Sperm Mitochondrial DNA

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Abstract

Human mitochondrial DNA (mtDNA) is 16.6 kb in size and resides in the mitochondrion. It encodes 13 of the subunits of the electron transfer chain that generates the vast majority of cellular ATP through the process of oxidative phosphorylation (OXPHOS). The importance of OXPHOS to sperm motility and function has been controversial. However, we present a case for the importance of OXPHOS in sperm function based on the effects that pathogenic mtDNA mutations and deletions have on sperm motility and function and how they are descriptive of certain forms of male subfertility. We also describe patterns of inheritance for the mitochondrial genome and how the elimination of sperm mtDNA in mammals prevents the transmission of mutant/deleted mtDNA to subsequent generations but when there is leakage it leads to a severe phenotype. This is also portrayed in the light of how mtDNA copy is reduced during the later stages of spermatogenesis and how reduced mtDNA copy number in the mature spermatozoa is indicative of good-quality, not poor-quality spermatozoa.

Keywords

Mitochondrial DNA • Replication • Oxidative phosphorylation • Polymerase gamma • Spermatozoa

What is mtDNA?

The human mitochondrial DNA (mtDNA) genome is approximately 16.6 kb in size [1] (Fig. 6.1) and is located in the inner membrane of the mitochondrion. It consists of a heavy (H) strand and a light (L) strand, which encode a total

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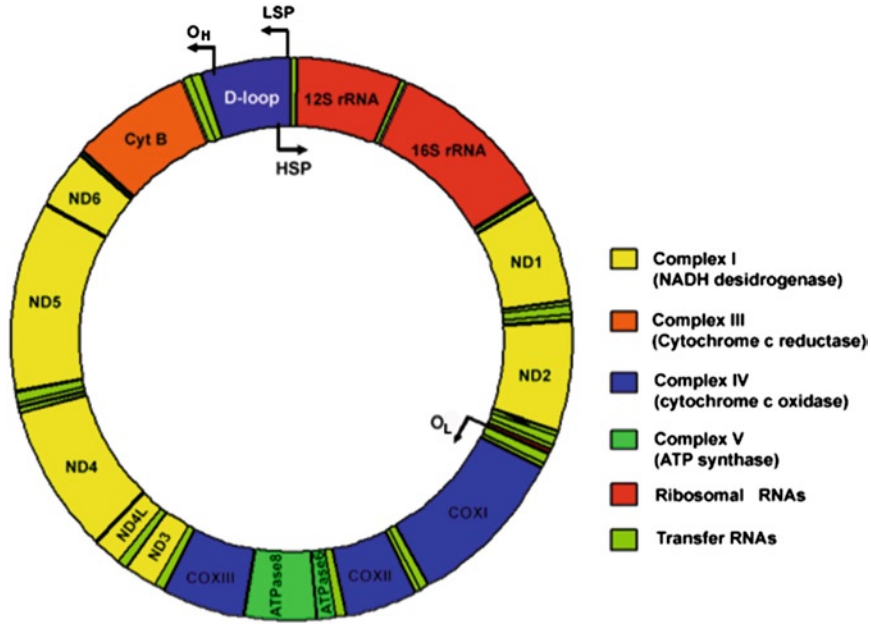


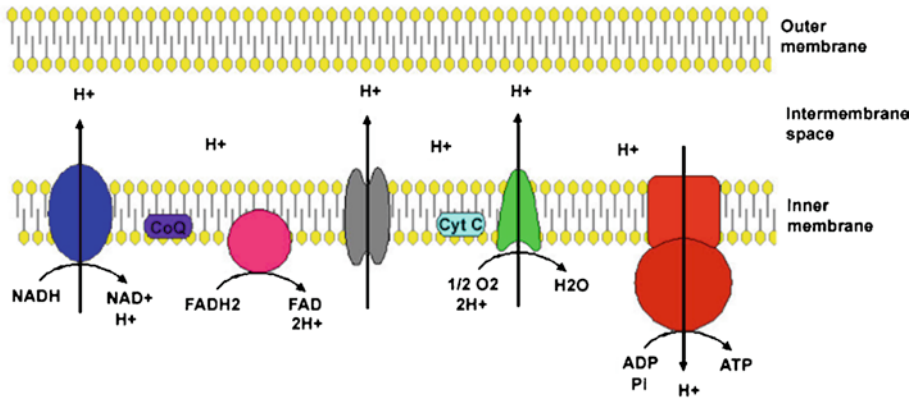
Fig. 6.1 The human mitochondrial genome. mtDNA encodes 13 of the subunits residing in four of the complexes of the ETC. It comprises a heavy (H) strand, which encodes 12 of these subunits along with 14 tRNAs and the 2 rRNAs, and a light (L) strand, which encodes one subunit (ND 6) and 8 tRNAs. The D-loop houses the H-strand origin of replication (O_H), the H- and L-strand promoters (LSP) and conserved sequence boxes. The D-loop is the only region of mtDNA that is not transcribed. However, it is the location of two hypervariable regions that can identify individuals from the same maternal lineage through molecular fingerprinting. O_L L-strand origin of replication

of 13 proteins associated with the subunits of Complexes I, III, IV and V of the electron transfer chain (ETC), the biochemical process that generates the vast majority of cellular ATP [2] (Fig. 6.2). The mitochondrial genome also encodes 22 tRNAs and 2 rRNAs (Fig. 6.1), thus contributing some, but not all of the transcription and translational machinery that is required for transcription and protein synthesis (Fig. 6.3). This demonstrates the importance of the symbiotic relationship between the cell and the mitochondria. The tRNAS are interspersed between most of the coding genes, while the coding regions for ATPase 6, ATPase 8 and ND4, and NDL4 overlap [1]. Furthermore, some of the genes do not have sequences for termination codons, which are thus generated through post-transcriptional polyadenylation [3].

There is one non-coding region of 1,121 bp, known as the displacement (D)-Loop (Fig. 6.1). This multifunctional control region is the site for interaction with the nuclear-encoded transcription and replication factors, which ensure efficient transcription and replication of this genome [4, 5]. Within the D-Loop (Fig. 6.1), there are two hypervariable (HV) regions, HV1 and HV2 [1] which contain specific sequences that distinguish distinct maternal lineages from one another. These regions are used by forensic scientists to determine perpetrators of crime [6] and to identify unidentified remains [7]. HV1 and 2 are also used to determine patterns of mtDNA transmission in offspring derived through fertilisation protocols and a range of assisted reproductive technologies including cytoplasmic transfer [8] and nuclear transfer [9–11].

Why is mtDNA Important?

The 13 subunits of the ETC encoded by the mtDNA genome are key components contributing to the process of oxidative phosphorylation (OXPHOS; Fig. 6.2). OXPHOS generates



Complex	I	II	III	IV	V
nDNA subunits	>18	4	8	10	10
mtDNA subunits	7	0	1	3	2
Inhibitors	Rotenone		Antimycin A	KCN	Oligomycin

Fig. 6.2 The electron transfer chain. The subunits for each of the complexes of the ETC, except for Complex II, are encoded by both the mitochondrial and chromosomal genomes. ATP is generated by electrons passing along each of the complexes. Protons are pumped across the inner mitochondrial membrane to establish an electrochemical gradient whilst molecular oxygen reacts with protons to generate H_2O. This process generates sufficient energy to support ATP synthesis. *nDNA* nuclear DNA; *mtDNA* mitochondrial DNA; *KCN* potassium cyanide

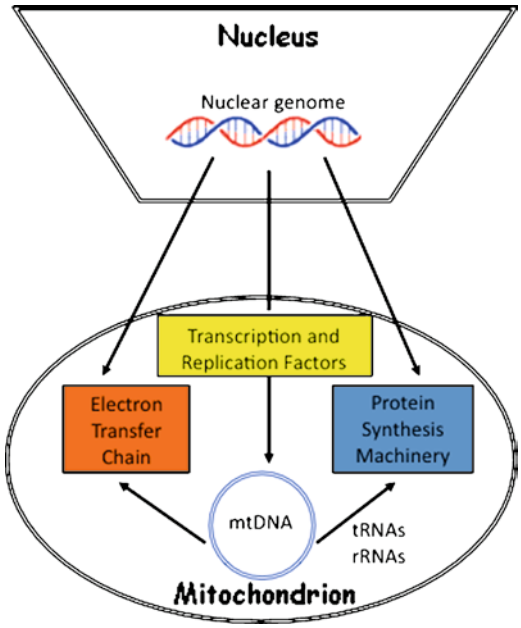


Fig. 6.3 Nucleo-mitochondrial interactions. MtDNA is reliant on nuclear-encoded transcription, replication and translation factors to generate proteins for the ETC. Likewise, the nucleus is dependent on the mitochondrial genes to contribute proteins to the ETC. This symbiotic relationship ensures that there is sufficient cellular energy so that the cell can perform its specific functions

32 molecules of ATP to every 2 produced through glycolysis but is highly dependent on substrates generated through the other anaerobic biochemical processes, such as the Krebs cycle and β-oxidation, and utilises these fuels in an O_2-mediated process [2]. This form of metabolism is especially essential for cells with high aerobic energy requirements, such as neurons and skeletal muscle [12]. The remaining 70+ genes of Complexes I, III, IV and V and all of the genes of Complex II are encoded by the chromosomal genome (Figs. 6.2 and 6.3), which translocate to the mitochondrion through a variety of import and chaperone proteins [13]. This again highlights the symbiotic nature of the mitochondria and the cell.

Until recently, it has been purported that all copies of mtDNA within an organism are identical and thus homoplasmic [14, 15]. However, the recent advances in sequencing technologies, and specifically deep sequencing, have demonstrated that many individuals have variable levels of polymorphic variants that contribute to wide-ranging levels of heteroplasmy [16]. Some of these mutations contribute to the genetic basis of

hereditary mitochondrial disorders. These include mitochondrial myopathy, encephalopathy, lactic acidosis and stroke (MELAS) syndrome [17], neuropathy, ataxia and retinitis pigmentosa (NARP; [18]), Leber's hereditary optic neuropathy (LHON; Wallace et al. [19]) and myoclonic epilepsy and ragged-red fibre (MERRF) syndrome [20]. Single point mutations in the mtDNA coding regions have been identified in all these disorders except MERRF syndrome [20], which results from an A→G substitution in the mitochondrial tRNA(Lys) gene. Furthermore, a single large-scale deletion of 4,977 bp is indicative of Kearns–Sayre syndrome [21], whilst multiple deletions ranging from 2 bp to >10 kb have been observed with ageing [22].

Generally, the phenotype for each of these diseases is determined by the degree of mutant to wild-type (WT) loading within the affected tissue, except in the case of LHON, where the mutation is usually homoplasmic and other factors, such as the sex of the individual, modify the phenotype, suggesting a role for *trans*-acting nuclear genetic factors in this disease [23]. Nevertheless, 10–15% of LHON carriers are thought to be heteroplasmic with the threshold for onset of the disease phenotype being 60% [24]. In MERRF, over 85% mutant loading is typical [25], while in severe multisystem disorder and respiratory chain deficiency syndrome, only 25% mutant loading is required to induce a dramatic phenotype [26]. These contradictory findings may be due to analysis of mutant loading in cybrids using mature differentiated cells, and thus, do not incorporate the period during differentiation, when mtDNA mass accumulates. Whilst studies in mouse models assess events during differentiation and development, they rarely include single cells or specific lineages, thus obscuring significant molecular events. However, one recent study using embryonic stem cell fusion approaches, whereby mutant mtDNA is transferred into mtDNA-depleted embryonic stem cells, has indicated that neuronal differentiation is affected by the mutant mtDNA loading [27], and this may have significant implications for spermatogenesis.

OXPHOS and Sperm Function

In comparison to the mature oocyte and somatic cells, mature mammalian spermatozoa have very few mitochondria, where 22–28 mitochondria are isolated in a helical manner in the midpiece [28]. This is unlike somatic cells where larger numbers of mitochondria are located in the cytoplasm and they have very dynamic roles which are influenced by, amongst other factors, the stage of the cell cycle [29]. Indeed, during spermatogenesis, mitochondria are located within the cytoplasm of these precursor cells [30]. However, a physical relocation takes place during spermiogenesis, just as when the transition between the acrosome and Golgi apparatus takes place [31]. Over the last 30 years, there has been a great deal of debate as to whether these few sperm mitochondria contribute greatly to sperm function, especially as they appear to be isolated in the mature spermatozoa that encapsulates them through rigorous disulphide bonding [32].

The significance of OXPHOS-derived ATP as opposed to anaerobically derived ATP in cells is generally determined using inhibitors that target the specific complexes of the ETC. In a number of classic experiments performed in the 1970s and 1980s, Storey and colleagues overcame the problem of isolating sperm mitochondria by rupturing the cytoplasmic membranes [33–38]. This enabled them to determine the respiration rates and levels of oxygen consumption to predict whether OXPHOS was vital for ATP production and thus motility. Their findings were species-specific, where the requirement for OXPHOS-derived ATP was dependent on the glucose concentration of the female reproductive tract. Nevertheless, in human spermatozoa, we have shown that by using the mitochondrial specific inhibitors, rotenone, potassium cyanide and oligomycin, and culturing spermatozoa in a 2-mM glucose environment, which is indicative of the glucose concentration in the female reproductive tract [39], sperm motility was significantly reduced [40]. However, when spermatozoa were cultured in classic sperm culture media, namely, with 5 mM glucose, it was evident

that sperm mitochondrial function was not severely hindered and that spermatozoa could utilise the glucose effectively for motility. Other investigators have also demonstrated an association between the OXPHOS inhibitors for respiratory Complexes I, III and IV and sperm motility [41], and an association with the performance of these complexes and asthenozoospermia [42]. Furthermore, biochemical studies on sperm from a patient harbouring a maternally inherited mtDNA mutation associated with Complex I have shown that the addition of succinate, which enters the ETC at Complex II, increases sperm motility significantly and bypasses the effects of the mutation [43].

The Mitochondrial Nucleoid

In somatic cells, it is thought that the mitochondrial genome persists in multimeric form within the mitochondrion. This would explain the large number of mitochondrial copies that have been observed in somatic tissues, such as cells from skeletal and cardiac muscle which possess 3,650 ± 620 and 6,790 ± 920 mtDNA copies/cell, respectively [44]. These multiple copies of the genome are anchored in the mitochondrial nucleoid, which in turn is likely to be anchored to the inner mitochondrial membrane through ATAD3 proteins [45]. In both spermatozoa [46] and oocytes [47, 48], mtDNA appears to exist in monomeric form in individual mitochondria. The mitochondrial nucleoid consists of not only one or more mitochondrial genomes but also approximately 30 nuclear proteins that are involved in the maintenance and packaging of the genome along with mediating transcription and replication of the genome [49]. In terms of transcription and replication, the key factors are as follows: mitochondrial transcription Factor A (TFAM), the mitochondrial specific Polymerase Gamma (Polg), which has both a catalytic (PolgA) and an accessory subunit (PolgB), the mitochondrial specific RNA Polymerase (mtRNApol), the mitochondrial specific single-stranded binding protein (mtSSB) and the mitochondrial specific helicase, Twinkle.

mtDNA Replication

Currently, two models have been described as mechanistic approaches for the replication of the genome. These are the asymmetric [50] and the coupled leading lagging strand synthesis [51] models. These two quiet distinct mechanisms are controversial with each party disputing each other's approach in the literature [52, 53]. Until recently, the asymmetric model provided the traditional understanding of mtDNA replication (reviewed in [50]). It is initiated from the H strand origin of replication, which is located within the D loop region. In this instance, TFAM interacts with the enhancer of the light strand promoter and this generates a conformational change that exposes the promoter region to mtRNApol. Once the RNA primer has been generated, it is then employed by PolgA to initiate mtDNA replication. Mitochondrial replication then progresses two thirds round the genome to the origin of L-strand replication, which in turn triggers synthesis of the L-strands in the anticlockwise direction. The coupled leading and lagging strands synthesis method proposes that both H- and L-strand synthesis are initiated from the same initiation cluster sites with each strand being replicated in a bidirectional fashion [51]. This model also incorporates the use of replication intermediates to fill gaps within replicating DNA on the lagging strand [54]. Although the proponents of this mechanism do not argue that it is the sole mechanism, they suggest that it operates in addition to the asymmetric model whereby one mechanism would be indicative of accumulation of mtDNA mass as might be the case during the early stages of spermatogenesis, whilst the other may be associated with mtDNA replenishment following mtDNA damage or transcription.

POLG, mtDNA-Type Disease, and Sperm Function

The human chromosomal POLG gene is located at 15q24-15q26 [55] and consists of a 140-KDa catalytic subunit (POLGA) and a 54-KDa accessory subunit (POLGB; [56]). POLGA possesses a 5'-3'

exonuclease domain that ensures effective proofreading and DNA repair [57], whilst POLGB is essential for promoting DNA binding and high levels of processivity and fidelity [58, 59]. It also has a putative role in recognising the RNA primers that initiate mtDNA replication [60]. A number of missense mutations have been identified in POLG, and these are associated with large-scale mtDNA deletions and/or mtDNA depletion-type syndromes. These include Progressive External Opthalmoplegia (PEO), mitochondrial neurogastrointestinal encephalomyopathy [61–63], testicular cancer [64], Alper's disease [65–67] and Parkinsonism and premature menopause [68, 69].

POLG activity is severely inhibited by nucleoside analogue reverse transcriptase inhibitors (NRTIs), the compounds that have been used to reduce viral load in HIV-positive patients [70]. For example, the frequently used 2',3'-dideoxycytidine (ddC) can mediate near mtDNA depletion of in vitro cultured cells within a few days [71]. As a result, many HIV-positive patients suffer from mtDNA-depletion type syndromes, such as mitochondrial myopathies and neuropathies [72]. We have also shown that sperm samples from HIV-positive men treated with NRTIs can, after a 12-month period, exhibit large-scale mtDNA deletions and, after a further 6 months, result in complete loss of sperm mtDNA content, rendering the male azoospermic [73].

Characteristic to human POLG, and not to other species, is a series of trinucleotide CAG repeats ($n = 10$), located at the 5′ end, that encode for a polyglutamine tract [74]. The variability of the number of CAG repeats in, for example, the androgen receptor gene, has been proposed as an indicator and putative cause of male infertility (reviewed in [75]). This approach has also been applied to POLG where a series of reports have debated whether it is accountable for some forms of male idiopathic infertility. The initial report suggested an absence of the common allele as the homozygous mutant genotype (not 10/not 10) was observed at an increased frequency in patients presenting with moderate oligozoospermia when compared with fertile men [76]. However, there was no association with extreme oligozoospermia and azoospermia. A subsequent study based on Danish patients identified an association between the loss of the common allele and idiopathic infertility [77]. However, this was not reproducible in two separate cohorts of Italian [78] and French [79] infertile and normozoospermic fertile men. Furthermore, the French study demonstrated that over 50% of the homozygous mutant men were able to produce offspring through intercourse or following assisted reproduction [79]. In addition, as a subsequent Italian study confirmed, there was no association between allelic frequency for oligozoospermia and normozoospermic [80].

As POLG is a mediator of mtDNA replication, it would be anticipated that, as with certain mtDNA depletion syndromes, there would be an increase in either the presence of mtDNA mutations or a decrease in mtDNA copy number in men presenting with the mutant genotype. This is especially in light of studies performed on POLG knockout mice where the homozygous null phenotype is embryonic lethal and the heterozygous knockout suffers from severe mtDNA-depletion type syndrome [81]. Nevertheless, it appears that there are no differences in the numbers of mtDNA nucleotide substitutions for the different POLG CAG genotypes in both normozoospermic and non-normozoospermic men, nor were any mutations identified in the three exonuclease motifs of POLG for such patients [82]. We have, however, taken this a step further by relating gene sequence variation to protein expression and determined that oligoasthenoteratozoospermic men had significantly higher incidences of heterozygosity for CAG repeats, which was coupled to a lower percentage of spermatozoa expressing POLGA [46]. Additionally, these men had higher numbers of mtDNA copy number, which is indicative of poor sperm quality.

TFAM, mtDNA Disease, and Its Role During Spermatogenesis

Human TFAM locates to chromosome 10q21 and its protein is 204 amino acids in size. It is a member of the High Mobility Group (HMG) of proteins and consists of two HMG boxes, a linker and a mitochondrial targeting sequence [83]. Knockout studies in the mouse demonstrate that

it has either a direct or indirect role as a regulator of mtDNA copy number. The heterozygous knockout exhibits reduced mtDNA copy number and myocardial OXPHOS deficiency [84]. Homozygous null mice suffer from severe mtDNA depletion and abolished OXPHOS and are embryonic lethal. Depletion of mtDNA in cultured cells also results in decreased expression of TFAM and mtRNApol [9, 85]. TFAM also acts as a regulator of mitochondrial gene expression, [86] but when overexpressed, binds to grooves within the mitochondrial genome, thus inhibiting transcription [87], and as transcription precedes replication, replication will also be inhibited. Other studies have demonstrated that it has a clearly defined role as a packaging protein, characteristic of its HMG family members such as histones [88]. Nevertheless, TFAM is dependent on interaction with other members of the nucleoid for it to be functional. These include mitochondrial transcription factor B1 and B2 [89] and Nuclear Respiratory Factors 1 (NRF-1) and 2 (NRF-2). Indeed, TFAM's promoter possesses recognition sites for NRF-1 and NRF-2 [90, 91], and these sites possess CpG islands, which may control mtDNA transcription and replication through their DNA methylation [92].

Sperm mtDNA Replication

As with oocyte precursor primordial germ cells, male primordial germ cells will have very few copies of mtDNA. However, whilst oocytes accumulate mtDNA mass later during maturation [93], the spermatogonial stem cells maintain higher numbers of mtDNA up to the spermatocyte stage [94]. These are then subsequently reduced once meiosis II has been completed so that, as the round spermatid differentiates into an elongated spermatid, the mature spermatozoa will have tenfold less mtDNA [94]. In the mouse, this loss in mtDNA copy number coincides with the loss of TFAM possessing the mitochondrial targeting sequence that will ensure its translocation to the mitochondria [95]. Instead, its expression is replaced by an isoform that does not possess this targeting sequence, and thus, ensures that TFAM remains located in the head of the spermatozoa and cannot interact with mtDNA. In the human, this is regulated in a somewhat different manner whereby TFAM simply ceases to be expressed [96]. Nevertheless, we have observed that significantly more good-quality spermatozoa express TFAM than poor-quality spermatozoa [46].

Clinically, the regulation of mtDNA copy number during early development may have significant implications for sperm quality. In spermatozoa collected from density gradients that were indicative of progressive motility, the mean mtDNA copy number per spermatozoa was 1.4 for normozoospermic samples, 6.1 when one abnormal sperm parameter was described and 9.1 for samples with two or more abnormal sperm criteria [97]. The spermatozoa present in lower gradient layers possessed higher levels of mtDNA copy number (17.1 copies/spermatozoa). However, another study reported to the contrary, whereby normozoospermics had a mean number of 74.1 DNA copies/spermatozoon, asthenozoospermics possessed a mean of 7.2 molecules [98]. Nevertheless, we have demonstrated that sperm samples from OAT patients exhibited significantly higher mtDNA (>46) content than normozoospermics and conversely they had a lower percentage of spermatozoa expressing POLG and TFAM [46]. On the contrary, good-quality spermatozoa possessed fewer mtDNA copies (<10) but had significantly more spermatozoa that expressed POLG, TFAM and mtDNA-encoded genes. The reduction in mtDNA content in normal samples is most likely indicative of normal spermiogenesis having ensued with the increases in POLG and TFAM expression being a compensatory mechanism for low mtDNA copy number and thus ensuring a form of mitochondrial homeostasis. Similar observations have been made from mtDNA-depletion studies in somatic cells [9].

mtDNA Inheritance

Under normal circumstances, mtDNA is inherited from the population present in the mature metaphase II oocyte just prior to fertilisation.

In mammalian crosses generated from the same strain or breed (intraspecific), sperm mtDNA appears to be eliminated prior to the onset of genome activation in the newly formed embryo, namely, the 2-cell stage in the mouse and [99, 100] and 4–8 cell stages in sheep [101] and non-human primates [102]. This targeted elimination of sperm mtDNA is thought to be through ubiquitination of the spermatozoa's mitochondria [103, 104]. To this extent, it has been proposed that spermatogonial cells maintain a ubiquitin label throughout development, which is recognised by the oocyte's ubiquitination machinery once fertilisation has been initiated [103–105]. This is very similar to an innate immune reaction where foreign particles would be destroyed and, in line with present thinking, indicating a role for mitochondrial or bacterial DNA being initiators of such innate immune responses [106]. Although the ubiquitin label is maintained throughout spermatogenesis, it appears to be suppressed during maturation of spermatozoa in the epididymis, and is then either re-expressed or unmasked in ejaculated spermatozoa [107]. Nevertheless, others have demonstrated in Japanese Medaka embryos the active digestion of sperm mtDNA just after fertilisation, which proceeds destruction of the sperm mitochondria [108]. However, it remains to be determined whether sperm mtDNA elimination is specific or targeted along with oocyte mtDNA elimination during the very early stages of preimplantation development [109, 110].

This process of targeted elimination does not appear to take place in interspecific crosses (i.e. crossings between different strains or breeds) as sperm mtDNA persists, although at low levels, in offspring from a range of mammalian species [99–102]. Interestingly, however, the original sperm mtDNA contribution does not persist in subsequent generations [100], thus indicating that it is not incorporated into the germ line. Interestingly, other species do transmit sperm and oocyte mtDNA in a heteroplasmic manner. *Drosophila* transmit sperm and oocyte mtDNA to their progeny following both intra- and interspecific crossing [111]. Uniquely, mussels transmit both male- and female-specific genomes to male offspring, but female offspring possess female-specific only molecules [112, 113]. Nevertheless, normal and abnormal human embryos can fail to eliminate their sperm mtDNA [114]. When such an outcome occurs, then sperm mtDNA can recombine with oocyte mtDNA resulting in the generation of a new hybrid mtDNA molecule that segregates randomly during development [115]. This has resulted in a male patient suffering from a muscle myopathy [116] and demonstrates the selective replicative advantage that was afforded sperm mtDNA based on its 1:30,000 contribution to the zygote.

Mitochondrial DNA Variants and Their Effect on Sperm Function

Following the initial hypothesis of Cummins et al. [117], it has been demonstrated that mutations associated with a clinical phenotype, such as the A3243G mutation, have effects on sperm quality and their motility [118]. Other studies have analysed large-scale deletions, such as the 4,977 bp common deletion, as an indicator of good- and poor-quality spermatozoa. One group demonstrated a correlation between an increase in the presence of this deletion and poor-quality spermatozoa; however, its incidence was at extremely low levels (0.0032% for the 80% Percoll fractions to 0.0708% for the <50% Percoll fractions; [119]). Two other studies demonstrated that it is not a general predictor for sperm function with the deletion being just as likely to be present at similar levels in semen [120] and sperm [121] samples from normozoospermic men and subfertile patients. Two further deletions, namely, the 7,345 bp and 7,599 bp deletions, were thought to be indicative of poor motility [122], though this was not substantiated in a subsequent study [121].

The long PCR, which allows long regions of the mtDNA genome to be amplified, has been used to identify a range of mtDNA deletions. This technique works on the basis that any deletions present within the region will be amplified and appear as shorter fragments when observed on DNA gels, with the large-scale deletions being

represented as the smaller fragments [22]. This approach has been used to analyse sperm samples from a male patient with multiple deletions associated with ptosis, who also exhibited subfertility [123]. This demonstrated a range of multiple deletions, which were symptomatic of poor sperm motility. Equally so, large-scale deletions have been identified in normozoospermic and oligozoospermic men [121, 124]. The presence of large-scale deletions in normozoospermic patients would not preclude the individual from having acceptable levels of motility, as they would still have significant numbers of spermatozoa with wild-type copies present. Nevertheless, it appears that poor-quality sperm samples appear to have a greater number of multiple deletions with oligoasthenoteratozoospermic men having the greatest proportion [121]. Equally so, mutations in the nuclear-encoded mtDNA replication factors, such as Twinkle, can also lead to multiple mtDNA deletions and dysfunctional spermatozoa [125]. However, for a true representation of the number of mtDNA deletions present within a sperm sample, pure populations of sperm mtDNA need to be isolated, as the ejaculate carries a range of somatic cells that would have significantly more copies of the mitochondrial genome, and if mainly WT in composition, it would bias the outcome, thus obscuring the deletions present in spermatozoa [121].

The mechanisms inducing sperm mtDNA deletions still need to be clarified. However, a multitude of studies have indicated a relationship between mtDNA deletions and the levels of mtDNA damage, as characterised by the levels of 8-OH-dG (see, for example, [126]). Many of the large-scale deletions that have been characterised lie between flanking direct repeats, where it has been hypothesised that inefficient proofreading mediates polymerase strand-hopping, i.e., from the heavy to the light strand, resulting in large regions of the genome not being incorporated during replication [127]. 8-OH-dG is a by-product of the hydroxyl (OH) free radical, which arises from H_2O_2 and has been associated with poor sperm quality and function due to increased levels of large-scale mtDNA deletions. In this respect, sperm samples from patients with diabetes mellitus appear to have increased levels of 8-OH-dG and large-scale mtDNA deletions [128]. This outcome is further supported by an increase in the frequency of nucleotide changes in the ATPase 6 and 8, ND 2, 3, 4 and 5 genes of the mtDNA genome in infertile men due to increased levels of free radicals [129]. The sperm mitochondrial genome is likely to be more susceptible to free radical activity, as it is less well-packaged than the chromosomal genome. Consequently, early signs of DNA damage are indicative in the mitochondrial genome rather than the chromosomal genome [130].

Whilst sperm mtDNA appears to be more susceptible to mtDNA deletions, this may not only result from the presence of free radicals present within the ejaculate and the testis, but could result from a decrease in mtDNA copy number during development, where those molecules that are selected for tend to be rearrangements mediated by the nuclear background of the cell [131], such as with other high ATP requiring cells, for example neuronal and muscle cells [12]. Such a mechanism of selection would have a twofold effect: [1] sperm motility becomes dependent on glycolysis, and [2] the mitochondria are rendered dysfunctional, and thus, once they enter the oocyte, are more susceptible to mechanisms such as apoptosis when challenged to generate ATP through the ETC. Consequently, these processes may be a mechanism for ensuring that the paternal genome is not transmitted to the offspring.

Furthermore, it is likely that any mutations and deletions affecting sperm function will arise from the spermatogonial cells, rather than spermatozoa, as they could only be incorporated into the mtDNA genome following mtDNA replication. These molecules would then be randomly selected for during the process of male gamete differentiation and not at later stages when copy number is reduced. We would also hypothesise that, in poor quality spermatogonial cells harbouring rearrangements, failure to regulate mtDNA copy number is indicative of inefficient nucleo-mtDNA interaction or attempts to rescue WT mtDNA at the expense of rearranged mtDNA.

mtDNA Haplotype

It has been argued that specific sequences within mtDNA have evolved and their origins can be traced back to several mitochondrial Eves. This has generated genetic diversity and has potentially provided individual populations with mitochondrial specific genotypes, otherwise known as haplotypes, which afford them specific advantage or disadvantage for survival and function [132]. For example, specific European type haplotypes are associated with tolerance to warmer and colder climates. Other haplotypes have been associated with fertility in a range of species such as pigs [133] and cattle [134], milk quality in cattle [135] and physical performance in mice [136]. A series of studies have indicated that male patients with haplotype H are not associated with asthenozoospermia, whilst individuals with haplotype T have such a predisposition [41]. Furthermore, additional differences in both sperm motility and vitality were identified in a number of sublineages of haplogroup U, perhaps arising from highly conserved missense mutations in the cytochrome C oxidase subunit III and cytochrome B genes [137]. However, similar analysis conducted on a population of Portuguese patients suggested that subpopulation studies can also influence haplogroup association studies, although they reported negative correlations with oligozoospermia when matched with geographic balanced controls [138].

Conclusions

It is evident that OXPHOS has a role to play in mediating sperm function and motility, as demonstrated from biochemical and genetic studies. However, this role needs further defining and characterisation. Specifically, we need to determine how and when rearranged mtDNA is incorporated into the male gamete, and we need to develop elaborate quantification protocols so that we can determine how much rearranged mtDNA is actually present in such samples. We further need to determine whether mtDNA damage is likely to prove a useful clinical diagnostic marker of early-onset DNA damage, which may enable us to warn patients to make lifestyle changes early on if they wish to conceive naturally.

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