Mitochondria in Human Reproduction: Novel Paradigm in the Onset of Neurodegenerative Disorders

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Summary

The disease progression of neurodegenerative disorders (NDD), including Alzheimer's, Parkinson's and Huntington's disease, is inextricably tied to mitochondrial dysfunction. However, although the contribution by nuclear gene mutations is recognised for familial onset of NDD, the degree to which cytoplasmic inheritance serves as a predetermining factor for the predisposition and onset of NDD is not yet fully understood. We review the reproductive mechanisms responsible for ensuring a healthy mitochondrial population within each new generation and elucidate how advanced maternal age can constitute an increased risk for the onset of NDD in the offspring, through the increased heteroplasmic burden. On the one hand, this review draws attention to how assisted reproductive technologies (ART) can impair mitochondrial fitness in offspring. On the other hand, we consider qualified ART approaches as a significant tool for the prevention of NDD pathogenesis.

Key words

Neurodegenerative disease, heteroplasmy, cytoplasmic inheritance, maternal age, assisted reproductive technologies

Introduction

Born *via* endosymbiosis of pro- and eukaryotic cells more than 1.5 billion years ago [1], the mitochondrion provides several essential functions to the eukaryotic cell, most notably the capacity for oxidative phosphorylation (OXPHOS) [1]. Although most genes moved into the nucleus during evolution, a few genes remain housed in mitochondrial DNA (mtDNA). Due to the mitochondrial essentials of energetic metabolism, it is not surprising that mitochondrial dysfunction is related to a range of different pathologies, including metabolic and NDD. However, rooted in the organelles' endosymbiotic origin is a unique pathway towards disease manifestation: the cellular accumulation of mutated mtDNA known as heteroplasmy. Together, mtDNA mutations and heteroplasmy are linked to the onset of several mitochondrial diseases, including neurodegenerative phenotype, often concurrent with ageing. Here we review the reproductive mechanisms responsible for ensuring the transfer of a healthy mitochondrial population within each new generation and, taking mitochondrial heteroplasmy as a predetermining factor, elucidate how the predisposition for the onset of a mitochondrial disease with neurodegenerative phenotype later in life becomes defined during early embryonic development.

Nativity and Life Cycle of the Mitochondrion, an Organelle with its Own Personality

During the course of mitochondrial evolution, the greater part of the mitochondrial genome has become part of the eukaryotic nucleus in order to utilise its advantages. A few genes remain in the circular mtDNA and represent the mitochondrial genome, following unique rules of non-Mendelian inheritance. Although only 37 genes are encoded within the mtDNA (22 tRNAs, 2 rRNAs, 13 protein subunits of complex I, III, IV and V of the electron transport chain), representing less than 0.1 % of all human genes, the cytoplasmic genome consists of multiple copies of mtDNA (16.5 kb), tailored to the specific requirement and function of the individual cell [2,3]. In humans, the mtDNA copy number typically varies from the tens or hundreds,

going up to a few thousands for specialised somatic cells, whilst the oocyte boasts a mtDNA copy number in the range of 100 000 [4]. The cytoplasmic genome thus represents a significant bulk of genetic material with unique signs: hundreds or thousands of alleles of a mere 37 genes, disseminated throughout the mitochondrial network of the cell.

The mtDNA is packaged into a nucleoid inside the mitochondrial matrix by the multifunctional protein Transcription Factor A, Mitochondrial (TFAM) [5], which also serves as the main transcriptional activator of the mitochondria leading to mtDNA replication [6,7]. This packaging of mtDNA into nucleoid structures is thought to provide the mtDNA with protection against reactive oxygen species (ROS) and mutagens, akin to the function of the histones in the nucleus [5,7]. Leastwise this simple protection of mtDNA is so necessary, due to the proximity of mtDNA to the cellular sources of ROS. However, unlike the nucleus-carried mitochondrial genes, which are subjected to the sophisticated mechanisms of eukaryotic DNA damage repair, mtDNA has to contend with less efficient mechanisms of mtDNA repair, leaving it more prone to accumulate mutations [8]. The quality of the mitochondria-housed cytoplasmic genome thus becomes decisive regarding proper mitochondrial function and cell fitness.

With several copies of mtDNA present in the same cell, mtDNA mutation will inevitably give rise to heteroplasmy – the state of two or more different versions of mtDNA present in the same cell [9,10]. As a single mitochondrion will, on average, contain several copies of mtDNA, varying with cell type, the segregation of mtDNA nucleoids becomes a crucial mechanism for cells to identify and selectively degrade damaged mtDNA. This becomes possible through the dynamic events of mitochondrial fission and fusion. The cycle of these opposing processes results in the even distribution of mtDNA throughout the cells' mitochondrial network [10,11]. It is therefore not surprising that fission/fusion dynamics have a profound effect on cellular heteroplasmic levels [10]. The proper functioning of mitochondrial fission becomes of crucial importance, due to governing both autophagy and biogenesis [12]. The process of mitochondrial autophagy includes sophisticated machinery capable of distinguishing and destroying inferior mitochondria and, indeed, not allowing them to replicate their possibly mutated mtDNA. Mitochondrial autophagy is in turn subjected to strict co-regulation with the process of mitochondrial biogenesis, to match the cellular needs for essential mitochondrial functions [13]. In general, sufficient total mitochondrial material, and mtDNA in particular, is required for successful mitochondrial biogenesis. Factors controlling mtDNA replication are thus extremely important, the failure of which is a frequent cause of mitochondrial disease, including neurodegenerative phenotype [6,14,15]. At the same time, mtDNA replication becomes a source of mtDNA mutations [8], commonly including deletions of large segments of essential mitochondrial genes [8]. Thus, failure during either mitochondrial autophagy or biogenesis can result in the cellular accumulation of heteroplasmic variants. A mitochondrial disorder is manifested when a critical threshold of heteroplasmy is exceeded, presumably due to improper assembly of the electron transport chain and the resultant bioenergetic deficiency [9,10,16].

Whereas mitochondrial dysfunction determines the health of both the individual cell and subsequently the organism based on the life span of somatic cells [17], mitochondrial fitness is especially crucial in gametes, oocytes and sperm. Indeed, mitochondrial biogenesis is critical for oocyte fitness, while the cytoplasm increases its volume many times through oocyte growth, and later developmental competence, due to the importance for fertilisation and the first few days of embryonic development [18]. In addition, another unique feature accompanies the oocyte: the mitochondrial cytoplasmic genome becomes a subject of inheritance, working outside of common rules and undergoing non-Mendelian genetics. Due to all these facts, the oocyte becomes remarkable for developmental biology, reproductive biology, and

medical genetics. Considering the mitochondrial contribution to the health of the individuum and cytoplasmic inheritance, this review presents a novel insight into the pathogenesis of NDD from the reproductive point of view.

Winner Takes All: Maternal Mitochondria Decide on Oocyte Quality

Mammalian mitochondria are naturally inherited through the maternal lineage [19], whilst the sperm mitochondria are degraded following fertilisation [20]. This fact requires a high quality of oocyte-born mitochondrial population, including cytoplasmic mtDNA genome [21]. Indeed, the abundance and quality of the oocyte mitochondrial population decide on oocyte health and fitness, fertilisation success, and early embryonic development [22]. On one hand, the quality and quantity of mitochondria in the oocyte are a valuable biomarker of oocyte quality and essential indicators for successful fertilisation and embryo growth [23,24]. On the other hand, the study and acknowledgement of the mitochondrial contribution are essential for defining the causes of the onset and inheritance of mitochondrial disease.

The number of mitochondria naturally varies within the developmental stage of oocyte and embryo [25]. Whilst the primordial oocyte carries hundreds of mitochondria, the mitochondrial population increases massively within oocyte growth following follicle recruitment [26]. Thus, the replication of mtDNA and mitochondrial biogenesis is maintained along with the follicle growth [27,28]. Accordingly, essential factors of mtDNA replication and mitochondrial biogenesis, such as TFAM, DNA polymerase subunit gamma 1 (POLG), dimethyladenosine transferase 1, mitochondrial (TFB1M), and Twinkle mtDNA helicase (all encoded in nuclear genome) are apparently expressed in growing oocytes [22,29,30]. During oocyte growth, the mitochondrial population multiplies itself more than 1 000 times and, finally, achieves approximately 100 000 mitochondria in the human mature oocyte. As the transcription is silenced in the fully-grown oocyte, the mitochondrial biogenesis is dormant as well [31]. Surprisingly, the mitochondrial renewal is not even re-initiated as the embryonic genome is activated, and remains apparently quiet until the blastocyst stage, although mitochondrial transcription is awakened in the earlier stages of eight-cell to morula [32]. Thus, with the exception of a slight decrease in mtDNA copy number immediately following fertilization [33], a result of the process known as embryo fragmentation [33,34], the total embryo mtDNA copy number remains unchanged up until the blastocyst stage where mitochondrial biogenesis is reinitiated [33,35]. Hence, a blastocyst has about 1 000 mitochondria per blastomere, corresponding to the mitochondrial input on behalf of the ooplasmic pool.

The unique dynamics of mitochondrial biogenesis require a high quality of the mitochondrial population, covering all the essential functions: OXPHOS [36], intracellular Ca^{2+} deposit [37,38], induction of apoptosis [37,38], biogenesis of steroids [37,38], etc. Indeed, the fully-grown oocyte, matured oocyte, fertilised oocyte, zygote, and embryo until blastocyst depend on impeccable mitochondrial equipment. It is not surprising that mtDNA copy number as well as mitochondrial viability positively correlate with oocyte developmental competence [39,40]. Similarly, mitochondrial fitness is associated with an adequate copy number of mtDNA [14]. On the other hand, in the blastocyst, an increase in mtDNA copy number is suprisingly correlated with inferior mtDNA quality and lower implantation rates [41].

In addition to a suitable enzymatic apparatus of the aforementioned mitochondrial functions, the quality of mtDNA regarding oocyte fitness is decided from two points of view: i) mutation can affect the essential gene of OXPHOS and the mitochondrial capacity is impaired [42,43]; ii) mtDNA renders a maternal genetic

legacy that undergoes non-Mendelian inheritance [19]. Thus, the self-preservation of mitochondrial genetic health is crucial, especially in the mitochondrion where ROS are produced in large numbers,and a limited DNA repair mechanism is available [8]. Furthermore, as mtDNA is more susceptible to mutations during replication [7], with large-scale mtDNA deletions attributable to the replication event [8], the massive expansion in mtDNA copy number during oocyte growth offers a new possibility of incorporating damaged mtDNA into the mitochondrial pool. In addition to the mitochondrial reproduction discussed above, mitochondrial autophagy in the oocyte [44] remains the fundamental machinery to eliminate mitochondria suffering from mtDNA mutations [35].

Mitochondrial autophagy represents the well-tuned approach of a cell to marking and destroying mitochondria, using the autophagosome [45]. At first, extra-organelle labelling of transmembrane proteins functions selectively and effectively, mostly working via protein ubiquitination. Ubiquitin ligase Parkin, and PINK1, the protein responsible for recruiting it, seems to be crucial in these processes. Accordingly, point mutations of some of these mitochondrial autophagy regulators lead to deleterious disease manifestations*, e.g.* Parkinson's and Alzheimer's disease, whilst the highly-sensitive nerve system and/or skeletal muscle are affected [46-48]. The failure of mitochondrial autophagy may be due to the improper anterograde signalling of nucleus-encoded proteins. However, even a mutation of mtDNA-stored genes can be responsible for a neurodegenerative phenotype [49-50] as a mutated population of mitochondria exceeds the threshold and heteroplasmy arises [9].

Whilst heteroplasmic somatic cell/tissue may induce severe disorders with nerve and/or muscle cells suffering [51], oocyte heteroplasmy has no clinical manifestation in the oocyte donor. On the other hand, oocyte heteroplasmy becomes the subject of inheritance and oocyte-born heteroplasmy can lead to sub-/infertility, post-implantation embryo loss, or mitochondrial disease onset in offspring. Therefore, a mitochondrial bottleneck [52] seems to have been established through evolution as a tool for eliminating mutated mtDNA *via* oocyte exclusion [53]. Notably, a mitochondrial bottleneck contributes to physiological atresia during follicle development, whilst dozens of primary follicles are recruited and one oocyte is mostly ovulated [54].

In addition to the measures in place to prevent oocyte-born heteroplasmy, the oocyte is further tasked with the removal of sperm mitochondria, thus defending against biparental mitochondrial inheritance: the second source of embryonic heteroplasmy.

Persecution of Sperm Mitochondria, Maternal Inheritance and Mitochondrial Bottleneck

There is currently still major controversy over how sperm energy requirements are supplied, mainly because the mechanism is highly species-specific [55]. In humans, while many studies support glucose, the main glycolytic substrate, as the primary component for sperm ATP supply, others clearly demonstrate OXPHOS as a prerequisite for motility and sperm function [53,57]. Despite not finding consensus, the mitochondrial metabolism is superior to glycolysis in terms of ATP production, making mitochondria paramount for sperm function and thus for fertilisation to occur. Sperm mitochondria are localised in the mid-section of the tail and their activity has been traditionally associated with motility and with the acquisition of fertilising ability within capacitation [58]. However, the mitochondrial contribution ends after fertilisation and becomes an inconvenient component in the newly produced embryo.

The removal of the mitochondria that the fertilising spermatozoa contributes to (i.e. sperm mitophagy), prevents the propagation of sperm mitochondrial genes that would otherwise cause issues when two significantly different mitochondrial genomes are found in a single organism [59,60]. In addition, since mtDNA integrity is guarded by a less efficient DNA repair apparatus than the nuclear genome, mutations in its sequence are more abundant [8,61]. This becomes a casual component of degenerative diseases, ageing and cancer [61]. Therefore, eliminating the potentially corrupted paternal mtDNA not only offers developmental advantages to the preimplantational embryo, but also determines the fate of the offspring.

The process of sperm mitophagy is intricately orchestrated by the combination of two principal components: the ubiquitin proteasome system (UPS) and autophagy pathway. The UPS drives the overall protein degradation in eukaryotes and, in particular, after fertilisation, works to eliminate those proteins of the sperm mitochondrial sheath that were ubiquitinated within spermatogenesis [62,63]. This occurs via the presentation of the ubiquitinylated to the 26S proteosome by valosine-containing protein (VCP) [64,65]. Concurrently, Sequestosome-1/p62 binds to poly-ubiquitination sites on the outer mitochondrial membrane and initiates the recruitment of the autophagosome [64,65]. Once engulfed by the autophagosome, lysosomes can fuse with the mitochondria and start their digestion.

As early in the development as at the two- or four-cell stage, human embryos lose the paternal mitochondria [20,66]. However, in recent years, there have been controversial reports on the prevalence of paternal mitchondria as representing a cause of disease. The authors presented these cases as examples of bi-parental inheritance of mtDNA, but there has been considerable debate on this issue in the literature. The first case points out a mitochondrial disease whose origin was the deletion of two base pairs of mtDNA on a paternal haplotype [59]. Similarly, the transmission of a paternal haplotype through several generations has recently been reported. Intriguingly, up to 40 % of the alleles observed corresponded to that paternal haplotype without any reduction through subsequent generations [67]. Possibilities other than biparental inheritance have been proposed, where the transmission of nuclear-encoded mitochondrial sequences, instead of mtDNA alleles, created the impression of heteroplasmy [68].

Even with the elimination of paternal mtDNA, the embryo receives the oocyte-born heteroplasmic burden and, therefore, additional mechanisms become necessary for the preservation of a healthy mitochondrial pool. Accordingly, the mitochondrial bottleneck constitutes the main tool for ensuring heteroplasmy clearance occurring within embryonic development in the previous maternal generation. The clearance of the harmful mtDNA is mediated by several divisions of the germ cells and, as a result, oocytes with mitochondria containing an inferior quality of DNA are targeted for degradation [52,69]. This mechanism enables a rapid shift towards homoplasmy within a single generation [52, 70-72]. However, failures to reduce heteroplasmic levels are probable, even more so if a mutated mtDNA shows a replicative advantage. As a result, instead of the heteroplasmy burden being reduced, the level increases as women age, possibly exceeding the threshold that leads to disease progression [73].

Embryonic Heteroplasmy and maternal age on the Road to Neurodegenerative Disorder

Taken together, two means of the origin of mitochondrial heteroplasmy in the early embryo are considered: i) oocyte-born heteroplasmy that carries the risk of onset of mitochondrial disease; ii) biparental mitochondrial inheritance whilst sperm mitochondria survive. Obviously, mitochondrial autophagy and oocyte-driven sperm mitophagy, respectively, fail, both due to the incapacity of the responsible element – the egg. Therefore, we pay attention to the maternal age as a main risk factor of oocyte quality, such as has been previously discussed many times [74-77]. However, mtDNA mutations and embryonic heteroplasmy are not as much in the spotlight as these phenomena deserve, even though advanced maternal age brings about a higher risk of mitochondrial disease onset with neurodegenerative phenotype, due to the increased heteroplasmic burden that may be transferred to the offspring [78, 79].

An individual carries on average one heteroplasmy with an allele frequency of 1% or higher [78, 80], and at least 20% of all individuals carry disease associated heteroplasmies [80]. The detrimental effect of advanced maternal age includes an increase in the prevalence of disease associated mtDNA mutations in the offspring [78], and the mutational rate of mtDNA across the entire female germline has been estimated, at 1×10^{-8} mutations per site per year, an order or magnitude higher than for nuclear DNA [78]. Furthermore, a large-scale study conducted in mice found that the variance in the heteroplasmy levels of both oocytes and offspring increased with maternal age [79], in effect increasing the risk of the offspring being born with heteroplasmies at pathological threshold levels [9,16,79].

A broad definition of NDD, mostly based on the observed phenotype, allows the inclusion of mitochondrial disease into this set of diseases. Similarly, mitochondrial disease is widely defined as a disorder caused by mitochondrial dysfunction, regardless of the real cause and pathogenesis. To distinguish a mitochondrial disease from NDD seems to be impossible, as mitochondrial failure leads to the damage of nerve tissue, one of the most sensitive systems to energy insufficiency [81]. Accordingly, several mentioned disorders are induced by mtDNA mutations, often preceding many years before the phenotype is manifested [44,82]. Thus we consider, based on our best knowledge and the facts recorded to date, the relevance of the mitochondrial genome of the oocyte regarding several factors: firstly, the mitochondrial genome renders a huge mutation-prone population, whilst the risk increases in a time-dependent manner; secondly, cell-cycle-arrested oocytes are long-lived, unlike most somatic cells; furthermore, since the maternal age can be increased through assisted reproductive technologies (ART), the accumulated mtDNA mutations is similarly increased; finally, the egg and embryonic heteroplasmy do not mean any burden for parents in the reproductive age, in contrast to the enormous risk to offspring, making prevention exceedingly elusive.

Indeed, mitochondrial diseases, such as Leber's hereditary optic neuropathy, Pearson's syndrome, and Leigh syndrome, affect paediatric patients with severe NDD and are accompanied by high morbidity [83]. While mitochondrial dysfunction is linked to several NDD's [84], the extent to which it should be viewed as a cause or result of disease manifestation is in many cases being debated by the scientific community. In the case of the dreaded multifactorial Parkinson's and Alzheimer's Disease, several studies suggest a link between mtDNA haplogroups and risk of disease onset [46,50,85], and mtDNA mutations has been suggested as to play a role in the pathogenesis of these disease [85]. As we have outlined in this review, the mutation of nucleus-encoded genes *Tfam*, as well as *Parkin and Pink,* lead to the phenotype of neurodegeneration, underlining the importance of both mitochondrial biogenesis and clearance for

disease onset [6,46-48]. These gene-encoded NDD are called 'familiar' with earlier onset than 'sporadic' being achieved; although familiar forms are less common [86]. Thus, we take heteroplasmy and/or bioenergetic deficiency to be components of the aetiology of familiar NDD, and assume an increased risk of these with maternal age, absent of any form of ART treatment.

Thus, maternal age may offer an increased risk of NDD through accumulation of mutations and decreased fitness in oocyte, as outlined in figure 1.

Concurrently, with advanced maternal age, ART is often applied to cure subfertility or infertility. In addition to the advanced maternal age that is allowed *via* ART, the hormonal stimulation leads to non-physiological ovulation of more than one oocyte. It is assumed that the ovarian apparatus of follicle atresia is bypassed, and we can expect some deviation in both euploidy of these oocytes [87] and/or mitochondrial genetic quality [53]. Finally, it is still unclear how some form of ART, e.g. invasive intracytoplasmic sperm injection (ICSI), can modulate epigenomes and cause other subtle modifications of nucleic acid and proteins with impactful effect on the generation of offspring. Indeed, failure to eliminate paternal mitochondria in the embryo is observed when poor quality oocytes are used for ART [88] and sperm quality also may need to be reassessed as a contributing factor to embryonic heteroplasmy when using ICSI [89].

In summary, there is a need to acknowledge the origin of heteroplasmy and concomitant disease manifestation, using a proper model of knock-out mouse strains, human biomonitoring, and epidemiological studies. Experimental and biomonitoring data should be followed by the innovation of embryonic genome assessment when the mtDNA genome is taken into consideration. Likewise, mtDNA screenings of neonates may offer early opportunity for preventive and therapeutic strategies [84,90] absent ART. However, although ART can unknowingly promote the onset of mitochondrial disease, a proper approach to ART can eliminate it *via* genetic testing and selection.

Concluding Remarks and Perspectives

As discussed in previous sections, both nuclear and mtDNA inheritance must be considered as defining of an individual's predisposition to developing a mitochondrial disease. Furthermore, advanced maternal age must currently be recognised as a risk factor. Comprehensive epidemiological studies are recommended to further inform ethical decisions regarding pre-emptive ART treatments.

Mitochondrial gene editing in the oocyte pool has been proposed as a treatment to prevent mitochondrial disease in the offspring [91]. However, gene editing which targets the mitochondrial genome comprises several challenges that need to be overcome and is currently not feasible [91]. Instead, mitochondrial replacement therapy (MRT) involving nuclear transfer, or "3-parent babies", has already seen its use as advanced ART, whereby females with mitochondrial diseases can produce a healthy child [92,93]. Earlier attempts at MRT had included mitochondrial donation via ooplasmic transfer, whereby the ooplasm of a donor oocyte was transferred into the oocyte of a patient [18,77,94]. However, while this technique was briefly in use as a fertility treatment after repeated embryo implantation failure in a patient undergoing IVF treatment [18], it was later suspended by the FDA due to concerns about mitochondrial disease in the offspring [18]. Indeed, a significant drawback of ooplasmic transfer and mitochondrial donation techniques such as autologous germline mitochondrial energy transfer (AUGMENT) [77] is that the

heteroplasmies of the patient is not removed. In contrast, nuclear transfer techniques involve the transfer of either the nuclear genome from a patient oocyte into the enucleated oocyte of a donor, as is the case for polar body transfer (PBT), maternal spindle transfer (MST), and germinal vesicle transfer (GVT), or the transfer of a zygote pronuclei into an enucleated donor zygote, known as pronuclear transfer (PNT) [18]. However, while these methods offer a chance for women with mitochondrial disease to have a family, they also raise several sociological and ethical concerns [94,95], and have seen limited use since the first baby was born by MST in 2016 [92]. Critics mainly point to the modification of the human germline that the technique involves [93,95], although to ignore the long-term consequences on the health of the offspring represents a compelling reason for distrust as well [93,95]. From a legal perspective, this technology remains forbidden or heavily restricted in the USA, New Zealand, Australia, Singapore and most of Europe, while the United Kingdom authorises the procedure when women a deemed at high risk for transmitting mutations leading to mitochondrial disorders [92]. While MRT still remains questionable, the inherited risk of using ART as a fertility treatment in cases of advanced maternal age must thus be acknowledged, and properly addressed by including the mtDNA in genetic screenings. On the other hand, preimplantation genetic testing of the embryo, already routinely performed as part of ART [96], offers an advantageous opportunity to screen for mtDNA heteroplasmy, since the heteroplasmic burden can be evaluated for each embryo individually.

Thus, qualified ART approaches constitute a powerful tool to prevent NDD pathogenesis and should be considered as a pre-emptive treatment, along with ethical implications, when a significant risk of mitochondrial disease is expected.

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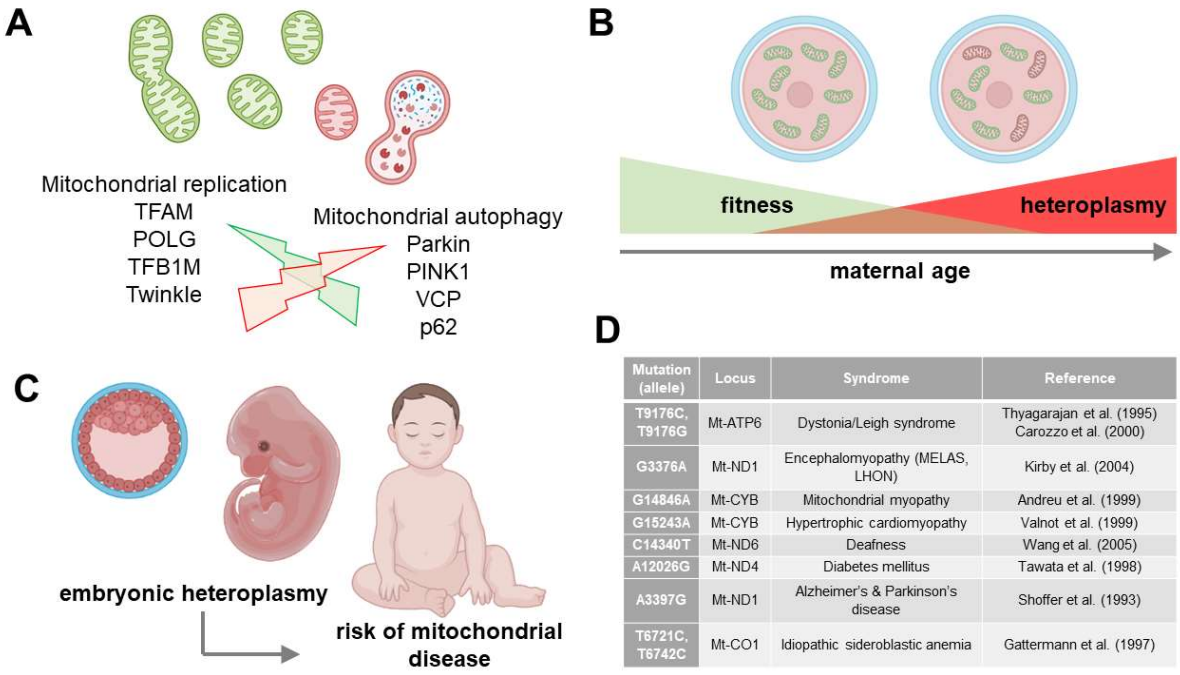

Mutation (allele)	Locus	Syndrome	Reference
T9176C, T9176G	Mt-ATP6	Dystonia/Leigh syndrome	Thyagarajan et al. (1995) Carozzo et al. (2000)
G3376A	Mt-ND1	Encephalomyopathy (MELAS, LHON)	Kirby et al. (2004)
G14846A	Mt-CYB	Mitochondrial myopathy	Andreu et al. (1999)
G15243A	Mt-CYB	Hypertrophic cardiomyopathy	Valnot et al. (1999)
C14340T	Mt-ND6	Deafness	Wang et al. (2005)
A12026G	Mt-ND4	Diabetes mellitus	Tawata et al. (1998)
A3397G	Mt-ND1	Alzheimer's & Parkinson's disease	Shoffer et al. (1993)
T6721C, T6742C	Mt-CO1	Idiopathic sideroblastic anemia	Gattermann et al. (1997)

Figure 1: Maternal age increases risk of NDD through accumulation of mutations and decreased fitness in oocyte. A) Genetic mitochondrial health is sustained due to mitochondrial biogenesis and autophagy. These two divergent strengths are responsible for the maintenance of mtDNA copy number and the elimination of mitochondrial mutations, respectively. Damage of fine network of signal pathways increases the risk of mtDNA mutation; therefore, advanced maternal age and genetic burden are considered as factors of oocyte-born heteroplasmy. B) Oocyte heteroplasmy increases with age as mutations accumulate over time, along with the failure of regulative mechanism of mitochondrial biogenesis and autophagy. Although a decrease in oocyte fitness with heteroplasmy onset is assumed, the subliminal mutation level and/or ART allows heteroplasmies to pass through the bottleneck. C) Heteroplasmy may be further worsened by the embryonic bottleneck due to replicative advantage of fused mtDNA plasmids. Finally, embryonic heteroplasmy leads to increased baseline tissue heteroplasmy, potentially resulting in mtDNA-encoded mitochondrial disease. D) Majority of mitochondrial diseases show NDD phenotype once the threshold of heteroplasmy for disease manifestation is reached, mostly in nerve and muscle tissue [42-44,49,50,97-100]. The scheme was created with BioRender.com.