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MITOCHONDRIAL DYSFUNCTION IN MALE SUB-FERTILITY: WHERE DO NUTRACEUTICALS STAND?

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ABSTRACT

Mitochondria plays a crucial role in male fertility by contributing to energy synthesis, meiosis, spermatogenesis, sperm maturation, and capacitation. Mitochondrial dysfunctions, in particular mitochondrial DNA (mtDNA) mutation, generation of reactive oxygen species (ROS), and lowered antioxidant capacity are associated with male sub-fertility. Under physiological conditions, sperms produce basal level of ROS necessary for sperm function. However, increased production of ROS causes DNA fragmentation and peroxidative damage to sperm plasma membrane, inducing cell death and causing a decline in fertility. Antioxidants could be employed to control the oxidative damage caused by excessive ROS during spermatogenesis. Mitochondrial medicine was introduced into clinical practice based on pathologies caused by abnormal functioning of mitochondria. Since then, various strategies have been implemented to develop mitochondrial therapeutics, including use of vitamins, co-factors, and antioxidants. The major supplementary antioxidants approved to manage male sub-fertility are L-carnitine, CoQ10, vitamin C, vitamin E, and Lycopene, as well as micronutrients such as folate and zinc. Evidence suggest favourable effects of nutraceutical-based antioxidant supplementation, alone or in combination, on sperm parameters such as sperm concentration, motility, and morphology. In this direction, future studies designed to precisely measure dosage, type, and duration of mitochondria-targeted treatments will encourage widespread use in clinical practices.

KEYWORDS: Mitochondria, antioxidants, spermatogenesis.

INTRODUCTION

Male infertility is a global health issue affecting at least 30 million people.[1] About 40–50% of infertility cases are associated to male sub-fertility worldwide. Approximately 7% of all men are infertile and 2% of men present suboptimal sperm parameters.[2] The term infertility is used interchangeably with sub-fertility in clinical practice, though, in a consensus, experts agreed to prioritize use of the term infertility to assess or diagnose an individual or couple.[3] As per the WHO, the overall prevalence of primary sub-fertility in India varies from 3.9% (Age-standardised 25-49 years) to 16.8% (Age-standardised 15-49 years).[2,4,5] A study conducted over a period of 13 years in South India reported 30.31% decline in sperm count whereas, 22.92% and 51.25% reduction was observed in sperm motility and morphology, respectively.[6] Another study performed in an infertility center of North Karnataka observed that the major fertility issues in the study sample (N=432 men with sub-fertility) were asthenozoospermia (130 cases, 30.09%), azoospermia (94 cases, 21.75%), oligoasthenoteratospermis (72 cases, 16.66%), and asthenoteratozoospermia (70 cases, 16.20%).[7]

A review that analysed trends of male sub-fertility indicates that almost 50% of cases in India are associated to male reproductive anomalies or disorders,[8] which generally manifest as deficiencies in sperm formation,

concentration, quality, or transportation.[9] Etiologically, decreased sperm motility, also known as asthenozoospermia, contributes to almost 19% of sub-fertility cases.[10,11] Approximately, 30%–80% of male sub-fertility cases are a result of damaging effects of oxidative stress on sperm that causes sperm dysfunction.[12] Genetic factors such as aberrant chromosomes, single gene mutation, and polygenic disorders also play an important role in male sub-fertility.[13,14,15]

However, a large proportion (~50%) of male sub-fertility cases are idiopathic, owing to limited understanding of spermatogenesis (maturation of male germ cells, transformation of spermatogonia to sperm cells/spermatocytes, spermatozoa) and its associated regulatory mechanisms.[16,17] During the early phases of fertilization, sperm flagellar motility and other physiological functions require a high amount of energy generated through mitochondrial oxidative metabolism and glycolysis.[15,18,19,20,21] Being the cellular energy generation hub, the integrity of mitochondrial DNA (mtDNA) plays a crucial role in the functioning of spermatozoa, and any quantitative or qualitative aberration in the mitochondrial genome may affect sperm physiology and motility.[19] Furthermore, oxidative stress generated by reactive oxygen species (ROS) in the male reproductive tract damages proteins and lipids in mitochondrial membranes, affecting motility of sperms and damaging the sperm DNA.[22,23,24,25] Oxidative stress can also reduce the ability of spermatozoa to fertilize the oocyte by inducing chemical and structural modifications in mtDNA.[26] Hence, use ofantioxidants (ROS scavengers) is perceived as an effective approach to prevent oxidative damage to gonadal cells and mature spermatozoa.[18,24] Micronutrients such as coenzyme Q10 (CoQ10) and L-carnitine are important components of the mitochondrial energy metabolism and provide protection from free radicals and oxidative damage.[27,28] Hence, micronutrients as antioxidants may help in maintaining the integrity of sperm, and improve male fertility and fertilization capacity.[18,24,28]

In this article, we review the current knowledge and gaps in the understanding of mitochondrial spermatopathy and the role of important nutraceuticals such as CoQ10, L-Carnitine, and Carotenoids as mitochondrial medicine in male sub-fertility.

Mitochondrial physiology

Mitochondria are vital intracellular organelles of eukaryotic cells that serve as principle sites for energy production.[20] During mitochondrial respiration, adenosine triphosphate (ATP) is synthesised through oxidative phosphorylation (OXPHOS — which involves transfer of high- energy electrons via a chain of protein complexes embedded in the inner-membrane of mitochondria, known as the electron transport chain [ETC]).[20,29,30] In addition to ATP production, mitochondria are also involved in cell proliferation, epigenetic regulation, cell cycle control, calcium homeostasis, and intrinsic apoptosis pathway.[19,24,31,32] Unlike other cell organelles, mitochondria have a double stranded circular DNA made of 16569 base pairs that contain 37 genes,encoding 2 ribosomal ribonucleic acids, 22 transfer ribonucleic acid, and 13 proteins.[19] Each cellular mitochondrion carries 2–10 copies of mitochondrial genome. Polypeptides encoded by mitochondrial gene form subunits of key complexes of the ETC, namely complex I, complex III, complex IV, and complex V. The subunits of complex III and complex IV contain enzymes cytochrome C reductase and cytochrome C oxidase, respectively, whereas complex V comprises enzyme ATP synthase.[19,31] The remaining proteins in the ETC complex are encoded by nuclear genes.[17] During OXPHOS, electrons obtained from electron donors (nicotinamide adenine dinucleotide dehydrogenase [NADH] or flavin adenine dinucleotide [FADH2]) are transferred through ETC via redox reactions coupled with the transfer of protons (H+ ions) across the mitochondrial inner membrane. The transfer of electron through ETC ends with complete reduction of oxygen into water and the release of energy in the form of ATP.[33] Instead of being transferred through the ETC to oxygen, if electrons are leaked from the complexes, they prematurely or partially reduce oxygen to form oxygen radicals (superoxide anion, hydroxyl radical) collectively known as ROS.[30,31,34] The partial reduction of oxygen by an electron produces superoxide anion ($O^{-}\bullet$) and dismutation of $O^{-}\bullet$ (spontaneously or due to catalytic reaction by superoxide dismutases) produces hydrogen peroxide (H_2O_2), that may either get fully reduced to water or partially reduced to hydroxyl radical ($OH\bullet$).[34] *In vivo*, ROS generated through mitochondrial ETC act as signalling molecules for a variety of cellular functions; however, increase in ROS levels are detrimental to mtDNA, proteins, and cellular integrity.[17,19,35] Physiological levels of ROS facilitate sperm maturation, capacitation, and sperm-oocyte interaction; whereas; supraphysiological levels of ROS induce mtDNA damage and apoptosis and adversely affect sperm concentration, motility, and morphology.[36]

Mitochondrial modulation during spermatogenesis

Modulation of mitochondrial physiology

Immature male germ cell mitochondria undergo changes in size, shape, location, and count duringspermatogenesis. Three different morphological forms of mitochondria have been described during spermatogenesis: orthodox mitochondria (cristae-type present in spermatogonia and early spermatocytes), intermediate mitochondria, and metabolically more efficient condensed mitochondria (late spermatocytes, spermatids and sperm).[37 Mitochondrial count reduces during spermiogenesis, as some mitochondria is lost in residual bodies along with cytoplasm.[31] The remaining mitochondria transform into a tubular structure and wind helically around the filamentous mid-piece of the sperm.[37] Concurrently, a considerable number of germ cells are lost (36%–45%)

throughout the meiotic division cycle of spermatogenesis.[37] As the quality of germ cells determines the number of mature sperms produced, degeneration of germ cells may be a mechanism to eliminate cells with genetic aberrations and to ensure that mature sperms are of good quality.[38] Mitochondria play an important role in this degeneration process probably by causing an increased release of cytochrome C, changes in the oxidative machinery, or morphological alterations.[39]

Modulation of mtDNA

The mtDNA copy number and integrity are linked to sperm health and could be indicators of overall male fertility status.[40] During spermatogenesis, the number of mtDNA copies is reduced (by down-regulation of the mitochondrial transcription factor A [mtTFA], a key regulator of mtDNA replication) to a critical threshold appropriate for sperm function.[41,42,43] This is reflected by a high difference in mtDNA copy numbers between mammalian sperm (~100) and oocyte (150,000).[44] Experimental studies on mice have shown that a threefold reduction in mtDNA copy number in sperm do not significantly impair it's function, whereas 40,000–50,000 copies of mtDNA are required in the mature oocyte to support critical post-implantation embryonic development.[44] These findings indicate that downregulation of mtDNA copy number is essential for normal sperm function, whereas, a high mtDNA copy number in the mature oocyte is responsible for distributing mitochondria and mtDNAs to the early implanting embryo before onset of mtDNA replication and mitochondrial biogenesis.[44]

mtDNA is more susceptible to oxidative stress than nuclear DNA due to dense packing, and lack of protective histones, efficient DNA repair system and exposure to anti-oxidant-rich cytoplasmic environment.[16,40,45,46] Under oxidative stress conditions, ROS leads to persistent mtDNA damage through polymorphisms and mutations; an accumulation of damaged mtDNA affects overall oxidative phosphorylation and deteriorates mitochondrial function.[17] Mutation rate in mtDNA is about 10 to 20 times higher than the nuclear DNA.[47] Hence, down-regulation of mtDNA copy number may strategically decrease the odds of ROS-mediated damage to mtDNA and thus, effectively evades potentially harmful effects on sperm function.[17] In trans-mitochondrial mouse model (mito-mice model), mtDNA mutations in sperm causing defects in mitochondrial ETC resulted in cell cycle arrest during meiosis along with morphological abnormalities and induced oligospermia and asthenozoospermia.[48] Likewise, the mtDNA copy number was found significantly high in men with oligozoospermia (low sperm count) and asthenozoospermia.[49] Another study revealed that mtDNA copy number in sperms was lower in normozoospermic donors than in infertile men with clinical varicocele.[50] In a large cross-sectional study in young Chinese men, the mtDNA copy number was found to be inversely associated with sperm concentration, count, morphology, and motility.[40]

Sperm cells have fewer mtDNA than somatic cells and are susceptible to early and more detrimental effects because of pathogenic mtDNA mutations. Abnormal phenotype manifests when the ratio of mutated or deleted mtDNA and wild mtDNA exceeds a critical threshold.[16] The levels of mutated and pathogenic heteroplasmic mtDNA are low in actively dividing cells and highin non-dividing or arrested cells.[17,51] This indicates that factors other than mitotic activity may probably modulate mtDNA mutational load and thereby, affect the function of the spermatozoa.[17,52]

Sperm cells containing damaged DNA could be eliminated by mitochondria-mediated ROS generation and induction of apoptosis-like phenomena.[31] High levels of ROS impair mitochondrial intergrity, induce release of cytochrome C, and activate caspases cascade that potentially damage and disrupt DNA, and ultimately result in apoptosis like phenomena.[53] Following seminal oxidative stress, sperm DNA damage and apoptosis chain of events lead to infertility. Apoptosis in semen could serve as a useful marker of semen quality.[54] Similarly, apoptosis can be triggered in ejaculated human sperm cells by critical events of the signalling cascade, including caspase activation, disturbance in mitochondrial membrane potential (MMP), externalisation of phosphatidylserine, and DNA fragmentation.[55]

Mitochondrial proteins, sperm maturation, and capacitation

Capacitation is the process in which biochemical and physiological changes in the female reproductive tract maintains the maturation of spermatozoa before fertilization process. Capacitation augments sperm activity and prepares the acrosome cap for acrosome reaction (exocytosis process after binding to zona pellucida of the egg). It involves alterations in sperm membrane, modulation of enzyme activities, and protein tyrosine phosphorylation that stimulate multiple signalling pathways essential for hyperactivation and the acrosome reaction.[56] Protein tyrosine phosphorylation is achieved by cyclic adenosine monophosphate (cAMP) dependent protein kinase A (PKA) activation. PKA (a serine/threonine kinase) activation regulates membrane potential, up-regulates intracellular calcium concentration, phosphorylates several proteins on serine and threonine residue, regulates several protein kinases and/or protein phosphatases, and finally produces an increase in the phosphorylated tyrosine residues downstream of PKA activation.[57] The flagellum (principal and mid piece) and acrosomal region are the major components of sperm cell that undergo tyrosine phosphorylation so as to develop hyperactivation and acquire fertilizing ability.[58,59,60] Thus, tyrosine phosphorylation augments sperm binding capacity to zona pellucida. Variations in tyrosine phosphorylation have been identified in sub- fertile subjects indicating its

physiological role in fertilization.[57] Tyrosine phosphorylation occurs in several mitochondrial proteins, located within the mitochondria in the mid-piece of sperm (ETC proteins, phospholipid hydroperoxide glutathione peroxidase [PHGPx], and voltage-dependent anion channel [VDAC]) and those located in the acrosome, head and/or principal piece of sperm (dihydrolipoamide dehydrogenase [DLD], pyruvate dehydrogenase [lipoamide] alpha 2 [PDHA2] and glycerol-3-phosphate dehydrogenase 2 [GPD2])[61] (**Table 1**). Studies on humans have shown that ETC proteins complexes play important roles in capacitation, while animal studies have indicated role of VDAC and DLD in acrosomal reaction, PDHA2 in capacitation, and enzymatic activity of GPD2 in hyperactivation and acrosome reaction.[61] Though PHGPx, a mitochondrial capsule protein, is tyrosine phosphorylated during capacitation, its function with respect to capacitation is not yet clearly established.[61,62] The expression of PHGPx was found significantly decreased in the spermatozoa of sub-fertile men with oligoasthenozoospermia.[63]

Table 1: Tyrosine phosphorylated mitochondrial sperm proteins involved in capacitation.

Protein	Localisation	Pathway	Function
Extracellular signal-regulated kinase 1 (ERK1)	Mid-piece, principal piece and equatorial segment	Signalling	Hyperactivation, acrosome reaction
Extracellular signal-regulated kinase 2 (ERK2)			
Phospholipid hydroperoxide glutathione peroxidase (PHGPx)	Mid-piece	Detoxification	Maturation, motility
Glycogen synthase kinase-3-beta protein (GSK3B)	Head, mid-piece and principal piece	Signalling	Hyperactivation
ATP synthase beta subunit (ATPB)	Acrosome, equatorial segment and mid-piece	Oxidative phosphorylation	Motility
A kinase anchoring protein (AKAP)	Acrosome, mid-piece and principal piece	Scaffolding protein	Hyperactivation
Voltage-dependentanion channel (VDAC)	Head, acrosome and mid-piece	Ion channel	-

Adapted from Shivaji et al.[61]

Mitochondrial dysfunction leads to mitochondrial spermatopathy

Mitochondrial dysfunction primarily affects cellular energy generation and/or balance in ROS levels and manifests in the form of various chronic and metabolic diseases, including male sub- fertility, cardiomyopathies, neuromyopathies, and skeletal myopathies.[61,64,65] Loss of mitochondrial function results from mutations in mtDNA and nuclear DNA that encode mitochondrial proteins as well as from mitochondrial abnormalities acquired during one's lifetime. This causes a decrease in the number of mitochondria or impairs mitochondrial physiological actions.[66,67]

Specific mutations and deletions in mtDNA are associated with poor semen quality as well as inadequate sperm function.[68] Defects in mtDNA primarily disrupts energy production and impair spermatogenesis and flagellar movement. Point mutations, single nucleotide polymorphisms (SNPs), and presence of haplogroups in mtDNA affect semen quality.[19] Point mutations or multiple mtDNA deletions have been reported in males with asthenozoospermia or oligoasthenozoospermia. Mutation in the *ND4*, *ATPase6*, and *cytb* gene, deletions in *4977bp* in mtDNA and 4216 single nucleotide polymorphisms (SNPs) have been reported in men with sub- fertility.[69,70,71,72,73] Large-scale deletions and several mutations have been found in mtDNA genes encoding OXPHOS pathway *(COX-II, ATPase 6, and ATPase 8 enzymes)*, resulting in a decline in sperm motility and partial or complete spermatogenesis arrest.[68] An association exists between sperm parameters like concentration, vitality, and motility and the activity of sperm mitochondrial enzymes, including ETC complexes.[17,19,71,74] A biochemical evaluation of sperms obtained from a patient with mitochondrial disease by[75] showed that motility in motile sperms improved three-fold with addition of substrates of ETC complex (pyruvate and succinate) in the presence of glucose. This finding demonstrated the importance of mitochondrial respiration in sperm motility.

Multiple copies of mtDNA are present in every cell and mutations in mtDNA are present either in all mtDNA copies (homoplasmy) or in a fraction of mtDNA copies (heteroplasmy).[67] Mitochondrial disease associated with sperm mtDNA may begin to be clinically apparent when the ratio of mutated or deleted mtDNA and wild mtDNA reaches certain critical level ('threshold expression').[16] However, the threshold for phenotypic expression of sperm mtDNA is not well studied. The total mtDNA copies, type of mutation and the type of affected cell greatly influence the threshold expression.[76] Animal

studies affirmed that a decrease in mtDNA copy number worsens mitochondrial anomalies in spermatocytes and spermatids, whereas an increase in mtDNA copy number alleviates the severe disease phenotype resulting from mtDNA mutations.[77] Thus, an increase in total mtDNA copy number (without increasing the mtDNA mutation load) may serve as an intervention for developing future strategies for the treatment of mitochondrial dysfunctions caused by mtDNA mutations.

ROS are produced predominantly during mitochondrial respiration or during exposure to xenobiotics, cytokines, and bacterial invasion.[78] Intrinsic origins of ROS include damaged or deficient sperms, varicocele, cryptorchidism, testicular torsion, infection/inflammation, and aging.[36] Increased levels of ROS along with antioxidant deficiency can cause oxidative stress, which can lead to DNA damage (nuclear and mitochondrial), telomere attrition, epigenetic abnormalities, and Y chromosomal microdeletions.[79] However, in balanced proportions, ROS function as signalling molecules in cell proliferation and survival. Similarly, ROS are critical mediators of normal sperm physiology and are essential for sperm hyperactivation to undergo acrosome reaction and egg fertilization.[17] Excessive levels of ROS are deactivated by an array of endogenous antioxidants, including enzymes such as superoxide dismutase, catalase, and the glutathione peroxidase, and non-enzymatic substances, such as vitamins E and C, folate, zinc, selenium, carnitine, coenzyme Q10, and carotenoids.[75] Oxidative stress occurs because of an increase in ROS levels or a decrease in cellular antioxidant capacity.[25] Oxidative stress generated in sperm damages mtDNA, mitochondrial proteins, and mitochondrial membrane lipids, reduces sperm motility, causes structural DNA damage, and apoptosis. ROS particularly targets mitochondrial membrane lipids through peroxidation as mitochondrial membranes have large deposits of polyunsaturated fatty acid and cause loss of membrane integrity with an increased in-membrane permeability.[25,80] The major source of ROS in sperm cells are mitochondria, whereas seminal leucocytes are main contributors of ROS in semen.[25,81]

Measuring levels of ROS, oxidative stress or total antioxidant capacity (TAC) may serve as an informative tool in sub-fertile men with abnormal semen analysis and men with normal semen parameters but idiopathic sub-fertility. Abnormal levels of oxidative stress or decreased levels of TAC in men can elicit the dominant role of male factor in conception failure; thus, supporting the use of antioxidant supplementation before proceeding with highly specialised and expensive assisted reproductive treatments (ART: in vitro fertilization or intracytoplasmic sperm injection) or during ART to improve the procedure outcomes.[36,82]

Mitochondrial medicine and the role of nutraceuticals in male sub- fertility

Various strategies have been evaluated in humans for treatment of mitochondrial disease including gene therapy (replacement or repair), controlled regulation of specific transcriptional regulators, metabolic manipulation, and altering the balance between wild-type and mutated mtDNA responsible for defects in OXPHOS; however, all at the single cell level. Despite advances in the understanding of pathophysiology and genetics of mitochondria, only symptomatic treatment is currently available.[83,84,85,86] The aim of metabolic manipulation is to correct the deranged biological process by preventing oxidative damage by ROS, inhibiting lipid peroxidation, restoring altered membrane potential, balancing calcium homeostasis, and regulating transcription interference.[84] Nutraceuticals (MTNs, a group of food nutrient products that claim to have a beneficial effect on health and medical conditions) have antioxidant properties and are mostly employed to counter oxidative damage.[84] Generally, people with mitochondrial disease have inadequate nutritional intake.[87] There has been an increase in the use of mitochondria-targeted dietary supplements or MTNs.[88] Intake of selective MTNs compounds could help intervene mitochondrial dysfunction or reinforce inherent defence against oxidative stress.[88] Micronutrients such as zinc, folate, and antioxidants are required for sperm maturation, DNA synthesis, repair and transcription.[89] Oral supplementation of nutraceuticals with antioxidants properties are widely used as therapeutic intervention for male sub-fertility.[90,91,92] Oral antioxidant intake improve sperm parameters, such as motility and concentration, and decrease DNA damage; while, addition of antioxidants to ART media was found to decrease lipid peroxidation and improve reproductive outcome by extending sperm preservation.[93] However, evidence demonstrating enhancement in fertility rates and live birth is inadequate. Supplementary antioxidants that are widely used to treat male sub-fertility are L-carnitine, CoQ10, vitamin C, vitamin E, and carotenoids, as well as the micronutrients folate and zinc.[94,95,96] In a systematic literature review of 26 studies,[97] explored the benefits of commonly used antioxidants (Vitamin E, vitamin C, carnitines, N-acetyl cysteine, co-enzyme Q10, zinc, selenium, folic acid and lycopene) in male sub-fertility. The antioxidant therapy demonstrated a significant positive effect on basic semen parameters, advanced sperm function, outcomes of ART, and live-birth rate.

CoQ10

Coenzyme Q10 is a physiologically omnipresent, endogenous lipid-soluble antioxidant. It is found in a reduced (ubiquinol) or oxidized (ubiquinone) form. As a transfer molecule in all cells, CoQ10 transports electrons from complex I and II to complex III in mitochondrial ETC and thus prevents leakage of electrons to oxygen.[98,99] This restricts the excessive production of ROS and protects the cell and its components from

damage due to abnormal oxidative stress. The CoQ10 in blood plasma and seminal fluid is an important metabolic biomarker for diagnosis and treatment of male sub-fertility.[100]

Being a strong antioxidant, CoQ10 is the most extensively used natural supplement.[98,99] Studies have reported low levels of CoQ10 in infertile men, and it is known that an increase in CoQ10 concentration improves semen quality.[101,102] Exogenous administration of CoQ10 in infertile men with idiopathic oligoasthenoteratozoospermia increased sperm concentration and motility, pregnancy rate, and antioxidant status.[12,98,102,103] A study of seminal fluid samples from 77 males with sub-fertility, previous phlogosis, or varicocele demonstrated that CoQ10 levels in total seminal fluid had a linear correlation with sperm count and motility, whereas CoQ10 in the pellet of spermatozoa correlated inversely with sperm parameters, indicating that CoQ10 may have a pathophysiological role in human semen fluid.[104] In contrast, another study elicited a large difference in CoQ10 levels in semen of fertile men (n=23, 37.1±12.2 ng/mL) and infertile men (n=195, 48.5 ± 20.4 ng/mL).[105] Another study shows that dietary CoQ10 intake from foods alone may be insufficient to optimize semen parameters; the mean dietary intake of CoQ10 in this study was 10-fold lower than the supplemental dose used in clinical trials showing improved sperm motility.[106] Such conflicting data necessitate further studies to deduce a conclusion on the effect of CoQ10 supplementation on sperm parameters and male fertility. In idiopathic oligoasthenozoospermia patients, CoQ10 was evaluated along with L-carnitine, and a significantly higher sperm motility and improved outcome of clinical pregnancy was observed.[107] In another study, CoQ10, Vitamin C, and Vitamin E were co-administered. This combined supplementation improved the qualitative and quantitative parameters of the seminogram in patients with oligoasthenoteratozoospermia and resulted in pregnancies in more than one third partners (28.4%).[108] A case-control study by Alahmar et al,[109] showed significant improvement in sperm concentration ($p<0.05$), progressive motility ($p<0.05$), total motility ($p<0.01$), seminal fluid CoQ10 concentration ($p<0.001$), and TAC ($p<0.001$) in patients with idiopathic oligoasthenozoospermia who received CoQ10 200 mg/day orally for three months. The study also identified a positive correlation between CoQ10 levels and sperm concentration ($r=0.48$, $p=0.01$) and total motility ($r=0.59$, $p=0.003$); whereas, a negative correlation was observed between sperm DNA fragmentation and sperm motility ($r=-0.54$, $p=0.006$). A meta-analysis was conducted by Lafuente et al[12] to assess the role of CoQ10 in live birth and pregnancy rates. Although sperm concentration and motility improved, there was no significant evidence confirming CoQ10 increases either live birth or pregnancy rates.

Various preclinical and clinical risk assessment studies indicate that CoQ10 is safe for use as a dietary supplement with no adverse effects even at high doses. Preclinical toxicity studies showed that CoQ10 has low toxicity in animals, wherein the lethal dose for rats was greater than 5000 mg/kg.[110] In in-vitro genotoxicity studies, ubiquinol acetate neither possessed genotoxic potential nor did it show clastogenic effect at dose of 5000 µg/mL or aneugenic effect at 62.5 µg/mL.[111] Based on various clinical trial data, the observed safety level (OSL) of CoQ10 was found to be 1200 mg/day/person.[110] Furthermore, pharmacokinetic evidence suggest that exogenous CoQ10 neither impacts the biosynthesis of endogenous CoQ9/CoQ10 nor does it accumulate into plasma or tissues after stopping the supplementation.

L-Carnitine

Carnitine is an essential amino acid derivative. It is produced endogenously or absorbed from dietary sources. L-carnitine, an active isomer of carnitine, transports and facilitates long-chain fatty acid oxidation (β-oxidation) in the mitochondrial matrix and thus increases energy supply necessary for sperm motility, sperm maturation, and spermatogenesis.[93] Concentration of L- carnitine is ~2,000 times more in epididymal lumen and in sperm cells than in circulating serum.[112,113] Low levels of carnitines cause a decline in fatty acids level in mitochondria, reducing energy production and thereby hampering sperm motility.[114,115] The active form of L-carnitine, L-acetyl-carnitine, via its antioxidant effects, protects mitochondria and cell membrane from damage induced by ROS.[113] Several clinical studies have elicited positivecorrelations between seminal carnitine concentration, sperm count and motility. In a placebo- controlled double-blind study, combination of L-carnitine and L-acetyl-carnitine effectively increased sperm motility in infertile men with oligoasthenoteratozoospermia, particularly in men with lower sperm motility.[116] Similar findings were obtained in patients with idiopathic asthenozoospermia; a daily dose of 3 g of carnitine for four months improved sperm motility, both qualitatively and quantitatively.[117] Another study demonstrated the beneficial effects of the combination of L-carnitine and L-acetylcarnitine with micronutrients on sperm motility, vitality, and sperm DNA fragmentation.[113] Taken together, these results suggest that optimum supplementation of L-carnitine can overcome problems in sperm motility and help achievefertility.

Risk assessment conducted in animals and humans exhibited strong safety evidence for L-carnitine supplementation at intakes up to 2000 mg/day (OSL in humans). Much higher doses of carnitine have also shown no adverse effects and were well tolerated in trials; however, there is not enough data for long-term safety of doses above 2000 mg/day in humans.[118]

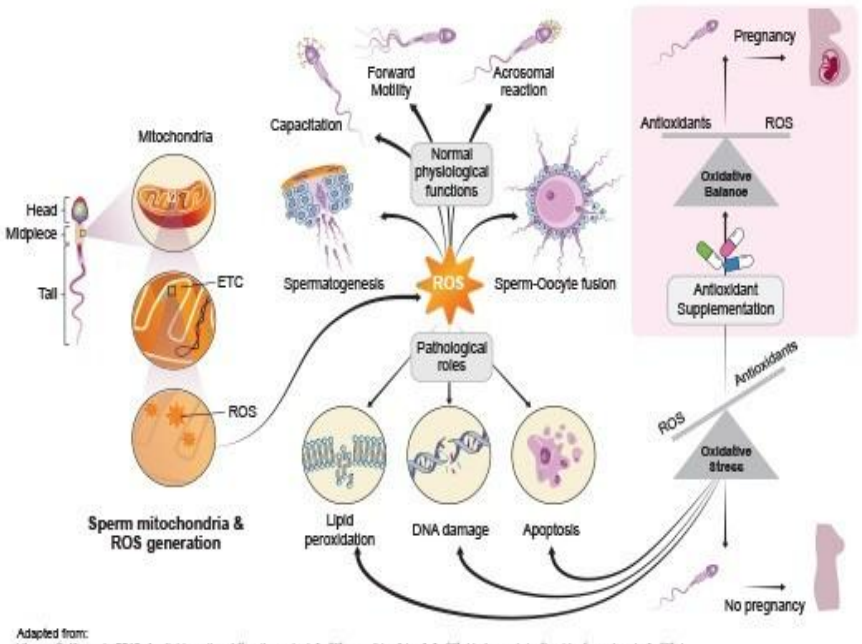
Carotenoids

Carotenoids are a group of more than 600 plant pigments and a part of human antioxidants mechanism. Lycopene, a red carotenoid pigment, is a highly potent ROS scavenger and can be a possible treatment option for patients with male sub-fertility.[119] Lycopene accumulates in higher concentration in testis and seminal plasma but found to be in lower concentration in individuals with sub-fertility.[115,120] A study conducted in men with idiopathic oligospermia, idiopathic asthenozoospermia, or idiopathic teratozoospermia showed that oral administration of lycopene led to significant improvement in sperm concentration, morphology, and motility, with maximum improvement in sperm concentration.[121,122] Incubation of human sperms cells with lycopene at optimum concentration has also demonstrated reduced oxidative damage to sperm mitochondria and sperm plasma membrane.[123] In addition, lycopene reduced sperm apoptosis and sperm DNA fragmentation when administered in combination or alone.[123,124] In another study, supplementation of lactolycopene in young healthy individuals improved sperm motility and morphology.[125] These results suggest that antioxidant activities of carotenoids such as lycopene can protect sperm from stress-induced oxidative damage and boost male reproductive functions.

On basis of the risk assessment studies, the OSL for carotenoids, lutein and lycopene was found to be up to intake of 20 mg/day and 75 mg/day, respectively. Even though higher doses of these carotenoids have been safe and tolerable in trials, limited data is available to predict long- term safety of higher doses.[126]

Combination of antioxidant supplementation

Molecules such as CoQ10, carnitines, and carotenoids are tolerable and safe, with common side effects such as nausea, gastrointestinal disorders, irritability, headache, and fatigue. CoQ10prevents peroxidative damage caused by superoxide ions and peroxides. Carotenoids protect cells and organism from ROS, and carnitines help in sperm maturation and development. A highly potent antioxidant, lycopene confers protection after oxidative damage. Use of multiple antioxidants, herbal therapies, and micronutrients increases sperm motility and concentration when compared with a single agent.[102,107,113,127] Hence, a combination of antioxidants would provide beneficial effect in achieving male fertility. A combination of antioxidant supplementation (CoQ10, L-Carnitine, Lycopene, and Zinc) administered to men with oligoasthenoteratozoospermia significantly improves sperm count and sperm total motility (both, $p<0.0001$ versus matching placebo).[28] A Cochrane analysis showed that men with sub-fertility andtaking oral antioxidant supplementation had more live-birth rate in comparison with those receiving placebo.[128] Furthermore, a combination of oral antioxidants (L-Carnitine, vitamin C, CoQ10, vitamin E, zinc, vitamin B9, selenium, and vitamin B12) improved sperm quality of patients with asthenoteratozoospermia by maintaining optimal seminal parameters and protecting from basal DNA damage; thereby, maintaining overall DNA integrity.[129] In an interventional study, a combination of antioxidants (L‑Carnitine, Lycopene, L‑Glutathione, CoQ10, selenium, and zinc) improved sperm concentration and motility, indicated by a reduction in oxidative stress markers in the seminal plasma.[92] Combination of antioxidants therefore, improve the overall sperm quality by targeting a variety of cellular mechanisms.



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Visvanath Karande 2019. Available online: https://www.invitrofertility.com/blog/blog/infertility/drvikarande/anti-oxidants-and-male-fertility/ (accessed on 17th march, 2020)

DNA, Deoxyribonucleic acid; ETC, Electron transport chain; ROS, Reactive oxygen species

CONCLUSIONS

Evidence shows that mitochondria-induced oxidative-stress is an etiological factor of male sub- fertility. Though the exact mechanism of oxidative stress in spermatogenesis is unclear, several studies corroborate that mitochondria is essential for sperm function and fertilization. Hence, further research to understand the pathophysiology of mitochondria in male fertility, mechanisms of generation of ROS and their effect on mtDNA mutation on causal relationship with sperm DNA fragmentation, low sperm count, and sperm motility should be conducted. The currently available treatments for male sub-fertility may be symptomatic and specifically targeted toward enhancing sperm functions by modulating mechanism of ROS though oxidative phosphorylation. Although mitochondrial medicine and nutraceuticals enhance sperm functions, evidence shows that they are not effective enough to rescue mitochondrial defects at the genetic level. Future studies specifically designed to explore male sub-fertility and roles of mitochondrial medicine and nutraceuticals will enhance our understanding.

Author Contributions

All the authors contributed substantially to the concept or design of the work and interpretation of the data. All authors contributed in drafting and revising the article critically for important intellectual content and approved the final version to be published.

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