or by employing a Sendai virus extract, that is, subsequently fertilised by ICSI.[44] In humans, spindle visualisation is achieved by polarised light microscopy.[45] Moreover, a cytoskeletal inhibitor is required in order to increase survival rates during spindle transfer manipulation. CB treatment can be used prior to enucleation in order to avoid second polar body (PB2) extrusion.[46]

Spindle transfer is less invasive than GV transfer, as condensed chromosomes can be easily aspirated with a smaller enucleation pipette and a minimal amount of cytoplasm,[37] due to its location at the periphery of the oocyte. Moreover, as spindle structure removal is a common procedure employed in cloning, there is more information available about this technique.[47]

Animal studies using spindle transfer offer promising results. In 2009, Tachibana and colleagues[48] demonstrated that monkey reconstructed oocytes following spindle transfer were capable of normal fertilisation and embryo development, and led to four live healthy offspring. However, the same group was unable to translate these successful results to humans. In this later experiment, around half the manipulated oocytes showed fertilisation failure, although those correctly fertilised proceeded to the blastocyst stage. The main reason proposed for this fertilisation failure was premature oocyte activation, which suggests human oocytes' marked sensitivity to spindle manipulation.[49]

The first human live birth by spindle transfer was reported in 2017 in a 36-year-old woman diagnosed with Leigh syndrome. By this procedure, the authors achieved a mtDNA carryover rate under 6%,[23] although it has been estimated that it can lower to less than 1%.[50,51]

Despite the promising results, human studies using spindle transfer need this technique to be further optimised and has been proposed as a potential treatment to reduce chromosomal abnormalities related to oocyte ageing. In 2010, Tanaka and colleagues transferred the spindle from *in vitro*–matured MII human oocytes as a model that resembles the age-induced aneuploidies present in oocytes from women of advanced age to enucleated cytoplasms from young oocytes. This procedure significantly increased the number of embryos that developed to the blastocyst stage.[52]

In 2020, Costa-Borges and colleagues published the proof of concept of the feasibility of spindle transfer to overcome massive embryo developmental arrest due to poor oocyte competence in a

sensitive mouse strain. Optimal enucleation and reconstruction procedures with minimal mitochondrial carryover have been achieved, and the group is currently working on the human translation of the technique (ISRCTN 11455145). To date, preliminary and encouraging results of a pilot study with 25 women, with a history of massive embryo development arrest, have been presented.[51]

Pronuclear transfer: Shortly after fertilisation, male and female pronuclei (PN) are visualised inside the zygote. Pronuclear transfer involves relocating the two pronuclei from the compromised zygote to an enucleated healthy zygote by either electrofusion or inactivated Sendai virus.[37] A cytoskeletal inhibitor is also required in PN transfer.[20]

PN transfer was the first manipulation to have been carried out in a mammalian embryo, and it was initially designed to study the mouse embryogenesis process.[53] Since then, PN transfer has been applied widely, and mice reconstructed zygotes have successfully proceeded to the blastocyst stage, to be implanted and they developed to full term in several studies.[54]

The first PN transfer in humans managed to reduce mtDNA carryover to less than 2% in most reconstructed zygotes.[20] However, PN transfer carryover is still higher than that of spindle transfer, probably due to the higher size of the PN and their central position within the zygote, meaning that more cytoplasm will likely be removed during enucleation. In any case, as described by Hyslop and colleagues[55] in 2016, it may be possible to reduce the amount of extra cytoplasm removed along with the pronuclei during manipulation.

Although later experimental studies have obtained healthy human zygotes after PN transfer,[56] the main limitation of this technique relates to the ethical concern of generating extra zygotes, which are subsequently discarded.[57] This issue has led to arguments both in favour and against the use of PN transfer. It has been discussed by many ethical committees and, for instance, the Nuffield Council on Bioethics in the United Kingdom has concluded that it would be ethical to offer PN transfer if it was shown to be safe, independently of the extra discarded zygotes.[58]

Polar body transfer (PBT): Polar bodies are residual structures deriving from oocyte meiotic divisions. PB1 appears after ovulation and, thus, contains a subset of bivalent chromosomes. PB2 appears after fertilisation and, hence, has a haploid set of chromatids. They are dispensable structures for subsequent embryo development because they usually degenerate within hours of forming.[59] However, these structures contain genetic material complementary to the oocyte,[60,61] with fewer mitochondria[62] and the advantage of being situated outside the oocyte surrounded by a membrane.[63] Indeed, their external position allows their manipulation without requiring any cytoskeleton inhibitor supplementation.

PBT consists in the relocation of the PB1 or PB2, instead of the nuclear genetic material from the oocyte, to a healthy cytoplasm.[64]

- PB1T: PB1 is isolated from the compromised MII oocyte and is relocated in the enucleated donor oocyte. This oocyte is subsequently fertilised with the partner sperm, which gives rise to a reconstituted zygote with healthy mtDNA, as well as the genetic material from the patient PB1 and sperm in the form of pronuclei.
- PB2T: the mature oocyte from the donor is fertilised with the same partner sperm. PB2 is then transferred from the reconstituted zygote previously generated into the healthy zygote after removing the maternal pronuclei. Therefore, a reconstituted zygote is generated with healthy mtDNA, as well as the genetic material from the patient's PB2 and sperm in the form of pronuclei.[22]

These two procedures can be singly performed, or done in two subsequent steps, to take advantage of both structures.

Both PB1T and PB2T have proven to produce offspring in mice,[60,61] and their feasibility can prevent the transmission of mtDNA diseases.[63] Wang and colleagues recorded undetectable mtDNA carryover levels after PB1T and around 2% of mtDNA carryover after PB2T. In humans, functional oocytes were produced after PB1T,[22,65] achieving minimum levels of mtDNA carryover (0.38%).[66] However, PB2T has not yet been successfully performed in human zygotes,[22] probably due to the difficulties surrounding the enucleation

of the maternal pronucleus alone from the donor's zygote. In 2019, Tang's group described a novel strategy for PB2T, which overcomes this issue by transferring the PB2 into a zygote previously enucleated at the MII stage. In this way, the zygote will have only one PN, and it will be of paternal origin.[67]

It is worth noting that these structures host the genetic material that, albeit complementary to that of the oocyte, has been discarded from it. This may be a reason why this material does not move to the oocyte in either the first or second meiotic division, and its movement to these residual structures was chosen instead. One possible explanation could be the lower recombination rates observed in the chromatids of PB2 due to meiotic drive to avoid their persistence in the human germline.[68]

Blastomere transfer: The last potential source of genetic material for nuclear genome transfer is the blastomere, which is one of the cells that shapes the early embryo in initial development stages. It presents a diploid load and is surrounded by a membrane. Following this technique, a blastomere from an embryo is transplanted into an enucleated healthy donor oocyte, which resumes meiosis and gives rise to a reconstituted embryo.[57]

The reconstituted embryo will also have mitochondria from the original blastomere and mitochondria from the donor's oocyte.[57] Indeed, high levels of mtDNA heteroplasmy have been registered in animal studies following this technique.[69,70] For this reason, and for the high resemblance of the blastomere transfer with the procedure for cloning, there are no clinical studies using this technique in humans.[57]

Concerns about total cytoplasm transfer. As well as partial cytoplasm transfer, techniques for total cytoplasm transfer have led to several ethical and safety concerns being voiced. These concerns have become barriers to further research, development and clinical translation of these techniques in humans and in some countries. Despite this, countries such as the United Kingdom have researched and translated mitochondrial replacement techniques to the clinic.

The main concern is related to the potential transmission of mitochondria from the patient along with the nuclear genetic material. As in the partial cytoplasm transfer, mtDNA heteroplasmy can lead to unknown consequences for the offspring and future generations, along with the ethically controversial so-called 'three-parent IVF' newborns.

Finally, potential mtDNA mutations in the donor are not taken into account when these techniques are applied. Against all the odds, the donor's recipient oocyte could carry a pathogenic mutation in the mtDNA associated with mitochondrial disease. This could be avoided by mitochondrial genome sequencing prior to mitochondrial donation, as it is currently performed in the UK mitochondrial donation programme, for example.

Table 1 summarises the main advantages and disadvantages of the different options for total cytoplasm transfer.

Autologous approach

The unpredictable detrimental consequences deriving from mitochondrial heteroplasmy have harmed the use of heterologous transfer for mitochondrial enrichment. In order to overcome these concerns, autologous transfer has arisen as a new methodological oocyte rejuvenation approach (Figure 2).

Autologous germline mitochondrial energy transfer (AUGMENT®). The oocyte pool found in female mammals is fixed after birth and progressively declines after puberty until only a residual pool of dormant follicles remains upon menopause onset.[71] Follicular endowment is considered non-renewable, but the existence of germline stem cells in the adult mammalian ovary of both mice[72] and humans[73] has been reported. Although their potential contribution to postnatal oogenesis remains questionable,[74] when isolated these ovarian stem cells constitute an autologous source of high-quality germline mitochondria[75] from the same cell lineage.[76]

Based on this finding, the autologous germline mitochondrial energy transfer (AUGMENT®) technology was proposed. This procedure involves injecting autologous mitochondria into the patient's oocyte at the time of ICSI, along with spermatozoa. Briefly, the patient undergoes laparoscopy for ovarian cortex retrieval to isolate egg precursor cells (EggPCs) by flow cytometry with the human VASA analogue DDX4 antibody, a

Table 1. Main advantages and disadvantages of the different total cytoplasm transfer techniques available.

Total cytoplasm transfer technique	Main advantages	Main disadvantages	Estimated % mtDNA carryover
Germinal vesicle	• Defined structure • Allows the correction of meiosis I errors	• *In vitro* maturation • Higher mitochondrial carryover	No data on humans
Spindle	• Less invasive • Well-developed technique • Peripheral location	• Possible premature oocyte activation • Human MII oocytes are very sensitive to spindle manipulation • Needs polarised light microscopy	• <6%[23] • <1%[50,51]
Pronuclear	• Membrane-enclosed structure • Easily visualised	• Half the generated zygotes will be discarded • Larger size and central location	<2%[20]
Polar body	• Dispensable structure • External membrane–enclosed structure • Low mitochondrial content	• Residual nature of these structures and unknown consequences • In PB2T: discarded embryos.	0.38% after PB1T[66]
Blastomere	• Membrane-enclosed structure	• No clinical studies in humans • Resembles cloning • High heteroplasmy levels	No data on humans

MII, metaphase II; mtDNA, mitochondrial DNA.

cell surface protein found in these cells. Finally, DDX4-positive EggPCs are centrifuged to release mitochondria.[77]

Since 2014, this technique has been tested in 166 women involved in three different studies performed by United Arab Emirates (UAE), Canada, Turkey, and Spain. Fakih[78] and Oktay and colleagues[79] have shown promising results, but their study design and results are controversial.

Fakih and colleagues reported the application of the AUGMENT technique in 59 patients from the Fakih IVF clinic in UAE, and 34 patients from TCART Fertility Partners in Canada. In this study, pregnancy rates rose above the historic IVF success rates for the same patients (11-fold and 18-fold increases in ongoing pregnancy rates in UAE and Canada, respectively). However, these results came from two different clinics and countries, with distinct IVF conditions and experimental designs reported together. In fact, the Fakih Clinic followed a prospective non-randomised cohort design, while the TCART Clinic did not include control samples.[78]

Oktay and colleagues tested the AUGMENT technique in 16 patients from Genart Ankara in Turkey. They reported higher fertilisation rates (78.3% vs 47.9%; $p = 0.036$) and better embryo quality (3.1% vs 2.3%; $p = 0.082$) than the results obtained in previous cycles from the same patients. Albeit promising, the small sample size, the retrospective intrapatient comparison, lack of a proper control group, and the differences in the IVF cycle protocol between patients are a major concern to properly establish the technique's real effectiveness.[79]

Despite some controversies about these studies' design, both claimed to favour the AUGMENT technique's efficacy. In this context, and in order to test its true efficacy, Labarta and colleagues performed a triple-blind, randomised, single-centre controlled experimental pilot study at IVIRMA Valencia, Spain. This study included 57 poor-prognosis patients with previous IVF failures and well-documented poor embryo quality. One of the main strengths of this study is its experimental design because in the same ovarian stimulation cycle for each patient, retrieved oocytes were randomised (1:1 ratio) to undergo standard ICSI or the AUGMENT protocol, which allows an intrapatient and intracycle comparison design to avoid potential bias.

Briefly, no differences were observed in the euploidy rate per biopsied blastocyst (43.8% in

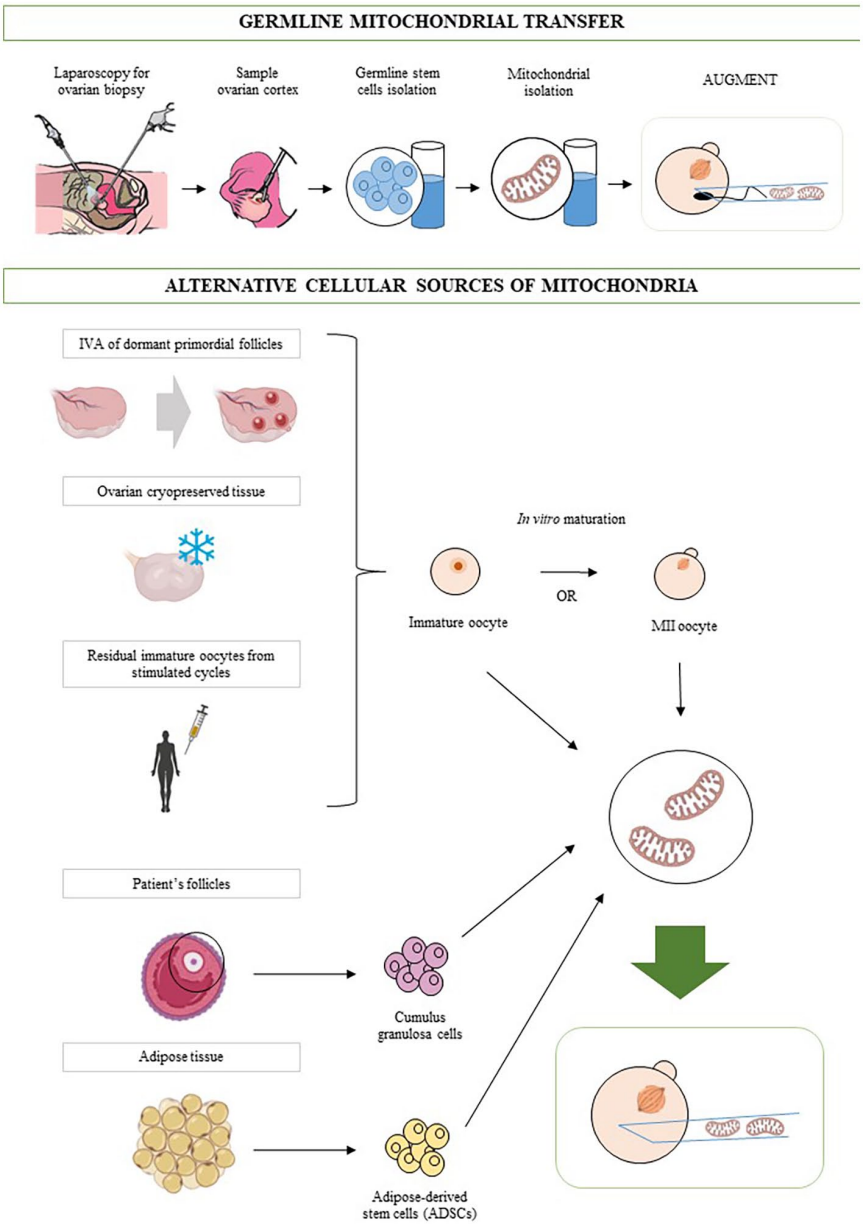

Figure 2. Schematic representation of the different autologous mitochondrial replacement techniques. Figure adapted from Labarta and colleagues' study.[44]

the AUGMENT group vs 63.8%; $p = 0.412$), the euploidy rate per MII (9.8% in the AUGMENT group vs 11.9%; $p = 0.541$), mitochondrial DNA content (21.8 (interquartile range [IQR] = 14.6–24.7) in the AUGMENT group vs 16.9 (IQR = 13.8–23.9); $p = 0.56$), or cumulative live birth rate per transferred embryo (41.2% in the AUGMENT group vs 41.7%; $p = 0.97$). Moreover, the technique did not improve the embryo development potential in this specific population as the day-5 blastocyst formation rate was significantly higher in the control group (23.3% in the AUGMENT group vs 41.1%; $p = 0.0001$). Hence, AUGMENT does not seem to improve prognosis in this population.[26]

Despite the evident strength of the study design, several comments have been made since its publication. On one hand, the majority of aneuploidies, particularly trisomies, usually occur during meiosis I,[80] and mitochondria in the AUGMENT treatment are injected into meiosis II oocytes. Hence, mitochondria may be injected too late, and their potential benefit to the developing

oocyte can be reduced at this development time.[81] On the other hand, the injection of isolated purified mitochondria may not be as beneficial as their injection in conjunction with other factors, such as the presence of the endoplasmic reticulum, which maintains the mitochondrial function, and many other functional small molecules might also benefit oocyte quality.[82] In addition, several authors question the reliability of an antibody-based method to isolate ovarian stem cells from the ovaries of adult humans and other animals.[83,84]

Hence, the AUGMENT technique did not offer any beneficial improvement in the clinical outcome of poor-prognosis patients following a well-designed randomised trial. Nonetheless, as several potential improvements have arisen since its publication, subsequent modified randomised trials should be performed to clarify these heterogeneous findings. Presently, it would seem that the AUGMENT technique, as it is currently designed, cannot be taken as feasible treatment to recover embryo quality in IVF.

Immature oocytes as a source of autologous mitochondria. Ovarian stem cells constitute a difficult cell population to obtain and contain relatively few mitochondria,[85] in addition to their unproven beneficial effect on oocyte quality.[26] Moreover, these stem cells have yet to pass the mitochondrial genetic bottleneck, which is thought to occur during postnatal folliculogenesis.[86] So they may contain multiple mtDNA variants related to the high mitochondrial replication rate in the germ line.

Immature oocytes have been proposed as an alternative source of autologous healthy mitochondria because these cells have already passed the genetic bottleneck, and there are several approaches that can be addressed to obtain these oocytes.

In vitro activation of dormant primordial follicles: In vitro activation (IVA) of dormant primordial follicles constitutes an experimental technique that has achieved several pregnancies and live births in premature ovarian insufficiency patients.[87,88] IVA consists in the activation and subsequent growth of these follicles by the up-regulation of the PI3K-AKT signalling pathway responsible for follicle quiescence.[89]

The large cohort of follicles yielded after IVA may result in more mature oocytes, although their competence would probably be compromised by the patient's age. However, increasing the cohort of mature oocytes would allow to use part of them as a source of healthy mitochondria to improve the oocyte quality of the remaining oocytes by enhancing not only the number, but also overall, gamete quality.[85]

In vitro matured or immature oocytes from ovarian cryopreserved tissue: Ovarian tissue cryopreservation constitutes a fertility preservation technique that is designed to restore ovarian function after thawing and subsequent transplantation.[90] It has been proven that many immature oocytes can be either obtained directly from antral follicles in the ovarian cortex or released into the medium during tissue preparation,[91] prior to cryopreservation. These immature oocytes can be matured *in vitro* and then vitrified to enhance patients' fertility preservation options.[92]

Both the immature and *in vitro* matured oocytes recovered from the ovarian cortex can also be employed as a source of autologous healthy mitochondria. Thus after cryopreserved ovarian cortex transplantation and subsequent ovarian stimulation, these additional organelles can be injected into the oocytes retrieved in an effort to improve their quality.[85]

Residual immature oocytes from stimulated IVF cycles: In stimulated IVF cycles, exogenous gonadotropins are administered to magnify the number of retrieved mature oocytes.[93] Of all the obtained oocytes, only MII oocytes can be fertilised, although around 5% and 20% of recovered oocytes are immature.[94] These immature oocytes may be compromised and are, thus, normally discarded, but their mitochondria may be of good quality.

In addition, immature oocytes in small antral follicles of less than 12–14 mm, which are not usually aspired, also constitute a promising source of autologous healthy mitochondria.[85]

Self-granulosa cell mitochondrial transfer. When considering other cell sources for mitochondrial transfer, granulosa is the closest related cell type to the oocyte. Tzeng and colleagues injected mitochondria that derived from autologous cumulus

granulosa cells into poor quality oocytes in patients with failed IVF/ICSI and compared the outcome with a non-mitochondrial transfer group. Mitochondrial transfer revealed higher fertilisation rates (data not published), improved day-3 embryo quality (data not published), higher pregnancy rates (35.23% vs 6.2%; $p < 0.05$) and lower abortion rates (15.4% vs 100%; $p < 0.05$).[24] Moreover, Kong and colleagues[95] observed improved embryo quality (59.4% vs 34.9%; $p < 0.05$) in the mitochondrial transfer group, but there was no difference in the fertilisation rates between both groups (74.4% vs 76.8%; $p > 0.05$).

Despite the bidirectional close communication between granulosa cells and the oocyte,[96] these two cell types do not share the same cellular lineage. In addition, granulosa cells go through ageing along with the oocyte as maternal age increases either physiologically or biologically.[25] Hence, an autologous source of mitochondria from granulosa cells would not solve poor oocyte quality cases due to ovarian ageing.

Non-ovarian stem cell mitochondrial transfer. Given the ageing process associated with granulosa cells' mitochondria and controversy about the existence of ovarian stem cells, stem cells from other lineages have been proposed as potential sources of autologous mitochondria.

Stem cells and early embryos present metabolic adaptation to their rapid proliferation, called the Warburg Effect. This effect is characterised by the metabolism of pyruvate into lactate, rather than into the tricarboxylic acid cycle, which indicates that glycolysis dominates in ATP production.[97] The metabolic similarity between early embryos (and mature oocytes) and stem cells is reflected in their mitochondrial morphology,[25] as both cell types have spherical mitochondrial with a few cristae.[98]

In this context, Wang and colleagues suggested employing adipose-derived stem cells (ADSCs) as a potential source of autologous mitochondria to improve oocyte quality. Animals are rich in this tissue and there is low risk, if any, of acquiring it. Moreover, it is acceptable to acquire it for women by liposuction, a widely used weight loss procedure.

They first tested mitochondrial morphology by transmission electron microscopy in mice oocytes and ADSCs. They saw the aberrant mitochondrial morphology of aged oocytes compared to the normal morphology visualised in young oocytes but did not see any morphological difference in mitochondria from ADSCs between young and aged mice, which suggests their potential utility for mitochondrial enrichment treatments. Second, they transferred these purified ADSC mitochondria from aged mice to their own GV oocytes, and cultured them to mature *in vitro* to evaluate oocyte quality. Finally, they transferred these mitochondria to MII oocytes to explore their effect on embryo development. In both procedures, some oocytes were injected with a placebo as the control group.

They observed increased mtDNA levels in the ADSC transfer group ($(12.47 \pm 4.16) \times 10^4$ vs $(8.38 \pm 1.99) \times 10^4$), better spindle organisation and chromosome alignment on the equatorial plate (5/5 vs 2/9), and lower aneuploidy rates (4/12 vs 11/18). They also observed higher blastocyst rates (30% vs 15%) and pregnancy outcome (eight pups after transferring 51 embryos to nine recipients versus one pup after transferring 50 embryos to seven recipients) in the ADSC transfer group. Therefore, ADSC mitochondria can improve oocyte quality, embryogenesis and fertility outcomes in aged mice.[25]

However, the procedure of allocating oocytes to the different treatment groups is not well described, and we are unaware if it was randomised or subjected to any possible bias. Moreover, the sample size is small, and no statistical assessment of the presented results has been made. Hence, ADSC mitochondria could be an alternative source for mitochondrial enrichment therapies, but further randomised trials are needed to prove this hypothesis, as are trials in humans. Other stem cell sources of mitochondria should be tested following this hypothesis.

Antioxidants

The techniques described so far in this review focus on oocyte rejuvenation through mitochondrial enrichment by an external source that is either autologous or heterologous. Antioxidant supplementation treatment, however, may constitute a feasible option to enhance the function of the mitochondria already present in the oocyte. These molecules may particularly solve the mitochondrial dysfunction related to poor oocyte

quality and quantity present in POR women by keeping cellular ROS levels balanced.[99]

Among others, there are several human clinical trials with very promising results that employ coenzyme-Q10 in young POR women,[100] melatonin in women undergoing IVF,[101,102] or a combination of melatonin, myo-inositol and folic acid in women with previous IVF failure due to poor oocyte quality.[103] However, further well-designed clinical and randomised controlled trials are still needed before this therapy can be incorporated into routine clinical practice.

Conclusion

Mitochondrial enrichment techniques have been proposed as an alternative to improve poor oocyte quality in either POR patients or patients with a poor embryo quality background, especially in those of advanced maternal age, given the association between mitochondrial activity and competence acquisition in human oocytes. The first strategies described for this purpose were based on transferring mitochondria from a heterologous origin, which is a healthy donor with good-quality oocytes. However, the presence of two different mitochondrial genomes in the resulting oocyte, and its unpredictable effects on offspring, led to the rejection of the partial cytoplasm transfer in favour of total cytoplasm transfer with the minimum mitochondrial carryover and ultimately to proposing mitochondrial replacement techniques of an autologous origin. In this new approach, the described mitochondrial sources include ovarian stem cells, adipose-derived stem cells, granulosa cells and immature oocytes. Although promising, these strategies are still under study, and only a few clinical trials have been conducted in humans. Therefore, further randomised clinical trials are needed before they can be transferred to clinical practice, and the already-proposed techniques require further technological improvement.

Funding

The authors received no financial support for the research, authorship and/or publication of this article.

Conflict of interest statement

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

ORCID iD

Cristina Rodríguez-Varela https://orcid.org/0000-0002-4273-4120

References

1. Esteves SC, Roque M, Bedoschi GM, *et al.* Defining low prognosis patients undergoing assisted reproductive technology: POSEIDON criteria – the why. *Front Endocrinol* 2018; 9: 461.
2. Chiang JL, Shukla P, Pagidas K, *et al.* Mitochondria in ovarian aging and reproductive longevity. *Ageing Res Rev* 2020; 63: 101168.
3. Sunkara SK, Rittenberg V, Raine-Fenning N, *et al.* Association between the number of eggs and live birth in IVF treatment: an analysis of 400 135 treatment cycles. *Hum Reprod* 2011; 26: 1768–1774.
4. Konstantinidis M, Alfarawati S, Hurd D, *et al.* Simultaneous assessment of aneuploidy, polymorphisms, and mitochondrial DNA content in human polar bodies and embryos with the use of a novel microarray platform. *Fertil Steril* 2014; 102: 1385–1392.
5. Wang L-Y, Wang DH, Zou XY, *et al.* Mitochondrial functions on oocytes and preimplantation embryos. *J Zhejiang Univ Sci B* 2009; 10: 483–492.
6. Zhao J and Li Y. Adenosine triphosphate content in human unfertilized oocytes, undivided zygotes and embryos unsuitable for transfer or cryopreservation. *J Int Med Res* 2012; 40: 734–739.
7. Lin DP-C, Huang C-C, Wu H-M, *et al.* Comparison of mitochondrial DNA contents in human embryos with good or poor morphology at the 8-cell stage. *Fertil Steril* 2004; 81: 73–79.
8. St John J. The control of mtDNA replication during differentiation and development. *Biochim Biophys Acta* 2014; 1840: 1345–1354.
9. Zinovkina LA. Mechanisms of mitochondrial DNA repair in mammals. *Biochemistry* 2018; 83: 233–249.
10. Hashimoto S, Morimoto N, Yamanaka M, *et al.* Quantitative and qualitative changes of mitochondria in human preimplantation embryos. *J Assist Reprod Genet* 2017; 34: 573–580.
11. Seli E. Mitochondrial DNA as a biomarker for in-vitro fertilization outcome. *Curr Opin Obstet Gynecol* 2016; 28: 158–163.

12. Fragouli E, Spath K, Alfarawati S, *et al.* Altered levels of mitochondrial DNA are associated with female age, aneuploidy, and provide an independent measure of embryonic implantation potential. *PLoS Genet* 2015; 11: e1005241.

13. Diez-Juan A, Rubio C, Marin C, *et al.* Mitochondrial DNA content as a viability score in human euploid embryos: less is better. *Fertil Steril* 2015; 104: 534–541.e1.

14. de Los Santos MJ, Diez Juan A, Mifsud A, *et al.* Variables associated with mitochondrial copy number in human blastocysts: what can we learn from trophectoderm biopsies? *Fertil Steril* 2018; 109: 110–117.

15. Wells D, Kaur K, Grifo J, *et al.* Clinical utilisation of a rapid low-pass whole genome sequencing technique for the diagnosis of aneuploidy in human embryos prior to implantation. *J Med Genet* 2014; 51: 553–562.

16. Özkan ZS. Ovarian stimulation modalities in poor responders. *Turk J Med Sci* 2019; 49: 959–962.

17. Herraiz S, Romeu M, Buigues A, *et al.* Autologous stem cell ovarian transplantation to increase reproductive potential in patients who are poor responders. *Fertil Steril* 2018; 110: 496–505.e1.

18. Van Blerkom J, Sinclair J and Davis P. Mitochondrial transfer between oocytes: potential applications of mitochondrial donation and the issue of heteroplasmy. *Hum Reprod* 1998; 13: 2857–2868.

19. Cohen J, Scott R, Schimmel T, *et al.* Birth of infant after transfer of anucleate donor oocyte cytoplasm into recipient eggs. *Lancet* 1997; 350: 186–187.

20. Craven L, Tuppen HA, Greggains GD, *et al.* Pronuclear transfer in human embryos to prevent transmission of mitochondrial DNA disease. *Nature* 2010; 465: 82–85.

21. Zhang J, Wang C-W, Krey L, *et al.* In vitro maturation of human preovulatory oocytes reconstructed by germinal vesicle transfer. *Fertil Steril* 1999; 71: 726–731.

22. Zhang SP, Lu CF, Gong F, *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: 563–571.

23. Zhang J, Liu H, Luo S, *et al.* Live birth derived from oocyte spindle transfer to prevent mitochondrial disease. *Reprod Biomed Online* 2017; 34: 361–368.

24. Tzeng CR, Hsieh RH, Au HK, *et al.* Mitochondria transfer (MIT) into oocyte from autologous cumulus granulosa cells (cGCs). *Fertil Steril* 2004; 82: S53.

25. Wang Z, Hao J, Meng T, *et al.* Transfer of autologous mitochondria from adipose tissue-derived stem cells rescues oocyte quality and infertility in aged mice. *Aging* 2017; 9: 2480–2488.

26. Labarta E, de Los Santos MJ, Herraiz S, *et al.* 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. *Fertil Steril* 2019; 111: 86–96.

27. Muggleton-Harris A, Whittingham DG and Wilson L. Cytoplasmic control of preimplantation development in vitro in the mouse. *Nature* 1982; 299: 460–462.

28. Cohen J, Scott R, Alikani M, *et al.* Ooplasmic transfer in mature human oocytes. *Mol Hum Reprod* 1998; 4: 269–280.

29. Levron J, Willadsen S, Bertoli M, *et al.* The development of mouse zygotes after fusion with synchronous and asynchronous cytoplasm. *Hum Reprod* 1996; 11: 1287–1292, http://humrep.oxfordjournals.org/

30. Huang C-C, Cheng T-C, Chang H-H, *et al.* Birth after the injection of sperm and the cytoplasm of tripronucleate zygotes into metaphase II oocytes in patients with repeated implantation failure after assisted fertilization procedures. *Fertil Steril* 1999; 72: 702–706.

31. Dale B, Wilding M, Botta G, *et al.* Pregnancy after cytoplasmic transfer in a couple suffering from idiopathic infertility. *Hum Reprod* 2001; 16: 1469–1472.

32. Zoon K. *Letter to sponsors/researchers – human cells used in therapy involving the transfer of genetic material by means other than the union of gamete nuclei*. Rockville, MD: Food and Drug Administration, 2001.

33. Brenner CA, Barritt JA, Willadsen S, *et al.* Mitochondrial DNA heteroplasmy after human ooplasmic transplantation. *Fertil Steril* 2000; 74: 573–578.

34. Barritt JA, Willadsen S, Brenner C, *et al.* Cytoplasmic transfer in assisted reproduction. *Hum Reprod Update* 2001; 7: 428–435.

35. Darbandi S, Darbandi M, Khorram Khorshid HR, *et al.* Ooplasmic transfer in human oocytes: efficacy and concerns in assisted reproduction. *Reprod Biol Endocrinol* 2017; 15: 77.

36. Sen A and Caiazza F. Oocyte maturation: a story of arrest and release. *Front Biosci* 2013; 5: 451–477.

37. Cree L and Loi P. Mitochondrial replacement: from basic research to assisted reproductive technology portfolio tool-technicalities and possible risks. *Mol Hum Reprod* 2015; 21: 3–10.

38. Tesarik J, Martinez F, Rienzi L, *et al.* Microfilament disruption is required for enucleation and nuclear transfer in germinal vesicle but not metaphase II human oocytes. *Fertil Steril* 2003; 79: 677–681.

39. Takeuchi T, Rosenwaks Z and Palermo GD. A successful model to assess embryo development after transplantation of prophase nuclei. *Hum Reprod* 2004; 19: 975–981.

40. Liu H, Wang CW, Grifo JA, *et al.* Reconstruction of mouse oocytes by germinal vesicle transfer: maturity of host oocyte cytoplasm determines meiosis. *Hum Reprod* 1999; 14: 2357–2361.

41. Li GP, Chen DY, Lian L, *et al.* Viable rabbits derived from reconstructed oocytes by germinal vesicle transfer after intracytoplasmic sperm injection (ICSI). *Mol Reprod Dev* 2001; 58: 180–185.

42. Sathananthan AH and Trounson AO. Mitochondrial morphology during preimplantational human embryogenesis. *Hum Reprod* 2000; 15(Suppl. 2): 148–159, https://academic.oup.com/humrep/article/15/suppl_2/148/619778

43. Neupane J, Vandewoestyne M, Ghimire S, *et al.* Assessment of nuclear transfer techniques to prevent the transmission of heritable mitochondrial disorders without compromising embryonic development competence in mice. *Mitochondrion* 2014; 18: 27–33.

44. Labarta E, de Los Santos MJ, Escribá MJ, *et al.* Mitochondria as a tool for oocyte rejuvenation. *Fertil Steril* 2019; 111: 219–226.

45. Keefe D, Liu L, Wang W, *et al.* Imaging meiotic spindles by polarization light microscopy: principles and applications to IVF. *Reprod Biomed Online* 2003; 7: 24–29.

46. Tachibana M, Sparman M and Mitalipov S. Chromosome transfer in mature oocytes. *Nat Protoc* 2010; 5: 1138–1147.

47. Iuso D, Czernik M, Zacchini F, *et al.* A simplified approach for oocyte enucleation in mammalian cloning. *Cell Reprogram* 2013; 15: 490–494.

48. Tachibana M, Sparman M, Sritanaudomchai H, *et al.* Mitochondrial gene replacement in primate offspring and embryonic stem cells. *Nature* 2009; 461: 367–372.

49. Tachibana M, Amato P, Sparman M, *et al.* Towards germline gene therapy of inherited mitochondrial diseases. *Nature* 2013; 493: 627–631.

50. Paull D, Emmanuele V, Weiss KA, *et al.* Nuclear genome transfer in human oocytes eliminates mitochondrial DNA variants. *Nature* 2013; 493: 632–637.

51. Costa-Borges N, Nikitos E, Spath K, *et al.* First registered pilot trial to validate the safety and effectiveness of maternal spindle transfer to overcome infertility associated with poor oocyte quality. *Fertil Steril* 2020; 114: e71–e72.

52. Tanaka A, Nagayoshi M, Awata S, *et al.* Metaphase II karyoplast transfer from human in-vitro matured oocytes to enuclueated mature oocytes. *Reprod Biomed Online* 2009; 19: 514–520.

53. McGrath J and Solter D. Completion of mouse embryogenesis requires both the maternal and paternal genomes. *Cell* 1984; 37: 179–183.

54. Kono T and Tsunoda Y. Effects of induction current and other factors on large-scale electrofusion for pronuclear transplantation of mouse eggs. *Gamete Res* 1988; 19: 349–357.

55. Hyslop LA, Blakeley P, Craven L, *et al.* Towards clinical application of pronuclear transfer to prevent mitochondrial DNA disease. *Nature* 2016; 534: 383–386.

56. Zhang J, Zhuang G, Zeng Y, *et al.* Pregnancy derived from human zygote pronuclear transfer in a patient who had arrested embryos after IVF. *Reprod Biomed Online* 2016; 33: 529–533.

57. Reznichenko AS, Huyser C and Pepper MS. Mitochondrial transfer: implications for assisted reproductive technologies. *Appl Transl Genom* 2016; 11: 40–47.

58. Nuffield Council on Bioethics. *Novel techniques for the prevention of mitochondrial DNA disorders: an ethical review*. London: Nuffield Council on Bioethics, 2012, pp. 18–97.

59. Li R and Albertini DF. The road to maturation: somatic cell interaction and self-organization of the mammalian oocyte. *Nat Rev Mol Cell Biol* 2013; 14: 141–152.

60. Wakayama T and Yanagimachi R. The first polar body can be used for the production of normal offspring in mice. *Biol Reprod* 1998; 59: 100–104, https://academic.oup.com/biolreprod/article/59/1/100/2740848

61. Wakayama T, Hayashi Y and Ogura A. Participation of the female pronucleus derived from the second polar body in full embryonic development of mice. *J Reprod Fertil* 1997; 110: 263–266.

62. Steuerwald N, Barritt JA, Adler R, *et al.* Quantification of mtDNA in single oocytes, polar bodies and subcellular components by real-time rapid cycle fluorescence monitored PCR. *Zygote* 2000; 8: 209–215.

63. Wang T, Sha H, Ji D, *et al.* Polar body genome transfer for preventing the transmission of inherited mitochondrial diseases. *Cell* 2014; 157: 1591–1604.

64. Farnezi HCM, Goulart ACX, Dos Santos A, *et al.* Three-parent babies: mitochondrial replacement therapies. *J Bras Reprod Assist* 2020; 24: 189–196.

65. Ma H, O'neil RC, Marti Gutierrez N, *et al.* Functional human oocytes generated by transfer of polar body genomes. *Cell Stem Cell* 2017; 20: 112–119, http://neomorph.salk.edu/PBNT1/browser.html

66. Yamada M, Emmanuele V, Sanchez-Quintero MJ, *et al.* Genetic drift can compromise mitochondrial replacement by nuclear transfer in human oocytes. *Cell Stem Cell* 2016; 18: 749–754.

67. Tang M, Guggilla RR, Gansemans Y, *et al.* Comparative analysis of different nuclear transfer techniques to prevent the transmission of mitochondrial DNA variants. *Mol Hum Reprod* 2019; 25: 797–810.

68. Ottolini CS, Newnham LJ, Capalbo A, *et al.* Genome-wide maps of recombination and chromosome segregation in human oocytes and embryos show selection for maternal recombination rates. *Nat Genet* 2015; 47: 727–735.

69. Steinborn R, Schinogl P, Zakhartchenko V, *et al.* Mitochondrial DNA heteroplasmy in cloned cattle produced by fetal and adult cell cloning. *Nat Genet* 2000; 25: 255–257.

70. Ferreira CR, Meirelles FV, Yamazaki W, *et al.* The kinetics of donor cell mtDNA in embryonic and somatic donor cell-derived bovine embryos. *Cloning Stem Cells* 2007; 9: 618–629.

71. Zuckerman S. The number of oocytes in the mature ovary. *Recent Prog Horm Res* 1951; 6: 63–109.

72. Zou K, Yuan Z, Yang Z, *et al.* Production of offspring from a germline stem cell line derived from neonatal ovaries. *Nat Cell Biol* 2009; 11: 631–636.

73. White YAR, Woods DC, Takai Y, *et al.* Oocyte formation by mitotically active germ cells purified from ovaries of reproductive-age women. *Nat Med* 2012; 18: 413–421.

74. Tilly JL, Niikura Y and Rueda BR. The current status of evidence for and against postnatal oogenesis in mammals: a case of ovarian optimism versus pessimism? *Biol Reprod* 2009; 80: 2–12.

75. Tilly JL and Sinclair DA. Germline energetics, aging, and female infertility. *Cell Metab* 2013; 17: 838–850.

76. Surani MA, Hayashi K and Hajkova P. Genetic and epigenetic regulators of pluripotency. *Cell* 2007; 128: 747–762.

77. Woods DC and Tilly JL. Autologous germline mitochondrial energy transfer (AUGMENT) in human assisted reproduction. *Semin Reprod Med* 2015; 33: 410–421.

78. Fakih MH. The AUGMENT treatment: physician reported outcomes of the initial global patient Experience. *J Fertil Vitr* 2015; 3: 3.

79. Oktay K, Baltaci V, Sonmezer M, *et al.* Oogonial precursor cell-derived autologous mitochondria injection to improve outcomes in women with multiple IVF failures due to low oocyte quality: a clinical translation. *Reprod Sci* 2015; 22: 1612–1617.

80. Chiang T, Schultz RM and Lampson MA. Meiotic origins of maternal age-related aneuploidy. *Biol Reprod* 2012; 86: 1–7.

81. Cozzolino M, Marin D and Sisti G. New frontiers in IVF: mtDNA and autologous germline mitochondrial energy transfer. *Reprod Biol Endocrinol* 2019; 17: 1–11.

82. Qi L, Chen X, Wang J, *et al.* Mitochondria: the panacea to improve oocyte quality? *Ann Transl Med* 2019; 7: 789–789.

83. Zhang H, Panula S, Petropoulos S, *et al.* Adult human and mouse ovaries lack DDX4-expressing functional oogonial stem cells. *Nat Med* 2015; 21: 1116–1118.

84. Hernandez SF, Vahidi NA, Park S, *et al.* Characterization of extracellular DDX4- or Ddx4-positive ovarian cells. *Nat Med* 2015; 21: 1114–1116.

85. Kristensen SG, Pors SE and Andersen CY. Improving oocyte quality by transfer of autologous mitochondria from fully grown oocytes. *Hum Reprod* 2017; 32: 725–732.

86. Wai T, Teoli D and Shoubridge EA. The mitochondrial DNA genetic bottleneck results from replication of a subpopulation of genomes. *Nat Genet* 2008; 40: 1484–1488.

87. Suzuki N, Yoshioka N, Takae S, *et al.* Successful fertility preservation following ovarian tissue vitrification in patients with primary ovarian insufficiency. *Hum Reprod* 2015; 30: 608–615.

88. Zhai J, Yao G, Dong F, *et al.* In vitro activation of follicles and fresh tissue auto-transplantation in primary ovarian insufficiency patients. *J Clin Endocrinol Metab* 2016; 101: 4405–4412.

89. Kawamura K, Cheng Y, Suzuki N, *et al.* Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment. *Proc Natl Acad Sci USA* 2013; 110: 17474–17479.

90. von Wolff M, Donnez J, Hovatta O, *et al.* Cryopreservation and autotransplantation of human ovarian tissue prior to cytotoxic therapy – a technique in its infancy but already successful in fertility preservation. *Eur J Cancer* 2009; 45: 1547–1553.

91. Yin H, Jiang H, Kristensen SG, *et al.* Vitrification of in vitro matured oocytes collected from surplus ovarian medulla tissue resulting from fertility preservation of ovarian cortex tissue. *J Assist Reprod Genet* 2016; 33: 741–746.

92. González C, Devesa M, Boada M, *et al.* Combined strategy for fertility preservation in an oncologic patient: vitrification of in vitro matured oocytes and ovarian tissue freezing. *J Assist Reprod Genet* 2011; 28: 1147–1149.

93. Lunenfeld B. Historical perspectives in gonadotrophin therapy. *Hum Reprod Update* 2004; 10: 453–467.

94. Cha KY and Chian RC. Maturation in vitro of immature human oocytes for clinical use. *Hum Reprod Update* 1998; 4: 103–120.

95. Kong L, Liu Z, Li H, *et al.* Mitochondria transfer from self-granular cells to improve embryos' quality. *Zhonghua Fu Chan Ke Za Zhi* 2004; 39: 105–107, https://europepmc.org/article/med/15059588 (accessed 29 October 2020).

96. Huang Z and Wells D. The human oocyte and cumulus cells relationship: new insights from the cumulus cell transcriptome. *Mol Hum Reprod* 2010; 16: 715–725.

97. Krisher RL and Prather RS. A role for the Warburg effect in preimplantation embryo development: metabolic modification to support rapid cell proliferation. *Mol Reprod Dev* 2012; 79: 311–320.

98. Houghton FD. Energy metabolism of the inner cell mass and trophectoderm of the mouse blastocyst. *Differentiation* 2006; 74: 11–18.

99. Gulcin İ. Antioxidants and antioxidant methods: an updated overview. *Arch Toxicol* 2020; 94: 651–715.

100. Xu Y, Nisenblat V, Lu C, *et al.* Pretreatment with coenzyme Q10 improves ovarian response and embryo quality in low-prognosis young women with decreased ovarian reserve: a randomized controlled trial. *Reprod Biol Endocrinol* 2018; 16: 29.

101. Eryilmaz OG, Devran A, Sarikaya E, *et al.* Melatonin improves the oocyte and the embryo in IVF patients with sleep disturbances, but does not improve the sleeping problems. *J Assist Reprod Genet* 2011; 28: 815–820.

102. Batioğlu AS, Sahin U, Grlek B, *et al.* The efficacy of melatonin administration on oocyte quality. *Gynecol Endocrinol* 2012; 28: 91–93.

103. Unfer V, Raffone E, Rizzo P, *et al.* Effect of a supplementation with myo-inositol plus melatonin on oocyte quality in women who failed to conceive in previous in vitro fertilization cycles for poor oocyte quality: a prospective, longitudinal, cohort study. *Gynecol Endocrinol* 2011; 27: 857–861.