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Sperm Mitochondria-Associated Male Infertility: *Sperm Quality Defects* and Mitochondria (mtDNA) Anomalies: Review

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The main functions of mitochondria in both somatic and germ cells are related with the cellular energy production (by ATP). However, these cellular organelles also have many other functions, depending of the cell life cycle and biological activities, by participation in cellular and molecular events, as cell signalling, proliferation, differentiation and epigenetic control. The injuries in the mitochondrial structure/ultrastructure, mitochondrial genome (mtDNA), transcriptome, proteome, as well as disturbances in mitochondrial membrane potential (MMP) or altered oxygen consumption, have been correlated with loss of sperm functions, which could lead to reproductive problems. Mutations in the mtDNA have been established to be often caused of oxidative stress as a result of free radicals accumulation, but also of other patho-physiological factors, connected with respiration defects in mitochondria and to mutations in the male germ cells.

Key words: mitochondria, mtDNA mutation, spermatozoa, sperm motility, male fertility/infertility

Introduction

The number and distribution of mitochondria on spermatozoa have been characterized as species-specific features [23]. In 1999 World Health Organization (WHO) determined three main criteria for stratification of human sperm quality: spermatozoa morphology, count and motility. Recently, as additional criteria for sperm quality were proposed mtDNA amplification and mtDNA/ß-globin ratio, which could be biomarkers in male infertility [30, 35].

The responsible for sperm motility "movement apparatus" – flagellum, appears in male gametes in the period of their cell differentiation (from spermatids to spermatozoa). In human spermatozoa the mitochondria (usually 10-12/per gamete) are grouped and helically arranged in the region of sperm neck and midpiece – around the outer dense fibers (ODFs) and axoneme [15, 16, 41, 66].

Specifically the internal mitochondrion forms deep cristae from lamellar type [24, 41]. Each sperm mitochondrion carries multiple copies of the paternal mitochondrial genome (mtDNA). In experimental conditions, this paternal mtDNA could be eliminated *in vitro* inside the fertilized ovum by micromanipulation - targeted proteolysis [60].

The anchorage of the mitochondrial sheath - a complex of filaments called sub-mitochondrial reticulum [38], seems to depend on kinesin light chain 3 expression (KLC3). Recent study [67] evaluated that in transgenic male mice, expressing mutant form of KLC3 protein, an abnormal sperm differentiation, low sperm quality and reduced fertility could be registered. On the other hand, the sperm outer mitochondrial membranes (OMMs) [61] are the male gamete structures protecting sperm mitochondria (mtDNA including), and probably contributing to the vitality and improved functions of these organelles. Having in mind that most of the cytoplasm is lost during the spermatogenesis, the quantity of mitochondrial proteins and of mtDNA molecules per cell could be also reduced (paralleled by an increase in the number of mtDNA copies) [5, 14, 26].

1. Morphological and physiological changes in the midpiece of the sperm flagellum related to male infertility

The changes in the mitochondrial integrity/functionality, namely defects in mitochondrial structure/ultrastructure, mitochondrial genome (mtDNA), transcriptome, proteome, as well as disturbances in mitochondrial membrane potential (MMP) or altered oxygen consumption, have been correlated with loss of sperm functions (particularly with decreased spermatozoa motility) [1]. Mitochondria in spermatozoa differ from these in somatic cells in their morphological and biochemical characteristics. The biochemical differences are mainly related to the existence of specific isoforms of enzymes. The morphological and ultrastructural studies of spermatids and spermatozoa

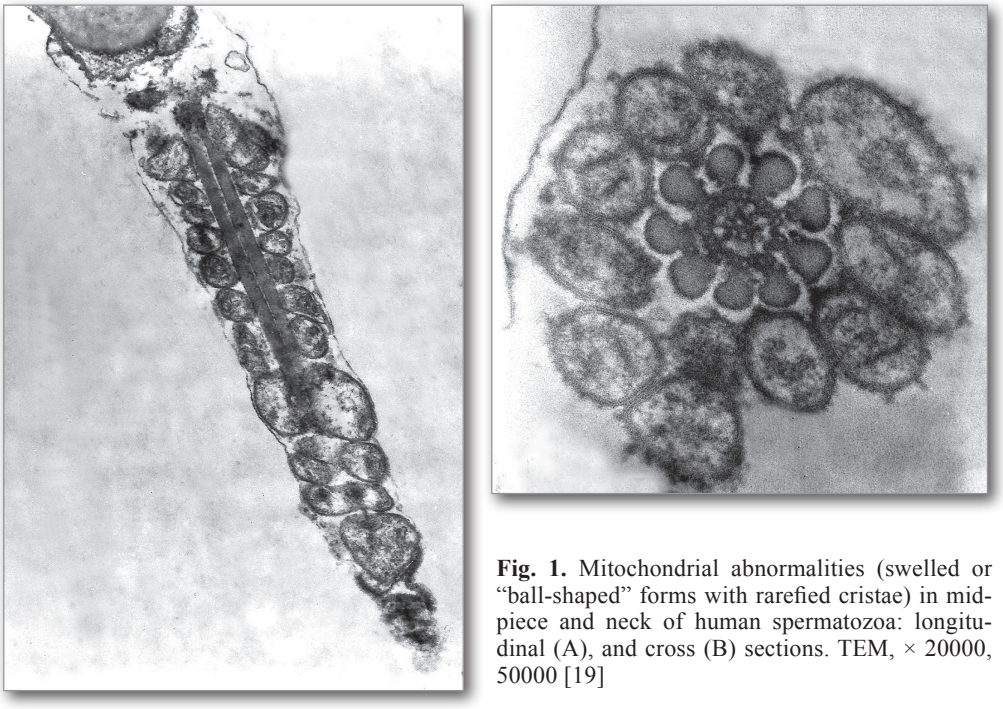

Fig. 1. Mitochondrial abnormalities (swelled or "ball-shaped" forms with rarefied cristae) in midpiece and neck of human spermatozoa: longitudinal (A), and cross (B) sections. TEM, × 20000, 50000 [19]

reveal many injuries, affecting the mitochondrial sheath formation. Disturbances in the ultrastructural components of spermatozoa midpiece were established: changes in the mitochondria number, loss of cristae, lack of mitochondrial membranes, asymmetry in the mitochondria size and distribution have been also observed (**Fig. 1**) [2, 19]. Morphological and biochemical changes lead to the sperm functional disturbances as male gamete hypokinesia and/or asthenozoospermia [8, 25]. The destructive changes in mitochondria could affect the spermatozoa motility, mainly as a result of ATP-synthesis or even because of its lack [25].

The spermatozoa malformations, as "short", "thickened" or "thinner" tails, could be usually related with mitochondria fission and fusion - leading to the increased mitochondrial mass in the spermatozoa midpiece, or with the decreased size - to the full lack of mitochondria [15, 19, 20]. Mundy et al. [34] and Pelliccione et al. [40] described spermatozoa from asthenozoospermic patients as male gametes having short midpieces and few mitochondrial gyres, disordered mitochondria - with swollen inter-membrane spaces, as well as with scattered and disorganised cristae, etc. The spermatozoa defects described in the region of tail [4, 8, 13, 19, 24, 34, 40] could lead to mitochondrial dysfunction as a reason for the low sperm motility and subsequent male infertility.

2. Mitochondria as spermatozoa energy sources and motility forces in male fertility/infertility

The main functional role of mitochondria in the somatic and/or germ cells is related to the cellular energy productions. Recently, mitochondria have been identified as organelles participating in cellular and molecular events, such as cell signalling, cell cycle - proliferation, differentiation, epigenetic control and regulation, etc. [36]. Sperm mitochondria also have specific functional characteristics, closely associated with the spermatozoa motility: spermatozoa resistance to hypotonic conditions and their ability to use lactate as an oxidative substrate [37].

Though sperm receives cellular energy by the glycolytic pathway, spermatozoa are also dependent on oxidative cellular metabolism for its normal physiology [54]. Oxidative metabolism, energy production (by ATP) and free radical generation (ROS) are the main biological reactions, occurring inside the sperm mitochondria. In addition, mitochondria participate in processes of apoptosis and Ca^{2+} homoeostasis. Important phenomenon for reproductive biology is that mitochondria participate in the steroid hormone biosynthesis [44]. As the mitochondrial energy metabolism is a key factor supporting several sperm functions, these organelles host critical metabolic pathways during germ cell development and fertilization [42].

According to data of many authors [3, 11, 47], mitochondria play a pivotal role as bioenergetic sources for spermatozoa vitality, and motility. The ability of mammalian spermatozoa "to swim" is acquired during their "epididymal transit", but it could be observed only upon sperm dilution with seminal plasma fluid at the time of ejaculation [64]. According to the studies of Mortimer [32], the spermatozoa motility force is generated mainly in the tail, in which the binding cytoskeleton components are responsible for both - modulation and performance of this process. The mitochondria provide necessary energy (by production of ATP and the protein dynein) [12, 27]. Glyceraldehyde 3-phosphate dehydrogenase-S (GPDHS) a sperm-specific glycolytic enzyme, appears to be responsible for up to 90% of the ATP synthesis in the spermatozoa, and thus, for their motility maintenance [31]. Confirming the importance of glycolysis in sperm motility, it was shown [28] that porcine and mouse sperm produces an important fraction of ATP anaerobically (mainly via the glycolytic pathway). According other studies [31, 33], the latter energy-generating pathway is more important for sperm motility. This unique feature of the spermatozoa to use different substrates and hence, to activate

different metabolic pathways is closely related to the mitochondrial functional plasticity and is very important for the process of fertilization (insemination) [42]. In some patients with astenozoospermia, the tail length of spermatozoa and their mitochondria volumes correlated with the intensity (frequency) of the vibrations (movements) of the flagellum. The positive correlation between the mitochondria number and spermatozoa motility has been also described [3].

The role of the NADH-tetrazolium-reductase system (NADH-TR) as a biomarker of mitochondrial activity was determined [7, 18, 63, 65]. The data from cytochemical studies on the NADH-TR activity in spermatozoa have been established as well as differences in the enzyme activity on dependence of mitochondrial structure and function [18]. According to the results, received by Edvinsson et al. [7], and Yonkov [65], the activity of NADH-TR system is stronger in spermatozoa from normospermic ejaculates compared with lower enzyme activity in male gametes of patients with astenozoospermia.

Biochemical studies on the activity of NADH-TR system in the sperm mitochondria (from ejaculates of patients with inflammatory diseases - mumps orchitis, prostatitis chr. and epididymitis chr.), indicated two bio-parameters, characterizing the functional status of the male germ cells [63]. First parameter shows the percentage of spermatozoa containing mitochondria with active enzyme systems, and the second one is related to the coefficient of spermatozoa deviation – according to the changes in their enzyme activity. The results demonstrated close relationships between spermatozoa motility and activity of NADH-TR system in their mitochondria.

The investigations, performed by Ruiz-Pesini et al. [47], also evaluated the relationship between the energy production by mitochondria and spermatozoa motility. The susceptibility of the male gametes to the influence of ROS [6], xenobiotics [51], mutations in the mtDNA [22], and other toxic factors influencing physiological status of spermatozoa have been analyzed in medical scientific literature. Spermatozoa, containing defective mitochondria and producing less efficiently ATP, generate reactive oxygen species, which may further cause oxidative stress and damage mitochondrial genome (mtDNA), leading to cellular (male gametes) energy crisis with subsequent decline of spermatozoa motility and male fertility. Additionally, the oxygen consumption (as a result from the effectiveness of the mitochondrial respiratory chains) [9, 55], as well as the influence of many different inhibitors of the electron transfer chains (ETCs) [49, 56], on the motility of spermatozoa, should be further clarified in relationships to male fertility/infertility.

3. Injuries in sperm mitochondrial DNA and male infertility

There is evidence that mitochondrial DNA anomalies in the sperm of mammalian and humans may lead to the male infertility. Point mtDNA mutations, deletions and the presence of mtDNA single nucleotide polymorphisms, as well as of specific mtDNA haplogroups have been associated with low sperm quality [17, 30, 49, 58]. In human sperm, the deletions in mtDNA are associated with a decline in sperm motility and fertility [10, 54]. On the cellular and molecular level, deletions in the mtDNA have been shown to influence the cellular homoeostasis, which could result in reduced sperm functionality and thus - male infertility in mammalian and humans [35, 56].

Folgero et al. [10] first reported data on the reduced sperm motility in individuals with mtDNA defects in spermatozoa: 4977bp deletion in the mitochondrial genome was described to correlate with spermatogenic failure [4].

Mutations at the level of the mitochondrial DNA-polymerase gamma (DNA-Pol-γ) locus were also typified as sperm quality defects associated with male infertility [46]. Concerning specific point mutations/deletions in spermatozoa, it seems consensual that

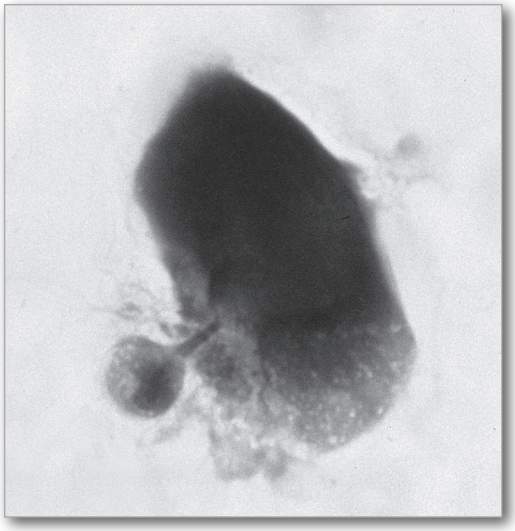

Fig. 2. Fertilization *in vitro:* fertilization cone (protuberance) was formed on the mouse ovum in response to sperm contact and penetration. Methylene blue-fast green (× 450) [*]

the accumulation of multiple mtDNA rearrangements could be associated with loss of normal sperm function. On the other hand, low-quality human sperm has shown an abnormal mtDNA copy number [56, 57]. MtDNA mutations in the ATP generating genes recently demonstrated that mtDNA changes could impair sperm motility [50]. Apart from single nucleotide base substitutions, large deletions in mitochondrial genome have been reported in infertile individuals. Increased levels of mtDNA point mutations and deletions reduce by apoptosis the spermatozoa lifespan [35, 62].

In opposite to the oogenesis, which is associated with a strong amplification of mtDNA copy numbers, the spermatogenesis is related with a drastic reduction in mtDNA content [14]. This mtDNA reduction mainly occurs when the rounded spermatids take on an elongated shape. On the molecular level, the reduction of mtDNA content is due to the down-regulation of the nuclear-encoded mitochondrial transcription Factor A, which is the main cellular factor controlling mtDNA copy number [26]. The reduction in the mtDNA content, together with the action of a specific (ubiquitination-mediated) mechanism of paternal mitochondrial destruction in the early embryo, could explain reduction and/or absence of paternal mtDNA transmission in the zygote and developing embryo [59]. Contradictory messages exist if the sperm midpiece tail is discarded outside the ovum in fertilization (**Fig. 2**) or the paternal mitochondria are degraded inside the zygote, following male gamete penetration [43].

According to May-Panloup et al. [30], the motile sperm from human normal sperm samples were found to contain only 1.4 mtDNA molecules on average (using real-time quantitative PCR). This means that the majority of sperm mitochondria are almost totally devoid of mtDNA, and that many sperm probably do not contain any mtDNA at all [30]. These values are similar and comparable to those established by Shitara et al. [53], who have found an average of 10 mtDNA copies per mouse sperm and 150 copies of mtDNA per mouse spermatid, by using a real-time quantitative PCR technique. Another important finding is the close correlation between the semen quality and the functionality of the respiratory chain in sperm mitochondria [48].

It has been reported that mtRNA transcripts remain highly stable in the mitochondria of sperm, despite the absence of mtDNA replication [45], but the sperm from asthenozoospermic patients have altered levels of specific mtRNAs [30]. The authors indicated that the mtDNA content of motile sperm is up to 28-fold higher in the sperm samples of poor quality, than in normal. Explanation of this epiphenomenon – closely related to abnormal (higher) mtDNA amplification in spermatozoa of low quality, was discussed in the scientific literature [14, 26, 30, 35]. The data show that a low respiratory chain activity of sperm mitochondria leads to the abnormal spermatozoa maturation/differentiation in mammalian and humans. Nakada et al. [35] evaluated that mitochondria respiration defects and genome (mtDNA) mutations in experimental mito-mice induced low sperm number (oligospermia), non-motile sperm (astenozoospermia) and

low sperm quality (sperm morphological abnormalities – preliminary in the midpiece and in the nuclei of male gametes). In addition, testes of the infertile mice showed meiotic arrest (at the zygotene stage) through spermatogenesis and enhanced sperm apoptosis. We described similar morphological changes in spermatozoa of infertile men [20].

Conclusions

Alterations in the mitochondrial genome (mtDNA), transcriptome, proteome or metabolome, as well as any cellular events resulting in compromised sperm mitochondria functionality during the time of sperm travelling and sperm-oocyte interaction (fertilization) (**Fig. 2**), may affect (suppress) sperm motility, functional activity and/or fertility, leading to male sub-fertility/infertility.

Several sperm mitochondrial proteins could be changed in asthenozoospermic patients [29, 39, 52, 68]. The micro-array analysis suggested differential mtRNAs in the sperm from asthenozoospermic patients [21].

ROS activity/oxidative stress and other pathophysiological factors are related to the respiration defects in mitochondria and to the mitochondrial genome (mtDNA) mutations in spermatozoa of patients with male infertility, as well as in other mitochondrial diseases. The data implied clinical applications in cases of male infertility associated to the mitochondrial sperm defects, as the new independent biomarkers of male infertility.

In the scientific literature existed disscussion on the topic: if the sperm midpiece tail is discarded outside the ovum in fertilization or the paternal mitochondria are degraded inside the zygote, following male gamete penetration [43]. The explanation of this interesting biological phenomenon needs of further investigations.

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