Open Access

*OBM Genetics*

Review

# Diagnosis and Treatment of Mitochondrial Abnormalities in Reproductive Medicine

Joep Geraedts *

Dept. of Genetics and Cell Biology, Maastricht University Medical Centre, 6202 AZ Maastricht, The Netherlands; E-Mail: joep.geraedts@mumc.nl

* **Correspondence:** Joep Geraedts; E-Mail: joep.geraedts@mumc.nl

**Academic Editor:** Khue Vu Nguyen

**Special Issue**: Treatment of Genetic Disease

*OBM Genetics*
2019, volume 3, issue 3
doi:10.21926/obm.genet.1903089

**Received:** April 8, 2019
**Accepted:** August 8, 2019
**Published:** August 14, 2019

**Abstract**

Mitochondrial diseases are among the most prevalent inborn errors of metabolism. The overwhelming majority of mitochondrial diseases (about 85%) result from mutations in nuclear genes involved in oxidative phosphorylation, while some (15%) are caused by mutations in mitochondrial DNA (MtDNA). The familial mtDNA mutations are exclusively inherited from the mother. There are four main methods available to prevent the transmission of mtDNA mutations: preimplantation genetic diagnosis (PGD) or prenatal diagnosis (PND) followed by the selection of the best embryo or fetus, the use of complete donor oocytes, mitochondrial replacement therapy (MRT), and genome editing. However, the latter two methods are not available in reproductive medicine yet.

Mitochondrial abnormalities can also disturb normal reproduction. The abnormalities of mitochondria in oocytes of older infertility patients are also believed to result in poor development. As a cure, ooplasm from fertile donor oocytes was injected into a group of patients to rejuvenate their developmentally compromised oocytes. However, this ooplasmic transfer series had to be discontinued, because two fetuses were affected by Turner's syndrome and an increased risk of mitochondrial heteroplasmy was apprehended. Subsequently, treatments with heterologous mitochondria and autologous transfer were

attempted. However, during the interim analysis of a controlled randomized trial, the transfer of autologous mitochondria to the maternal germline failed to improve embryo quality in IVF**.**

**Keywords**
Mitochondria; mtDNA mutations; infertility treatment; mitochondrial replacement therapy; gene editing

## 1. Introduction

Mitochondria are parts of all eukaryotic cells. These small cell organelles contain two membranes and a matrix. The inner membrane is a phospholipid bilayer that contains the enzyme complex for oxidative phosphorylation. The matrix is the location of many important biochemical pathways. Mitochondria play important roles in several cellular processes and the most important one is cellular respiration, i.e., energy production from oxidation of nutrients. Energy is generated in the form of ATPs, which are consumed by the cell to carry out different functions. Mitochondria are believed to descend from ancient prokaryotes, which still have their own DNA distinct from the DNA found in the nucleus of the cell. The mitochondrial DNA (mtDNA) is a circular DNA, exclusively present in the mitochondria. Furthermore, it is polyploid, which means that there are more than one mtDNA molecules per mitochondrion. Except for the red blood cells, all other cell types contain multiple mitochondria and their number depends on the cellular function and energy requirements [1]. Therefore, neurons and muscles have higher numbers of mitochondria. The mitochondrial genome consists of a circular double-stranded DNA molecule with 16,569 base-pairs and follows its own genetic code slightly different from the nucleus one. It codes for 13 mitochondrial proteins, 22 tRNAs, and 2 rRNAs. However, most essential respiratory chain proteins are encoded by the nucleus. Furthermore, many proteins required for mtDNA maintenance and replication are also coded by the nuclear genome [2].

## 2. Mitochondrial Disease

Mitochondrial diseases are among the most prevalent inborn errors of metabolism [3]. The majority of mitochondrial diseases (about 85%) are caused by mutations in the nuclear genes of oxidative phosphorylation, while the remaining (15%) originate from mtDNA mutations [4]. Nuclear gene mutations follow a Mendelian inheritance pattern. Mitochondrial diseases include a large heterogeneous group of disorders, which are found with an incidence of about 1 in 5000 live births [5]. Mutations have been identified in more than 250 genes [6]. The familial mtDNA mutations are exclusively inherited from the mother and almost always the mother carries a mixture of wild type and mutated mtDNA copies. This condition is called heteroplasmy. The transmission from the mother to a child is subject to the genetic bottleneck, where only a small number of mtDNA molecules are transmitted to the child and therefore the mutation levels in the offspring can vary considerably. Furthermore, the percentage of abnormal mtDNA molecules is not identical in different tissues and at different time points. Generally, there is a threshold, above which the mutant mtDNA leads to disease. This threshold is dependent on the disease type.

Mitochondrial diseases are often multi-system disorders since a decrease in cellular energy can affect several different organs, which can result in different clinical phenotypes. Furthermore, patients with a mitochondrial disease suffer from serious morbidity and mortality [7].

## 3. Ooplasmic Transfer to Improve Oocyte Quality

Mutated mitochondrial DNA can segregate in families and lead to mitochondrial diseases as mentioned in the previous paragraph. However, abnormal amounts of mtDNA can also lead to abnormalities during reproduction. The female germline can be affected by this issue; therefore, several ways have been developed to improve the quality of oocytes prior to IVF. The total number of mtDNA copies increases considerably during oogenesis but remains unchanged from the time of fertilization until the early post-implantation stages [8]. MtDNA replication starts only after the morula stage and the number of mtDNA copies per embryonic cell proportionally decreases after each embryonic cell division [5]. At fertilization, an oocyte contains a huge number of mitochondria and mtDNA copies per cell, more than any other cell type. The number of mitochondrial profiles per metaphase II (MII) stage oocyte varies from 150 000 to 300 000 or even more [9]. In older infertility patients, abnormalities of oocyte mitochondria might result in poor developmental competence of oocytes [10]. Furthermore, the oocytes of reproductive-aged women or those with diminished ovarian reserve have significantly lower mtDNA content than the oocytes from younger women or those with a normal ovarian reserve [11, 12, 13]. However, other authors have been unable to confirm this [8]. Long before the discussion on this controversial topic was started, a group of patients underwent ooplasmic transfer from fertile donor oocytes to rejuvenate their developmentally compromised oocytes. This was mainly done in patients with recurrent implantation failure [14]. The ooplasmic transfer technique was aimed at transplanting beneficial components from donor oocytes, with the aim to restore normal growth and viability [15, 16]. The transfer of a small volume of donor ooplasm included many different molecules and organelles along with mitochondria. In most cases, the transfer was efficient and simple, since it was a modification of an intracytoplasmic sperm injection (ICSI) procedure, where both the sperm and donor cytoplasm are simultaneously injected into a recipient oocyte [17]. The birth of the first baby resulting from ooplasmic transfer from fertile donor oocytes into potentially compromised recipient patient oocytes was published in 1997 [18]. Since the inception of this method, nearly 30 babies have been born after its worldwide introduction [19]. However, this approach was discontinued later for several reasons. One was that two fetuses were affected by Turner's syndrome [20]. The second issue was related to the risks of mitochondrial heteroplasmy. The simultaneous presence of donor and recipient mitochondria can interfere with the communication between the nuclear and mitochondrial genome [21]. Small proportions of donor mtDNA were indeed detected in some children born after the transfer of third-party cytoplasm [22]. The US Food and Drug Administration suggested in 2002 that the technique of donor cytoplasm transfer should be suspended unless it is proved successful under an Investigational New Drug Application [23]. In conclusion, on the one hand, it could not convincingly be shown that mitochondrial supplementation into oocytes can restore embryo development in human as well as non-human species. On the other hand, heterologous ooplasmic transfers in mice resulted in a range of abnormalities that can negatively affect outcomes of offspring [24-25].

## 4. Autologous Germline Mitochondrial Energy Transfer (AUGMENT)

After the prohibition of all treatments with heterologous mitochondria by the FDA, autologous transfer was introduced. This approach was based on the discovery that mammalian females are capable of postnatal oogenesis, reported first in mice [26] and later also in humans [27]. This made it possible to use patient-matched germline mitochondria, where autologous oogonic stem cells or egg precursor cells are used to obtain cytoplasm. However, the presence of adult oogonial stem cells has not yet been well established and is still under scrutiny [28]. The supporters of the presence of adult oogonial stem cells use patient-matched germline mitochondria to boost egg health and embryonic developmental potential instead of young donor eggs to obtain cytoplasm [29]. The delivery of an IVF patient's own mitochondria into her oocyte along with sperm by ICSI provides an autologous means of recapitulating the fertility-boosting benefits of heterologous cytoplasmic transfer without the drawback of having "foreign" mitochondria present in the resultant embryos and offspring [29]. This concept was patented as AUGMENT (Autologous Germline Mitochondrial Energy Transfer) [30]. Since 2014, a U.S. company, Ovascience, is obtaining cortical tissue by means of laparoscopic ovarian biopsy. The tissue is kept frozen until the day of the ICSI procedure. During the ICSI procedure, a tiny amount of mitochondrial suspension is injected along with the spermatozoon. In 2015, the birth of the first child using AUGMENT was reported and since then the number has increased. However, there have been only a few well-designed studies on the use of this method. Fakih and colleagues published pregnancy rates after the application of this technique in a total of 93 patients; however, their results were based on retrospective cohorts. Furthermore, they used descriptive analysis and compared the rates obtained after AUGMENT with historic IVF success rates for these patients [31]. Recently, a triple-blind, randomized, single-centered, controlled experimental pilot study was performed. A total of 59 patients were included, with a mean age of 36.3 years and 2.5 previously failed IVF cycles. The number of MII oocytes in the AUGMENT and control groups were 253 and 250, respectively. The day 5 blastocyst formation rates per zygote were significantly lower in the AUGMENT group (23.3 ±32.0%) in comparison to the control group (41.1 ±36.9%; P= 0001). This suggests that this technique did not improve embryo development in this population. Moreover, no differences were observed in the euploidy rates, mitochondrial DNA content, and cumulative live birth rate per transferred embryo [32]. After an interim analysis, this study was stopped, as the authors concluded that autologous germline mitochondrial energy transfer does not improve embryo quality in IVF.

## 5. Preventing the Transmission of Mitochondrial Disease

Mitochondrial diseases include Kearns-Sayre Syndrome (KSS) [33], myoclonic epilepsy, with ragged red fibres (MERRF) [34], mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) [35], neurogenic weakness with ataxia and retinitis pigmentosa (NARP) [36], Leber's Hereditary Optic Neuropathy (LHON) [37], and Leigh syndrome [38]. However, the clinical patterns of these diseases are heterogeneous, as sometimes their clinical symptoms overlap, while in some cases they may present as distinct clinical entities [39]. In order to prevent the transmission of mitochondrial diseases, which are caused by mtDNA mutations, there have been introduced several reproductive options. Women carrying a pathogenic mtDNA mutation can prevent the birth of an affected child by refraining from having children, by adoption, donation of

oocytes, preimplantation genetic diagnosis (PGD), prenatal diagnosis (PND), genome editing, and mitochondrial replacement [40]. Extensive genetic counseling is required since mitochondrial genetics is complex. At present, for the prevention of the transmission of mtDNA mutations, four methods are available: preimplantation genetic diagnosis (PGD) or prenatal diagnosis (PND) followed by the selection of the best embryo or fetus, the use of complete donor oocytes, mitochondrial replacement therapy (MRT), and genome editing. The first two methods are aimed at the selection of the best embryo. Therefore, strictly speaking, only the latter two methods are therapeutic with the aim to restore normal functionality. However, it should be noted that most of these modalities for preventing the transmission of mitochondrial diseases are either controversial or in very early stages of development and have not been proven beyond the experimental stage.

### *5.1 PGD or PND*

If a mtDNA mutation is not inherited from the mother but has arisen 'de novo" in the affected child, the risk of recurrence is expected to be low. In all other cases, maternal transmission has a risk of recurrence depending on the type of mutation [41]. The selection of the best embryos after PGD and PND can be used to lower the chances of having a severely diseased child. Technically, it is possible to accurately establish the mtDNA mutation load in different types of biopsies (blastomere biopsy, blastocyst biopsy, amniocentesis, etc). However, there is uncertainty that the mutation load found in the tested sample may not be a true representative of the rest of the embryo or fetus. Furthermore, there may be changes in mutation load during further embryonic or fetal development [42]. Only in a few cases, there is a consensus about a cut-off value for embryo selection, since there is enough correlation between the mutation load and the clinical symptoms. These percentages have been published for the following two mtDNA point mutations.

The most common heteroplasmic mtDNA point mutation is m.3243A>G in the mitochondrial MT-TL1 gene leading to the MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes) syndrome. The mutation load in oocytes and embryos shows large variations indicating that the level of mutant mtDNA is largely determined by random genetic drift [43-44]. Therefore, to be at a safer side a threshold level of 15% can be adapted [45].

On the other hand, m.8993T>G leads to NARP (Neuropathy, ataxia, and retinitis pigmentosa). This does not show random transmission but rather demonstrates skewing. This means that the mutation load of oocytes and embryos shows almost homoplasmy, i.e., 0 and 100%. In this case, the threshold value can be increased to 30% [46].

However, there is no predicted "safe heteroplasmy threshold level" for most of the mtDNA diseases. Furthermore, it should be noted that the threshold depends on mutation type, rather than the disease type.

PND is the best option for female carriers with low-level mutations demonstrating skewing to 0% or 100%. In other cases, the preimplantation selection of embryos with a mutant load below a mutation-specific or general expression threshold of 18% can prevent the transmission of mtDNA diseases. PGD is also the best reproductive option for familial heteroplasmic mtDNA point mutations [47]. However, the success rate, which means the chance of having a baby after the IVF procedure, is still a problem. It can be improved if blastomere biopsy is used to obtain the

diagnostic mtDNA, by the biopsy of a single-blastomere. It improves the live birth delivery rate and is applicable to all mtDNA mutations [48].

### *5.2 The Use of Complete Donor Oocytes*

Complete donor oocytes must be used if complete safety is desired. It will prevent the transmission of mtDNA disease and the risk of having an affected child will be equal to the general population risk. However, the child will be genetically related only to the father.

### *5.3 Mitochondrial Replacement Therapy*

Mitochondrial replacement therapy (MRT) is a very interesting approach to prevent the transmission in patients and especially those with higher mutant mitochondrial loads. From an oocyte or zygote that carries a mtDNA mutation, the nucleus is removed and transferred to an enucleated donor oocyte or zygote. Since the recipient donor oocyte or zygote contains mostly wild type mtDNA, the reconstituted oocyte or zygote will contain mostly normal mtDNA and therefore have a reduced risk of transmitting a severe mtDNA disease to the offspring [49]. In theory, there are three sources of genetic material that can be used for the transfer to emptied donor oocytes: pronuclei, meiotic spindles, and polar bodies. Many years ago, pronuclear transfer was demonstrated in mice but live offspring showed high carryover levels [50]. However, in the case of human, MRT can be combined with PGD as an extra safety test. When pronuclei are transplanted shortly after completion of meiosis rather than shortly before the first mitotic division, the carry-over of mtDNA was reduced [51]. The first clinical pronuclear transplantation was carried out in a 30-year-old nulligravida woman who had two failed IVF cycles characterized by all her embryos arresting at the two-cell stage. During her third IVF treatment, the pronuclei of the patient were transferred into enucleated donor oocytes resulting in seven zygotes with a maternal genome and a donor cytoplasm. Of the resulting embryos, five were transferred to the uterus resulting in a triplet pregnancy with fetal heartbeats, normal karyotypes, and nuclear genetic fingerprinting matching with the mother's genetic fingerprinting. The fetal mitochondrial DNA profiles were identical to those from the donor cytoplasm and the resemblance with patient's mitochondrial DNA was seen [52].

The spindle transfer technique was developed in the Rhesus monkey and its efficacy and safety were demonstrated by normal live births [53]. The feasibility of clinical mtDNA replacement by spindle transfer has also been shown, although some human ST oocytes displayed abnormal fertilization [54]. A successful spindle transfer into the cytoplasm of enucleated donor oocytes was reported in a case of Leigh syndrome (mtDNA mutation 8993T > G), with a long history of multiple undiagnosed pregnancy losses and deaths of offspring. A male euploid blastocyst was obtained from the reconstituted oocytes having only a 5.7% mtDNA mutation load. After transfer of this embryo, a pregnancy was established. This resulted in the delivery of a boy with a neonatal mtDNA mutation load of 2 to 9 percent in his various tissues [55]. The editors of RBM Online explained, in an editorial, why they decided to publish this manuscript despite its shortcomings, some of which were rectified after the authors were invited to correct them and include more details [56].

For a long time, it has been difficult to successfully achieve polar body transfer in mammals other than the mouse. Many scientists have tried to achieve this in various species including

primates. In most species, polar bodies are degraded and the DNA is fragmented soon after its synthesis due to the apoptotic pressure [50]. However, in 2017, the content of first polar bodies from metaphase II oocytes was successfully transferred into the enucleated donor MII cytoplasm. After transfer, de novo meiotic spindles were formed and, after fertilization, meiosis was completed and normal diploid zygotes were observed [57].

These approaches of Mitochondrial Replacement Therapy can prevent inheritance of mitochondrial diseases but at the same time, they are the subject of many ethical discussions. Owing to the combination of mtDNA and nuclear DNA from different mothers, the babies are sometimes referred to as three-parent babies. However, if one looks at the total amount of mtDNA from the donor (16,600 bp) in comparison to about 3 billion bp from the nuclear genome, such issues can be resolved.

### *5.4 Genome Editing*

Gene-editing can be applied somatically as well as in the germline. The first application is aimed at curing a diseased person, while the second is for reproductive purposes. Somatic gene therapy, which is the alteration of the genome of somatic tissues, is in general not controversial. Treatments are under development for many genetic diseases. In this respect, a revolution occurred after the introduction of CRISPR/CAS as a gene-editing technique [58]. However, CRISPR, the genome editor which has revolutionized molecular biology in many ways, does not seem to be an option for the correction of mitochondrial mutations. Many researchers doubt that mitochondria can take up the RNA strands that are needed to guide the DNA-cutting protein to the right spot in the genome. [59]. Therefore, it is interesting to note that several groups have tested two other approaches—zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). Both methods make use of DNA-cutting proteins that do not need to guide RNAs to find their way to the right place in the genome. Several recent studies have shown promising results for the treatment of mitochondrial diseases. In a mouse model, showing features of heteroplasmic mtDNA disease in cardiac tissue, the mutant mtDNA was specifically eliminated. This was done by the application of mitochondrially targeted zinc-finger nucleases (mtZFN) delivered by adeno-associated virus. This shows that a correction in the mtDNA heteroplasmy using programmable nucleases could provide a therapeutic route for many heteroplasmic mitochondrial diseases. According to the authors, in this way, therapy can be developed for many patients with mitochondrial diseases [60].

An alternative approach to realize the same outcome is the mitochondria-targeted TALENs (mitoTALENs). These were injected via different routes and both muscle and heart showed a robust reduction in mutant mtDNA, which was stable over time. The molecular defect, namely a decrease in the transfer of RNAAla levels, was restored by the treatment. The treatment based on mitoTALENs showed that the diseased tissues can be tailored to be less affected in diseased mice [61]. However, the efficiency of the TALENs to reduce the mutant load is low.

This approach is not only suitable for somatic therapy but the elimination of mitochondrial mutations from the germline of mice on a selective basis by genome editing has also been reported in 2015 [62]. In addition, using mitochondria-targeted TALEN (mito-TALENs) in mammalian oocytes, the authors also selectively eliminated mutated mtDNA responsible for Leber's hereditary optic neuropathy (LHOND), and neurogenic muscle weakness, ataxia, and

retinitis pigmentosa (NARP). The application of these methods, therefore, can potentially prevent the transgenerational transmission of human mitochondrial diseases caused by mutations in mtDNA. However, one of the limitations of genome editing is the resulting mtDNA depletion in case of high mutant levels. Another recent study has shown great potential for using mitoTALENs for specific targeting of mutant mtDNA both in iPSCs and mammalian oocytes [63].

These alternative genome editing approaches seem to be very promising. Germline modification using CRISPR-CAS9 is not only ineffective, but it is also very controversial since a scientist in China used this gene-editing tool to modify the nuclear genomes of twin baby girls [64]. Germline genome editing raises important bioethical and safety issues, but we should keep in mind that MRT itself is a germline modification per se. Furthermore, bans on germline editing will deprive patients with mitochondrial diseases from getting a cure as explained recently [65].

## Author Contributions

Joep Geraedts did all works.

## Competing Interests

The author has declared that no competing interests exist.

## References

1. Leary SC, Battersby BJ, Hansford RG, Moyes CD. Interactions between bioenergetics and mitochondrial biogenesis. Biochim Biophys Acta. 1998; 1365: 522-530.
2. Burgstaller JP, Johnston IG, Poulton J. Mitochondrial DNA disease and developmental implications for reproductive strategies. Mol Hum Reprod. 2015; 21: 11-22.
3. Thorburn DR. Mitochondrial disorders: Prevalence, myths and advances. J Inherit Metab Dis. 2004; 27: 349-362.
4. Smeets HJ. Preventing the transmission of mitochondrial DNA disorders: Selecting the good guys or kicking out the bad guys. Reprod BioMed Online. 2013; 27: 599-610.
5. Skladal D, Halliday J, Thorburn DR. Minimum birth prevalence of mitochondrial respiratory chain disorders in children. Brain. 2003; 126: 1905-1912.
6. Mayr JA, Haack TB, Freisinger P, Karall D, Makowski C, Koch J, et al. Spectrum of combined respiratory chain defects. J Inherit Metab Dis. 2015; 38: 629-640.
7. Chinnery PF, Hudson G. Mitochondrial genetics. Br Med Bull. 2013; 106: 135-159.
8. Cecchino GN, Garcia-Velasco JA. Mitochondrial DNA copy number as a predictor of embryo viability. Fertil Steril. 2019; 111: 205-211.
9. Jansen RP, de Boer K. The bottleneck: Mitochondrial imperatives in oogenesis and ovarian follicular fate. Mol Cell Endocrinol. 1998; 145: 81-88.
10. Babayev E, Seli E. Oocyte mitochondrial function and reproduction. Curr Opin Obstet Gynecol. 2015; 27: 175-181.
11. May-Panloup P, Boucret L, Chao de la Barca JM, Desquiret-Dumas V, Ferré-L'Hotellier V, Morinière C, et al. Ovarian ageing: The role of mitochondria in oocytes and follicles. Hum Reprod Update. 2016; 22: 725-743.

12. Duran HE, Simsek-Duran F, Oehninger SC, Jones HW Jr, Castora FJ. The association of reproductive senescence with mitochondrial quantity, function, and DNA integrity in human oocytes at different stages of maturation. Fertil Steril. 2011; 96: 384-388.
13. Murakoshi Y, Sueoka K, Takahashi K, Sato S, Sakurai T, Tajima H et al. Embryo developmental capability and pregnancy outcome are related to the mitochondrial DNA copy number and ooplasmic volume. J Assist Reprod Genet. 2013; 30: 1367-1375.
14. Huang CC, Cheng TC, Chang HH, Chang CC, Chen CI, Liu J, Lee MS. 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.
15. Cohen J, Scott R, Alikani M, Schimmel T, Munné S, Levron J, Wu L, Brenner C, Warner C, Willadsen S. Ooplasmic transfer in mature human oocytes. Mol Hum Reprod. 1998, 4: 269-280.
16. Van Blerkom J, Sinclair J, Davis P. Mitochondrial transfer between oocytes: Potential applications of mitochondrial donation and the issue of heteroplasmy. Hum Reprod. 1998; 13: 2857-2868.
17. Labarta E, de los Santos MJ, Escriba MJ, Pellicer A, Herraiz S. Mitochondria as a tool for oocyte rejuvenation. Fertil Steril. 2019; 111: 219-226.
18. Cohen J, Scott R, Schimmel T, Levron J, Willadsen S. Birth of infant after transfer of anucleate donor oocyte cytoplasm into recipient eggs. Lancet. 1997; 350: 186.
19. Barritt J, Willadsen S, Brenner C, Cohen J. Cytoplasmic transfer in assisted reproduction. Hum Reprod Update. 2001: 7: 428-435.
20. Barritt JA, Brenner CA, Willadsen S, Cohen J. Spontaneous and artificial changes in human ooplasmic mitochondria. Hum Reprod. 2000; 15: 207-217.
21. Stefano GB, Bjenning C, Wang F, Wang N, Kream RM. Mitochondrial heteroplasmy. Adv Exp Med Biol. 2017; 982: 577-594.
22. Brenner CA, Barritt JA, Willadsen S, Cohen J. Mitochondrial DNA heteroplasmy after human ooplasmic transplantation. Fertil Steril. 2000, 74: 573-578.
23. Chappel S. The role of mitochondria from mature oocyte to viable blastocyst. Obstet Gynecol Int. 2013; 2013: 183024.
24. Acton BM, Lai I, Shang X, Jurisicova A, Casper RF. Neutral mitochondrial heteroplasmy alters physiological function in mice. Biol Reprod. 2007, 77: 569-576.
25. Sharpley MS, Marciniak C, Eckel-Mahan K, McManus M, Crimi M, Waymire K, et al. Heteroplasmy of mouse mtDNA is genetically unstable and results in altered behaviour and cognition. Cell. 2012; 12; 151: 333-343.
26. Johnson J, Canning J, Kaneko T, Pru JK, Tilly JL. Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature. 2004; 428: 145-150.
27. Woods DC, Tilly JL. Isolation, characterization and propagation of mitotically active germ cells from adult mouse and human ovaries. Nat Protoc. 2013; 8: 966-88.
28. Albertini DF, Geicher N. A detour in the quest for oogonial stem cells: Methods matter. Nat Med. 2015; 21: 1126-1127
29. Woods DC, Tilly JL. Autologous germline mitochondrial energy transfer (AUGMENT) in human assisted reproduction. Semin Reprod Med. 2015; 33: 410-421.

30. Tilly JL, Woods DC. Compositions and methods for autologous germline mitochondrial energy transfer. US8,642,329. United States Patent and Trademark Office; United States Patent Number. 2014
31. Fakih MH, Shmoury MEl, Szeptycki J, dela Cruz DB, Lux C, et al. The AUGMENTSM treatment: Physician reported outcomes of the initial global patient experience. JFIV Reprod Med Genet. 2015; 3: 154.
32. Labarta E, Herraiz S, de los Santos M, Marzal A, Escriba M, Buigues A, et al. Autologous mitochondrial transfer as a complementary technique to ICSI to improve embryo quality in IVF patients. A randomized pilot study. Fertil Steril. 2019; 111: 86-96.
33. Kearns TP, Sayre GP. Retinitis pigmentosa, external ophthalmophegia, and complete heart block: Unusual syndrome with histologic study in one of two cases. AMA Arch Ophthalmol 1958; 60: 280-289.
34. Fukuhara N, Tokiguchi S, Shirakawa K, Tsubaki T. Myoclonus epilepsy associated with ragged-red fibers (mitochondrial abnormalities): Disease entity or a syndrome? Light-and electron-microscopic studies of two cases and review of literature. J Neurol Sci. 1980; 47: 117-133.
35. Pavlakis SG, Phillips PC, DiMauro S, De Vivo DC, Rowland LP. Mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes: A distinctive clinical syndrome. Ann Neurol. 1984; 16: 481-488.
36. Holt IJ, Harding AE, Morgan-Hughes JA. Deletions of muscle mitochondrial DNA in patients with mitochondrial myopathies. Nature. 1988; 331: 717-719.
37. 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: 1427-1430.
38. Ciafaloni E, Santorelli FM, Shanske S, Deonna T, Roulet E, Janzer C, et al. Maternally inherited Leigh syndrome. J Pediatr. 1993; 122: 419-422.
39. Craven L, Tang MX, Gorman GS, De Sutter P, Heindryckx B. Novel reproductive technologies to prevent mitochondrial disease. Hum Reprod Update. 2017; 23: 501-519.
40. Geraedts J. Healthy children without fear: Reproductive options for patients or couples carrying inherited diseases. EMBO Rep. 2017; 18: 666-669.
41. Sallevelt SC, de Die-Smulders CE, Hendrickx AT, Hellebrekers DM, de Coo IF, Alston CL, et al. De novo mtDNA point mutations are common and have a low recurrence risk. J Med Genet. 2017; 54: 73-83.
42. Hellebrekers DMEI, Wolfe R, Hendrickx ATM, de Coo IFM, de Die CE. et al. PGD and heteroplasmic mitochondrial DNA point mutations: A systematic review estimating the chance of healthy offspring. Hum Reprod. 2012; 18, 341-349.
43. Sallevelt SC, Dreesen JC, Drüsedau M, Spierts S, Coonen E, van Tienen FH et al. Preimplantation genetic diagnosis in mitochondrial DNA disorders: Challenge and success. Med Genet. 2013; 50: 125-132.
44. Monnot S, Gigarel N, Samuels DC, Burlet P, Hesters L, Frydman N, et al. Segregation of mtDNA throughout human embryofetal development: M.3243A>G as a model system. Hum Mutat. 2011; 32: 116-125.
45. Chinnery PF, Howell N, Lightowlers RN, Turnbull DM. Molecular pathology of MELAS and MERRF. The relationship between mutation load and clinical phenotypes. Brain. 1997; 120: 1713-1721.

46. White SL, Shanske S, Biros I, Warwick L, Dahl HM, Thorburn DR, Di Mauro S. Two cases of prenatal analysis for the pathogenic T to G substitution at nucleotide 8993 in mitochondrial DNA. Prenat Diagn. 1999; 19: 1165-1168.
47. Smeets HJ, Sallevelt SC, Dreesen JC, de Die-Smulders CE, de Coo IF. Preventing the transmission of mitochondrial DNA disorders using prenatal or preimplantation genetic diagnosis. Ann NY Acad Sci. 2015; 1350: 29-36.
48. Sallevelt SCEH, Dreesen JCFM, Coonen E, Paulussen ADC, Hellebrekers DME, de Die-Smulders CEM, et al. Preimplantation genetic diagnosis for mitochondrial DNA mutations: Analysis of one blastomere suffices. J Med Genet. 2017; 54: 693-697.
49. Richardson J, Irving L, Hyslop LA, Choudhary M, Murdoch A, Turnbull DM, et al. Concise reviews: Assisted reproductive technologies to prevent transmission of mitochondrial DNA disease. Stem Cells. 2015; 33: 639-645.
50. Wolf DP, Mitalipov N, Mitalipov S. Mitochondrial replacement therapy in reproductive medicine. Trends Mol Med. 2015; 21: 68-76.
51. 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: 383-386.
52. Zhang J, Zhuang G, Zeng Y, Grifo J, Acosta C, Shu 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.
53. 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: 367-372.
54. Tachibana M, Amato P, Sparman M, Woodward J, Sanchis DM, Ma H, et al. Towards germline gene therapy of inherited mitochondrial diseases. Nature. 2013; 493: 627-631.
55. Zhang J, Liu H, Luo S, Lu Z, Chávez-Badiola A, Liu Z. et al. Live birth derived from oocyte spindle transfer to prevent mitochondrial disease. Reprod. Biomed. Online 2017; 34: 361-368
56. Alikani M, Fauser BCJ, García-Valesco JA, Simpson JL, Johnson MH. First birth following spindle transfer for mitochondrial replacement therapy: Hope and trepidation. Reprod Biomed Online. 2017; 34: 333-336.
57. 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: 112-119.
58. Ho BX, Loh SJ, Chan WK, Soh BS. In vivo genome editing as a therapeutic approach. Int J Mol Sci. 2018; 19: E2721.
59. Leslie M. 'Old' genome editors might treat mitochondrial diseases. Science. 2018; 361: 1302.
60. Gammage PA, Viscomi C, Simard ML, Costa ASH, Gaude E, Powell CA et al. Genome editing in mitochondria corrects a pathogenic mtDNA mutation in vivo. Nat Med. 2018; 24: 1691-1695.
61. Bacman SR, Kauppila JHK, Pereira CV, Nissanka N, Miranda M, Pinto M, et al. MitoTALEN reduces mutant mtDNA load and restores tRNAAla levels in a mouse model of heteroplasmic mtDNA mutation. Nat Med. 2018; 24: 1696-1700.
62. Reddy P, Ocampo A, Suzuki K, Luo J, Bacman SR, Williams SL et al. Selective elimination of mitochondrial mutations in the germline by genome editing. Cell. 2015; 161: 459-469.
63. Yang Y, Wu H, Kang X, Liang Y, Lan T, Li T et al. Targeted elimination of mutant mitochondrial DNA in MELAS-iPSCs by mitoTALENs. Protein Cell. 2018; 9: 283-297.

64. The Lancet. CRISPR-Cas9: A world first? Lancet. 2018; 392: 2413.
65. Cohen IG1, Adashi EY2, Ravitsky V3. How bans on germline editing deprive patients with mitochondrial disease. Nat Biotechnol. 2019; 37: 589-592.