Review Article

A narrative review of mitochondrial dysfunction and male infertility

Zihang Mai[1#], Di Yang[1#], Dan Wang[2], Jingyi Zhang[1], Qi Zhou[1], Baoquan Han[1], Zhongyi Sun[1]

[1]Department of Urology, Shenzhen University General Hospital, School of Pharmacy, Shenzhen University Medical School, Shenzhen University, Shenzhen, China; [2]The University of Hong Kong-Shenzhen Hospital, Shenzhen, China

Contributions: (I) Conception and design: Z Mai, D Yang; (II) Administrative support: B Han, Z Sun; (III) Provision of study materials or patients: D Wang, J Zhang, Q Zhou; (IV) Collection and assembly of data: D Wang, J Zhang, Q Zhou; (V) Data analysis and interpretation: Z Mai, D Yang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Dr. Zhongyi Sun, MD; Dr. Baoquan Han, PhD. Department of Urology, Shenzhen University General Hospital, School of Pharmacy, Shenzhen University Medical School, Shenzhen University, 1098 Xueyuan Blvd., Nanshan District, Shenzhen 518055, China. Email: zhy.sun@szu.edu.cn; baoquan.han@szu.edu.cn.

Background and Objective: Recent investigations have highlighted mitochondrial dysfunction as a major component in reduced sperm function and male infertility. The creation of energy, control of reactive oxygen species (ROS), apoptosis, and sperm motility are all critically dependent on mitochondria. The health of the male reproductive system may be significantly impacted by any alteration of mitochondrial structure, function, or integrity. This review intends to open the door to better diagnostic methods, novel therapy strategies, and improved reproductive outcomes for infertile couples by clarifying the crucial function of mitochondria.

Methods: We searched PubMed, Google Scholar, and others for articles related to male infertility and mitochondrial dysfunction from 2014 to 2023. The articles related to the theme were preliminarily screened by abstract, and then the selected literature was read and summarized. In this essay, we examine the research on male infertility and mitochondrial malfunction. We investigate the intricate connection between sperm quality, deoxyribonucleic acid damage, oxidative stress (OS), and mitochondrial bioenergetics. We discuss about how spermatogenesis and sperm function are affected by mitochondrial mutations, deletions, and single nucleotide polymorphisms. We also explore the impact of age-related changes, lifestyle choices, and environmental factors on mitochondrial function and male fertility. This review also clarifies the mechanisms by which mitochondrial dysfunction impacts the viability, morphology, and capacitation of sperm, among other aspects of male reproductive health. Furthermore, we go over the recently developed field of mitochondrial treatments and possible therapeutic approaches that target mitochondrial malfunction to enhance male fertility.

Key Content and Findings: Mitochondria are important for sperm: The control of sperm motility, capacitation, and general quality is largely dependent on mitochondria. Deterioration of sperm motility and male infertility may result from disruption of the structure, function, or integrity of the mitochondria. Future studies should focus on figuring out the processes underlying mitochondrial dysfunction as fertility and reproductive health are significantly impacted by it.

Conclusions: We discuss the evaluation of infertile men mitochondrial function defects and difficulties, and make recommendations for further study in this area. This article provides a thorough resource for clinicians, researchers, and reproductive biologists to understand the underlying mechanisms of male infertility and explore potential therapeutic interventions.

Keywords: Mitochondrial dysfunction; male infertility; oxidative stress (OS); mitochondrial genetics

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Introduction

Background

A persistent issue in the entire world is male infertility. A husband was considered infertile if his wife was unable to conceive after at least 12 months of regular sexual activity without contraception. The evaluation of male infertility includes detailed history taking, focused physical examination and selective laboratory testing, including semen analysis. Treatments include lifestyle optimization, empirical or targeted medical therapy as well as surgical therapies that lead to measurable improvement in fertility (1). Male reproductive health is not in good shape right now (2). Hormonal imbalances, illnesses, trauma, obstructions of the reproductive anatomy, and sexual dysfunction in men can damage sperm either temporarily or permanently and impede pregnancy. Some disorders become more challenging to treat the longer they go untreated. The prevalence of male infertility grew by 0.291% per year between 1990 and 2017 (3). Infertility is a problem affecting one in six couples, which is approximately 15% of the population wishing to start a family (4). Globally, sperm count issues (oligospermia or azoospermia), an increase in abnormal sperm with morphologic defects (teratozoospermia), and decreased sperm motility (asthenospermia) affect about 10–15% of couples, with male factors accounting for about 50% of all infertile couples. After cancer and cardiovascular/cerebrovascular disease, the World Health Organization (WHO) estimates that 190 million people worldwide have infertility, making it the third most often diagnosed illness (5).

Rationale and knowledge gap

The "tadpole" shape of spermatozoa determines their structural and functional specificity; the acrosome, some cytoplasm, and a nucleus with dense deoxyribonucleic acid (DNA) make up the head of the spermatozoa, while ordered mitochondria and numerous longitudinal microfilaments make up the middle and terminal segments. Male infertility can result from dysfunctional sperm and abnormal cellular structure (6). The WHO reports that half of the incidence of infertility is caused by male factor infertility, which is characterized by poor sperm quantity, poor sperm motility, and morphological defects in at least one sample of two semen analyses and collected in an interval of 1 to 4 weeks apart (7). Semen quality can be assessed by factors including sperm count, morphology, DNA quality, and viability, which are important for fertility. Routine semen analysis is the initial step in evaluating a man's capability for conception. However, the accuracy of reference markers for semen analysis in assessing male fertility or forecasting reproductive success is restricted. Recent criteria for sperm quality added by the WHO include mitochondrial DNA amplification and the mitochondrial DNA-pearl protein ratio, which may be indications of male infertility.

Objective

The majority of male infertility cases are idiopathic among the recognized causes (3). It has been shown that male infertility is linked to higher mortality and worse general health. A deeper knowledge of sperm pathophysiology is crucial and should offer more insights into the management of male infertility, given its prevalence and potential complexity (5). We present this article in accordance with the Narrative Review reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-24-262/rc).

Methods

We searched PubMed, Google Scholar, and others for articles related to male infertility and mitochondrial dysfunction from 2014 to 2023. The articles related to the theme were preliminarily screened by abstract, and then the selected literature was read and summarized (*Table 1*).

Mitochondrial dysfunction and male infertility

Significance of mitochondrial dysfunction in male infertility

Mitochondrial dysfunction has been shown to be associated with asthenospermia or oligoasthenospermia, and mitochondrial abnormalities include short midsections,

Table 1 The search strategy summary

Items	Specification
Date of search	August 7, 2023
Databases and other sources searched	PubMed, Google Scholar
Search terms used	(mitochondrial dysfunction) And (male infertility)
Timeframe	From 2014 to 2023
Inclusion criteria	Mitochondria and male reproductive related will be incorporated into use, has nothing to do for the rest of the content
Selection process	We first read the abstracts of the collected literature and removed articles that were not related to mitochondrial dysfunction and male infertility. Then the selected articles were carefully read and the useful content was sorted out and summarized

complete absence of the mitochondrial sheath, defective assembly, or aggregation of mitochondria. Reproductive issues may arise from loss of sperm function due to damage to the mitochondrial structure, mitochondrial genome (mtDNA), transcriptome, proteome, disturbance of the mitochondrial membrane potential (MMP), or altered oxygen consumption. Changes in the mitochondria are one of the main characteristics that contribute to lower fertility. Organelles called mitochondria are present in practically all eukaryotic species and are crucial to energy production as well as other elements of cell life. Apart from providing energy to the cell, mitochondria also carry out tasks like force sensitivity, cell differentiation, signaling, and regulating the cell cycle and growth. According to population genetics theory, male reproduction is especially susceptible to mitochondrial malfunction brought on by mtDNA variations (8). Sperm function and male fertility are significantly regulated by mitochondria, which are distinct organelles in male gametes both physically and functionally (5,6). Spermatogenesis sperm motility, hyperactivation, and energization, acrosome response, and other physiological processes are all facilitated by mitochondria, which are essential for sperm energy conversion and metabolism. Apart from producing adenosine triphosphate (ATP), mitochondria are also involved in several critical cellular functions such as calcium homeostasis, apoptosis, reactive oxygen species (ROS) production, mitochondrial autophagy, and the creation of sex steroid hormones (5). Furthermore, during epididymal maturation, mitochondria regulate the concentration of sperm DNA and secrete protons into the lumen to maintain an acidic environment in the epididymis. These processes also result in the creation of testosterone (9). Sperm functions such as capacitation, acrosome response, oocyte contacts, and motility might be compromised by any anomalies in MMP, ultrastructural integrity or energy metabolism. Prolonged opening of the mitochondrial membrane permeability transition pore (mPTP) during mitochondrial malfunction causes the cytoplasm to fill with cytochrome C, apoptosis-inducing factor (AIF), calcium ions, and other components related to apoptosis (5). Following this, there is a disturbance of cellular structure and an increase in apoptosis due to activation of important members of the apoptosis-associated caspase protein family, disruption of nuclear chromatin, or effects on other Ca^{2+} dependent proteins. Research has verified that spermatozoa's mitochondria-mediated apoptosis can result in aberrant ATP metabolism and sperm cell membrane disintegration, which can ultimately lead to a decrease in sperm counts and infertility. It's interesting to note that mature spermatozoa from viable men did not contain apoptotic markers, while spermatozoa and immature spermatozoa from infertile men did. Furthermore, sperm nucleus DNA is damaged by signaling via the mitochondrial apoptotic pathway, which ultimately disrupts normal fertilization and results in aberrant embryo attachment following fertilization. Sperm survival in men is strongly inversely associated with age. Men who are older tend to have higher rates of sperm apoptosis. Male infertility can be linked to variations in sperm mitochondrial activity brought on by age-dependent changes in the epididymis. Among these, OS brought on by an excess of ROS in the mitochondria may be the primary cause of many illnesses. Overexposure to ROS can cause apoptosis, damage DNA, interfere with OXPHOS, disturb calcium homeostasis, and destroy sperm lipid membrane integrity (5). During spermatogenesis, mitochondria undergo continuous morphological and distribution changes with the development of germ cells. Defects in these

processes lead to mitochondrial dysfunction and abnormal sperm development, which can lead to male infertility (10).

Mitochondrial function and structure

Normal male fertility is predicated on the form and function of sperm. Sperm are not inhibited by common cytoplasmic organelles such as the Golgi apparatus or endoplasmic reticulum, in contrast to other cell types. The primary endoplasmic organelles in the sperm flagellum are mitochondria (5). Traditionally, mitochondria have been thought to have two main roles: the first is to control catabolism by producing ATP via OXPHOS, and the second is to promote anabolism by producing metabolites of the TCA cycle, like citrate and oxaloacetate, which in turn produce macromolecules like lipids and nucleotides (10). The primary energy source in a cell is produced when ATP molecules are converted into adenosine diphosphate (ADP) molecules through dephosphorylation. Cells need to employ nutrients to renew the ATP molecules used in this process to make it sustainable. Glycolytic catabolism breaks down a single glucose molecule into two glucose molecules and pyruvate ketone. The mitochondrial pyruvate carrier (MPC) allows pyruvate to enter the mitochondrial matrix. There, it undergoes oxidation, decarboxylation, and coupling to coenzyme A by the enzyme pyruvate dehydrogenase (PDH), resulting in the formation of acetyl-coenzyme A (11). Carbon dioxide (CO_2) and reducing agents, such as flavin adenine dinucleotide ($FADH_2$) and nicotinamide adenine dinucleotide (NADH), are produced during the oxidation of acetyl-coenzyme A. By sending electrons to the mitochondrial respiratory chain to start OXPHOS, NADH and $FADH_2$ act as fuel for the respiratory chain. Glycolysis is linked to the citric acid cycle, also known as the tricarboxylic acid cycle (TCA cycle) or Krebs cycle, an aerobic second route that occurs in mitochondria and maximizes ATP synthesis. The mitochondrial matrix is the site of the TCA cycle, a sequence of metabolic activities that catabolize proteins, lipids, and carbohydrates to create acetyl coenzyme A (ACA). Thus, two acetyl coenzymes produced from glucose Two CO_2 molecules are produced via a complex catabolic cycle called the Krebs cycle, which is triggered by molecules in the mitochondrial matrix. In the process, the electron equivalents of these molecules, or hydrogen atoms, are removed and used to create a total of six NADH, two $FADH_2$, and two guanosine triphosphates (GTP). This makes the molecules of $FADH_2$ and NADH accessible for the oxidative phosphorylation pathway. Most of the energy is eventually transformed to ATP during the process of oxidative phosphorylation, which uses the NADH and $FADH_2$ molecules. In this mechanism, electrons go from NADH and $FADH_2$ to the end acceptor oxygen via the electron transport chain (ETC). Cellular respiration is the term for this process, which uses molecular oxygen as an absorber of bound electrons. The production of ATP by ATP synthase, or complex V of the ETC, is linked to respiration. The cell uses the entire process of oxidizing one glucose molecule to create thirty ATP molecules (11). Furthermore, mitochondria are understood to function as signaling organelles, regulating the α NAD^+/NADH ratio, pyruvate metabolism, ROS, and TCA cycle metabolites to influence the destiny of stem cells. Male germ cell development is a multifaceted process wherein mitochondria undergo continuous modification to accommodate varying energy requirements of distinct cell types. In addition to being essential for the creation of cellular energy, mitochondria also control apoptosis, steroid hormone synthesis, and redox and calcium homeostasis. There are roughly 70 mitochondria in each spermatozoon. Because sperm motility depends mostly on ATP generated by oxidative phosphorylation in the mitochondrial sheath, abnormalities in mitochondrial respiration will lead to decreased motility and fertility. To move the flagellum during the early phases of fertilization, sperm need a lot of ATP. Any quantitative or qualitative abnormalities in the mitochondrial DNA may have an impact on the cellular activity of the sperm, since the bioenergetic function of the mitochondria is essential for sperm motility. For sperm to swim quickly enough to reach the fallopian tube during fertilization, they need a lot of energy, which is why healthy mitochondria are essential for male infertility (12). An increasing amount of data points to the significance of mitochondria in spermatogenesis, and suggests that perturbations to the dynamics and function of mitochondria impact spermatogenesis and ultimately result in infertility in men.

The mitochondria in sperm have special shapes and physiological roles. Inner and outer membranes, membrane gaps, cristae, and matrix are the different functional sections that make up mitochondrial organelles. The outer mitochondrial membrane (OMM) and the inner mitochondrial membrane (IMM), which separate the organelle into the matrix compartment and the intermembrane space (IMS), are two structurally and functionally unique phospholipid membranes found

in mitochondria. The morphologically complex inner membrane, which can be further divided into two parts: the inner boundary membrane (IBM), which is tightly apposed to the OMM, and the cristae membrane (CM), which, depending on the morphogenetic origin and metabolic state, generates laminar or tubular protrusions into the interior of the organelle, surrounds the organelle and directly junctions with the cytoplasmic lysate. The intercristal space (ICS), which is defined by the CM, the innermost stroma, and the IMS between the outer and inner border membranes are the three aqueous compartments depicted in this bilaminar system. The cristae junction (CJ), an oval or circular structure that connects the cristae to the IBM, and the cristae tip (CT), which is distal to the cristae, are two further functionally significant structures that can be further resolved from the cristae themselves (13). While the inner membrane has limited permeability and includes the enzymes necessary for electron transport, the outside membrane and IMS are very permeable. Because the organelles are endosymbiotic, there are considerable differences between the two membranes in terms of permeability, shape, characteristics and roles of transmembrane proteins, and lipid composition (11). The IMM is mostly involved in energy conversion, whereas the OMM serves as the primary platform for mitochondrial signaling. The ETC and ATP synthase, which are both found in the IMM and account for 90% of cellular energy production, must work together for OXPHOS to occur. To create cristae that extend into a protein-dense matrix containing mtDNA, the IMM folds. It expands the membrane surface to hold 85% of total cytochrome c, which is crucial for the OXPHOS system, as well as 94% of the OXPHOS complex and ATP synthase (9). The OXPHOS machinery and numerous other proteins are embedded in the cristae, and it has been suggested that the folding of the IMM increases the surface area that is accessible for the production of energy. The IBM is the section of the IMM that runs parallel to the OMM. CJs are small tubular or slit-like structures that connect the cristae and IBM. CJs are enriched for protein input systems required for nuclear-encoded proteins and mitochondrial fusion. Internally arranged into cristae, or invasions of the IMM, the mitochondria can dynamically reorganize and become more or less compact in response to a variety of stimuli, including shifts in the energy demand or signals from the cell death machinery (11). Throughout their life cycle, mitochondria engage in a variety of processes including biogenesis, fusion and fission, modification of the cristae, clearance, and apoptosis. The preservation of regular cellular function depends on these processes, and their interference causes bioenergetics to deteriorate and apoptosis to be triggered quickly, particularly in cells that require a lot of energy, such neurons and myocytes. Mitochondrial dynamics refers to the overall flexible and adaptable shape and subcellular division of these organelles. Maintaining a healthy balance of mitochondrial dynamics is crucial since mutant fusion/fission proteins have been linked to a number of human illnesses. Complex signaling networks and gene expression across a range of functions, including metabolism, cell cycle, differentiation, and cellular senescence, are impacted by mitochondrial dynamics. In reaction to cellular stress, mitochondria first modify their structure and makeup through processes such as folding and degradation of proteins, DNA repair, and antioxidant activity. When damage surpasses the capability of these processes, damaged mitochondria initiate quality control systems, like cytophagy, to eliminate them (10).

Mechanisms of mitochondrial dysfunction in male infertility

The role of mitochondria in energy production and ATP synthesis

One of the most important sperm characteristics linked to fertility is frequently acknowledged to be motility. Insufficient energy availability results in decreased sperm motility (1). Sperm motility activity, one of the primary markers of male fertility, depends heavily on mitochondrial bioenergetics performance because sperm movement requires a significant amount of energy, which is produced by mitochondria in the form of ATP. Each spermatozoon in an adult mammal has between 70 and 80 mitochondria, all of which are gathered at the center of the tail (2). The homologous organelles in somatic cells are not morphologically like the mitochondria found in human spermatozoa. The primary location for regulating sperm motility, fertilization, and other processes is the flagellum of mature spermatozoa. It is mostly made up of a telotail, a main segment, and a middle segment with a mitochondrial sheath. Because the mitochondrial sheath produces ATP, the primary energy product required for sperm activity, there is a tight correlation between male fertility and mitochondrial abnormalities. Sperm need the ATP produced by mitochondria for a number of biological functions, such as hyperactivation, energy acquisition,

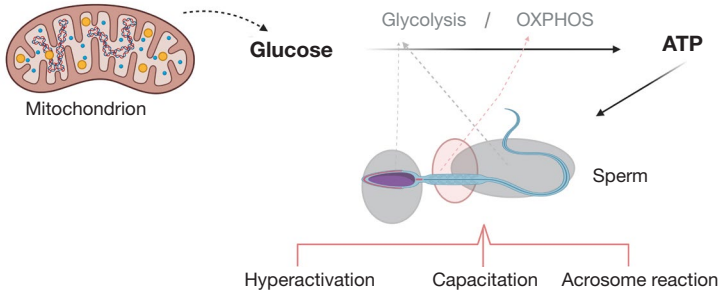

Figure 1 ATP in sperm is produced by only two metabolic pathways, glycolysis and OXPHOS. The former occurs mainly in the sperm head and the main part of the flagellum, whereas the latter occurs mainly in the region of mitochondrial distribution in the middle segment of the flagellum. Sperm consumes ATP provided by mitochondria primarily to maintain motility, but also for various cellular events, including hyperactivation, capacitation, and acrosome reaction. ATP, adenosine triphosphate.

acrosome response (14), and oocyte penetration, in addition to primarily sustaining motility. Both anaerobic glycolysis and oxidative phosphorylation are available to sperm. In spermatozoa, OXPHOS and glycolysis are the only two metabolic processes that create ATP. The former is primarily found in the spermatozoon head and most of the flagellum, while the latter is primarily found in the mid-flagellum's mitochondria-distributed region (*Figure 1*). It is still debatable, nevertheless, whether kind of energy metabolism spermatozoa primarily use. It is widely acknowledged that because glycolysis involves fewer connections and proceeds more quickly than OXPHOS, it is a more suitable energy source for sperm. Furthermore, the inter-sliding process of the "9+2" microtubule structure of the sperm flagellum necessitates a rapid source of ATP supply, meaning that sperm activity demands a significant amount of ATP. It is significant that mitochondria are situated far from the tip of the sperm flagellum, in the middle of the structure. Nevertheless, it is thought that their ATP production is not fast enough to be translocated to the tip of the axoneme. Mice with impaired mitochondrial OXPHOS capacity have less spermatozoa that can fertilize. OXPHOS activity rises during meiotic prophase I, lactate and pyruvate are necessary for spermatocyte survival, and mitochondrial fusion proteins are essential for metabolic changes. OXPHOS hence encourages mammalian spermatogenesis, particularly meiosis (10). These findings imply that OXPHOS-mediated ATP synthesis is still a significant energy source necessary for sperm survival. Aging modifies the bioenergetic profile of mitochondria. The reproductive cycle is correlated with respiratory activity and OXPHOS function, which peaks in young adult animals but declines with age. Testicular homeostasis maintenance may be impacted by this energetic crisis caused by disruptions in ATP synthesis caused by these alterations in testicular mitochondria (5). Future research, however, ought to investigate this method by developing more models for the genetic deletion of different mitochondrial proteins (10).

Mitochondria are involved in ROS regulation and oxidative stress (OS)

In the mid-segment of spermatozoa, there are about 80 mitochondria. They perform a variety of tasks, such as controlling cell division and proliferation to preserve male fertility and producing steroid hormones in the testis (5). Apart from producing ATP, mitochondria are crucial for regulating the lifespan of sperm because they serve as the hub for the interplay between the production of ROS and the initiation of molecular pathways that result in alterations akin to apoptosis. ROS are another consequence of electron leakage in the ETC (5). During normal conditions, ROS play a crucial role as second messengers by oxidatively activating proteins, receptors, kinases, phosphatases, cysteine asparaginase, ion channels, and transcription factors. They are also essential for tyrosine phosphorylation, cholesterol efflux, and sperm-egg interactions in spermatozoa. Conversely, during pathological processes, an imbalance between ROS and antioxidant defenses induces OS. Numerous illnesses, such as type II diabetes, chronic inflammation, local ischemia, neurological disorders, and male infertility, can be brought on by OS via ROS generation. Superoxide (O^{2-}) and hydrogen peroxide (H_2O_2) are examples of ROS that are created when oxygen receives insufficient electron reduction (9). Several locations in the ETC, including

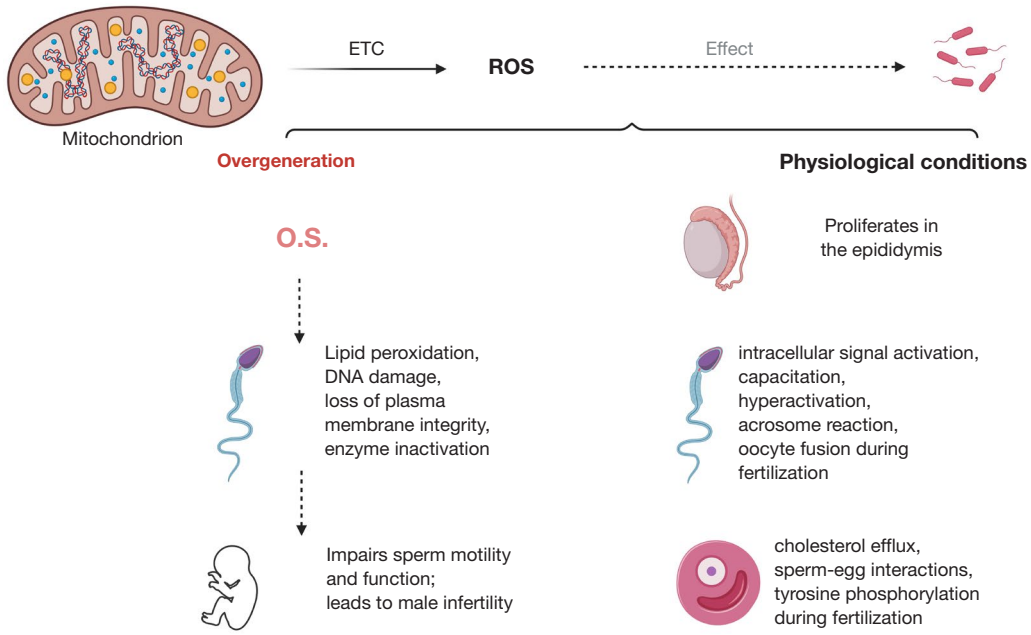

Figure 2 Under physiological conditions, sperm produce appropriate levels of ROS, which are required for sperm proliferation and maturation in the epididymis, important for intracellular signaling activation, capacitation, hyperactivation, acrosome reaction and fusion with oocytes during fertilization, and critical for cholesterol efflux, sperm-egg interaction and tyrosine phosphorylation during fertilization. However, excessive accumulation of ROS in sperm may lead to oxidative stress, which in turn leads to sperm lipid peroxidation and DNA damage, or loss of plasma membrane integrity, or enzyme inactivation. All of these may further impair sperm motility and function and lead to male infertility. ETC, electron transport chain; ROS, reactive oxygen species; O.S., oxidative stress.

complex I and complex III, allow electrons to "escape". The departing electrons combine with O_2 to make O^{2-}, which is produced when O_2 is reduced to water by COX. Two distinct indicators of sperm malfunction are mitochondrial respiratory capability and ROS produced by mitochondria. Furthermore, there is mounting proof that ROS produced by mitochondria are necessary for spermatozoa's structural integrity and regular operation. The "double-edged sword" that controls sperm activity is ROS generated by sperm mitochondria (5). Under physiological conditions, sperm production of ROS at the right amounts is essential for sperm maturation and proliferation in the epididymis; it also plays a crucial role in cholesterol efflux, sperm-egg interaction, and tyrosine phosphorylation during fertilization, as well as for intracellular signaling activation, capacitation, hyperactivation, acrosome reaction, and fusion with the oocyte (9). O^{2-} in, phosphorylates target proteins' serine and tyrosine residues and activates protein tyrosine kinase (PTK), hence initiating the cAMP/PKA cascade that modulates the signaling activity of multiple downstream pathways. The induction of sperm capacitation and modulation of sperm viability by these signaling cascades dramatically improves sperm's capacity to bind zona pellucida. Furthermore, ROS can facilitate sperm-egg fusion and improve membrane fluidity at the physiological level. By blocking protein tyrosine phosphatase activity, which separates secondary fatty acids from membrane phospholipids and increases membrane fluidity, ROS during capacitation prevent phospholipase A_2 (PLA_2) from becoming inactivated. But an overabundance of ROS in spermatozoa can cause OS, which can then cause lipid peroxidation, DNA damage, loss of plasma membrane integrity, and enzyme inactivation in spermatozoa. All these processes can worsen sperm viability and function and result in male infertility (*Figure 2*). The DNA fragmentation index (DFI) of the nuclear and mitochondrial DNA in sperm is one of the most significant markers of male fertility. Sperm DNA fragmentation and fertility have a strongly inverse relationship (DFI >25%) (15). ROS can harm many components of spermatozoa, such as mitochondrial and nuclear DNA; however, unlike somatic cells, mature spermatozoa do not contain cytoplasm.

Since cytoplasm is the primary source of antioxidants, problems in endogenous repair processes and antioxidant defense result from spermatozoa lacking cytoplasm (16). Multiple unsaturated fatty acids are present in sperm cell membranes, which increases their susceptibility to lipid peroxidation caused by ROS. Furthermore, it has been demonstrated that OS induced lipid peroxidation in human spermatozoa produces electrophilic aldehydes such acrolein and 4-hydroxynonenal. Targeting succinate dehydrogenase, these substances interact with mitochondria to alter the function of the respiratory chain and trigger the apoptotic pathway, both of which increase ROS generation. Furthermore, spermatozoa may experience DNA damage because of high ROS generation, which may result in transcription and translation mistakes that impair sperm motility. High amounts of ROS have been found in the semen of 25–40% of male infertile patients, according to numerous clinical investigations. Proposing that OS and elevated ROS in sperm mitochondria could be responsible for *in vitro* fertilization failure and that ROS originating from mitochondria is a separate biomarker of atypical male factor infertility, when diagnosing male infertility, particularly infertile male infertility that is not explained, ROS levels in semen can be utilized as a biomarker (6). ROS are produced when the quantity of free radicals and endogenous antioxidants in spermatozoa differs. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) are examples of specialized antioxidant enzymes found in the antioxidant sperm system. Non-enzymatic antioxidants that are also present include glutathione (GSH), pantothenic acid, N-acetylcysteine (NAC), melatonin, coenzyme Q_{10} (CoQ_{10}) or ubiquinone, steroid hormones, flavonoids, folic acid, carnitine, zinc, selenium, lycopene, vitamin A, vitamin E, vitamin K, vitamin C, vitamin D, and chelator proteins. Various parts of the cellular antioxidant system that prevent overabundance of free radicals and keep the system functioning at physiologically suitable levels. OS is caused by disruption of oxidative homeostasis, which includes excess free radicals synthesis, underactivation, and depletion of antioxidant system components. Reduced ejaculate fertilization, sperm pathology (oligozoospermia, weak spermatozoa, dyszoospermia), mitochondrial damage (mitochondrial dysfunction and mutations in mitochondrial DNA) in germinal cells, and damage to the organs of inheritance are all caused by elevated levels of ROS in semen (15). Because OS has a strong correlation with male fertility loss, doctors often give various antioxidants to patients to boost the antioxidant defense system's scavenging ability and promote sperm motility and function. Exogenous antioxidant therapy has been shown in numerous studies to mitigate OS, or OS, and enhance sperm viability and DNA integrity in male infertile. In order to lower OS and prevent male infertility, a healthy lifestyle and regular exercise are crucial (5).

Mitochondrial genetics and male infertility

Mutations and deletions of mitochondrial DNA

Cellular bioenergetics depends critically on the control and integrity of the mtDNA. The genetic material known as mtDNA is unique to each mitochondria (11). Both of mtDNA's strands are transcribed to produce functional proteins, making it an unadorned molecule devoid of histones and introns. The mtDNA has a 10- to 20-fold greater mutation rate than nuclear DNA because to asexual replication, rudimentary repair mechanisms, absence of protective histones, and closeness to areas where free radicals develop (17). Reference is known that ROS can originate from and target mtDNA. Since mtDNA is not as densely packed as nuclear DNA, it is more vulnerable to OS than nuclear DNA (16). Since the oocyte, not the sperm, contributes nearly all the mitochondria in a fertilized egg, germline mutations in mitochondrial DNA are passed on from the mother to the next generation in sexual creatures where mtDNA is maternally inherited. It keeps all the usual features of bacterial DNA, including being polycistrons, circular, and having 16,569 base pairs (bp) of double strands. It also lacks introns. Each gene is continuous with the next despite some partial overlap, except for a non-coding area known as the substitution loop, or D loop. In addition, in contrast to nuclear DNA, mtDNA exists in multiple copies in the cell, between 100 and 10,000 copies, in accordance to the energy requirements of a given tissue. Furthermore, the genetic code of mtDNA differs slightly from that of nuclear DNA due to the presence of two termination codons and distinct codons that encode methionine and tryptophan. Most mitochondrial genes were either lost or moved to nuclear DNA over evolution, leaving mitochondria with a 16.6-kb maternally transmitted genome today. Only 37 genes are encoded by mtDNA: 22 tRNAs, 2 ribosomal RNAs (rRNAs, 12S and 16S), and 11 messenger ribonucleic acid mRNAs, which are translated into 13 electron-transferring strand proteins. Second, while each nuclear gene is found in only two copies per cell, each mtDNA molecule is found in numerous copies per

cell. Homogeneity is the state in which these duplicates can have the same sequence. However, heterogeneity can result from mutations in mtDNA in a specific percentage of copies due to inheritance of mutant copies, replication mistakes, OS, or ineffective DNA repair. The development and severity of the pathogenic phenotype are significantly influenced by the ratio of mutant DNA to the wild-type variation (11). A mixture of nuclear-encoded proteins, such as mitochondrial transcription factor A (TFAM), which has been demonstrated to nonspecifically encapsulate mtDNA, and DNA polymerase gamma (POLG) regulate mtDNA. Since mtDNA lacks antioxidant-rich cytoplasm, protective histones, and DNA repair systems, it is more prone to damage and deletion than genomic DNA. To maintain energetic homeostasis and regular functions, which in turn causes the motor apparatus to activate, mitochondria are essential. Numerous infertile males have abnormalities in their mitochondrial DNA in genes that control the oxidative phosphorylation pathway, according to several studies (15). It is hypothesised that spermatozoa with high frequency of these mtDNA mutations produce less energy, which in turn causes severely impaired sperm motility and decreased fertility (18). Research on the ultrastructure of sperm has demonstrated that morphological defects and a reduction in motor activity are the results of damage to the sperm mitochondria caused by mutations in their DNA. According to some experts, an overabundance of sperm forms with aberrant morphology is a sign that DNA integrity and chromatin packing have been compromised. The number of mtDNA copies per cell is higher in spermatozoa with poor motility than in spermatozoa that are motile and progressive1. The number of copies of mtDNA per copy of nuclear DNA is known as the mitochondrial DNA copy number (mtDNAcn), or mitochondrial DNA content. Since mtDNA turnover is tightly controlled, tissue type affects it, and it is not affected by the cell cycle, mtDNAcn is thought to be an indicator of overall mitochondrial dysfunction. Sperm mtDNAcn reproduction has been linked to aberrant spermatogenesis, increased mitochondrial autophagy in mature spermatozoa, and excessive proliferation of faulty mitochondria. One measure of mtDNA damage and integrity is mtDNA deletion (mtDNAdel). Sperm mtDNAcn and mtDNADEL are linked to a higher chance of clinical infertility in addition to sperm concentration, count, viability, and morphology. While sperm mtDNAdel exhibited a moderate prediction accuracy for clinical infertility, sperm mtDNAcn had a high predictive accuracy for clinical infertility serial diagnosis. Sperm mtDNAcn is a potentially useful diagnostic test since it provides a continuous measurement of aberrant semen parameters (19).

It is widely acknowledged that decreased energy production causes cellular malfunction and clinical symptoms when the proportion of mtDNA defects rises above a threshold. One crucial mitochondrial quality control mechanism that removes damaged mitochondria from sperm is selective autophagy of mitochondria, or cytophagy. While the paternal mitochondria and their genomes are typically removed in the embryo by an autophagy-associated degradation process known as mitochondrial autophagy, maternal mtDNA is passed on to the progeny. An essential "housecleaning" process for protecting cells, mitochondrial autophagy controls mitochondrial quality, upholds intracellular homeostasis, and promotes perpetual mitochondrial renewal. During spermatogenesis, mitochondria become ubiquitinated, which encourages the breakdown of sperm mitochondria during fertilization as well as the breakdown of damaged spermatozoa in the epididymis. The form, location, and function of mitochondria change with increasing spermatogenic weakness, and the quantity of normal mitochondria eventually declines. In these circumstances, sperm viability may benefit from mitochondrial autophagy. To sum up, the function of mitochondrial autophagy is to promptly eliminate damaged mitochondria to shield spermatogenic cells from mitochondrial malfunction and thereby lower the rate of spermatogenic cell apoptosis (5). Consequently, understanding the unknown causes of male infertility may be aided by researching and evaluating changes in the mitochondrial DNA genes of sperm, which encode oxidative phosphorylation complexes and are in charge of producing the energy (ATP) needed for normal sperm motility.

Age-related changes in mitochondrial function

A growing number of researches have begun to focus on the implications of increased paternal age on male fertility and offspring health as the global age of males at childbearing grows. Age at conception >40 years is the most widely used criterion for identifying advanced paternal age. As people age, male fertility decreases. Age has a variety of detrimental effects on semen parameters, some of which have been shown in numerous studies to include decreases in semen volume, sperm counts, viability, and normal morphology; other effects include increases in DFI and methylation, which can all result in male

infertility and have a negative impact on the progeny (5). Men over 50 exhibited a 3–22% drop in semen volume, a 3–37% reduction in sperm motility, and a 4–18% loss in normal morphology compared to men under 30 years of age, according to a study that examined the impact of age on semen characteristics (5). Simultaneously, the elder age group experienced a 23–38% relative decrease in pregnancy rates. Another 2013 study examined semen data from 5,081 men ranging in age from 16.5–72.3 years (5). It discovered that while spermatozoa total, sperm viability, and normal morphology decreased with age, semen parameters remained relatively constant until age 34. Similar findings from the study also indicated that a rise in the DFI and a decrease in semen volume, total sperm count, motility, and normal morphology sperm are associated with male aging. All the researches point to a steady loss in male fertility with aging. The male reproductive system ages due to a variety of factors. One of the most noticeable factors that lower fertility is mitochondrial changes. Specifically, the build-up of oxidative damage brought on by damage to the mitochondria. Aging modifies the structure and function of mitochondria. Reduced sperm MMP and oxidative damage to mtDNA are two consequences of aging. Research has also demonstrated that aged men's spermatozoa have a decreased capacity for antioxidants, although their levels of ROS and lipid peroxides are elevated (5).

Diagnostic tools to assess mitochondrial function

Reduced ATP generation can result from electron leakage, elevated ROS, OS, and reduced MMP caused by mitochondrial malfunction. Assays for measuring ATP and MMP are two crucial markers for evaluating mitochondrial activity. It can be utilized as a diagnostic and treatment tool for infertile men (20). The oxidation of NADH or succinate produces electrons in this process, which are then transported via the ETC. Four respiratory enzyme multimeric complexes (I–IV) make up the mitochondrial ETC (2). The complexes I–IV cooperate to establish an electrochemical proton gradient in the IMM, which is necessary for the production of ATP via OXPHOS (9). Protons are pumped into the IMS by Complexes I, III, and IV, creating a potential difference across the IMM that is also referred to as the MMP. The MMP can reflect OXPHOS and mitochondrial electron transport activities, and it serves as a kind of intermediate energy storage during ATP generation. The physiological underpinning for preserving chromatin integrity and sperm acrosomal enzyme activity is normal sperm MMP, and sperm with low MMP are less likely to experience the acrosome reaction. Studies have indicated a strong correlation between sperm density, viability, and vitality such as spermatozoa with body and tail aberrations and the sperm MMP and degree of apoptosis. The favorable association between MMP and sperm viability has been validated by numerous investigations. To sustain proper sperm activity, mitochondria must remain structurally intact and have a normal MMP. Decreased membrane potential in the mitochondria can result in apoptosis and necrosis of spermatozoa, as well as insufficient ATP synthesis and problems with energy metabolism. Spermatozoa travel forward due to tail wagging, and the presence of an energy source ensures that the mitochondrial sheath structure remains intact. Research has indicated that sperm loss of motility is primarily caused by damage to the mitochondrial structure in the middle of the sperm tail; other pathological changes, such as alterations in the dense fibers of the axoneme within the sperm tail, result in inefficient use of ATP, which ultimately causes reduced motility or death of the sperm. Therefore, the foundation for good sperm motility is a normal MMP and a normal structure of the mitochondria located in the middle of the sperm tail (2). To maintain sperm motility, mitochondria produce ATP, which helps to conserve metabolic energy. Elevated superoxide concentrations in mitochondria could result in damage to the inner membrane of the mitochondria and a decrease in MMP (21). It has recently been suggested that MMP can be utilized as a marker to predict the ability of sperm to fertilize during IVF and natural conception, which is significant for the clinical assessment of male fertility. More specifically, the combination of MMP and DFI is a valuable tool for the clinical assessment of idiopathic male infertility and is even more effective than traditional semen measures in predicting the success of natural conception in females (5).

Conclusions

Male infertility is a common reproductive health issue that impacts a large proportion of couples globally. Male infertility may be caused by a variety of causes, but recent studies have shown that mitochondrial dysfunction plays a crucial role in sperm function and reproductive outcomes. Frequently called the "powerhouse of the cell" mitochondria are essential for the generation of cellular energy, the control of ROS, apoptosis, and several metabolic processes. The control of sperm motility, capacitation,

and general quality is largely dependent on mitochondria. Deterioration of sperm motility and male infertility may result from disruption of the structure, function, or integrity of the mitochondria. Male infertility can result from lifestyle choices that negatively affect mitochondrial health, including smoking, drinking alcohol, eating a poor diet, and being exposed to poisons and pollution in the environment. Male fertility tends to decrease with age, and age-related alterations in mitochondrial activity may have a negative effect on sperm quality, viability, and DNA integrity. There is much interest in the role of mitochondrial malfunction in male infertility, and diagnosing and evaluating the health of the mitochondria in infertile males present diagnostic problems. In light of this, it is critical to create therapeutic strategies to reverse and stop more oxidative damage in addition to precise and focused techniques for relevant mitochondrial ROS testing. Specific therapies like gene therapy and antioxidants have the potential to repair mitochondrial function (22). Optimizing diagnostic accuracy and improving outcomes can be achieved through individualized therapy methods and precision medicine. Future studies should focus on figuring out the processes underlying mitochondrial dysfunction as fertility and reproductive health are significantly impacted by it. While the effects of mitochondrial dysfunction on male reproductive outcomes and sperm quality are widely known, the precise molecular pathways behind these effects are still not fully understood. Developing focused treatment approaches depends on elucidating the molecular connections and signaling pathways causing mitochondrial dysfunction in male infertility. The creation of reliable and consistent diagnostic instruments is one of the main obstacles to determining the role of mitochondrial malfunction in male infertility. Because of the limitations, current methods, including detecting MMP or analyzing enzymes, may not fully reflect the intricacy of mitochondrial function. Future studies should focus on developing novel biomarkers and refining current diagnostic techniques to offer a thorough and accurate evaluation of mitochondrial health. To sum up, tackling the issues surrounding mitochondrial malfunction and male infertility and exploring new avenues for research will yield important knowledge about possible processes, methods for diagnosis, and treatment strategies. By overcoming these obstacles, we can enhance infertile couples' reproductive outcomes and diagnostic, therapeutic, and treatment outcomes, thereby advancing reproductive health and the welfare of future generations.

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Footnote

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