Chinese Medical Journal®

Mechanism of mitochondrial oxidative phosphorylation disorder in male infertility

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Abstract

Male infertility has become a global concern, accounting for 20–70% of infertility. Dysfunctional spermatogenesis is the most common cause of male infertility; thus, treating abnormal spermatogenesis may improve male infertility and has attracted the attention of the medical community. Mitochondria are essential organelles that maintain cell homeostasis and normal physiological functions in various ways, such as mitochondrial oxidative phosphorylation (OXPHOS). Mitochondrial OXPHOS transmits electrons through the respiratory chain, synthesizes adenosine triphosphate (ATP), and produces reactive oxygen species (ROS). These mechanisms are vital for spermatogenesis, especially to maintain the normal function of testicular Sertoli cells and germ cells. The disruption of mitochondrial OXPHOS caused by external factors can result in inadequate cellular energy supply, oxidative stress, apoptosis, or ferroptosis, all inhibiting spermatogenesis and damaging the male reproductive system, leading to male infertility. This article summarizes the latest pathological mechanism of mitochondrial OXPHOS disorder in testicular Sertoli cells and germ cells, which disrupts spermatogenesis and results in male infertility. In addition, we also briefly outline the current treatment of spermatogenic malfunction caused by mitochondrial OXPHOS disorders. However, relevant treatments have not been fully elucidated. Therefore, targeting mitochondrial OXPHOS disorders in Sertoli cells and germ cells is a research direction worthy of attention. We believe this review will provide new and more accurate ideas for treating male infertility.

Keywords: Mitochondria; Oxidative phosphorylation; Spermatogenesis; Male infertility

Introduction

Mitochondria play a key role in the generation and regulation of cellular bioenergetics, producing most adenosine triphosphate (ATP) via oxidative phosphor ylation (OXPHOS).[1] OXPHOS refers to the process in which nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide lose electrons through respiratory chain oxidation accompanied by adenosine diphosphate (ADP) phosphorylation to produce ATP. Mitochondrial OXPHOS is involved in many cellular processes, including the synthesis of most of the ATP required by organisms, the production of ROS, and regulation of apoptosis and ferroptosis.[1,2] The testis includes various cells, such as Sertoli cells, germ cells, Leydig cells, and myoid cells. Sertoli cells and germ cells are two main types of cells that coordinate with each other during spermatogenesis. Germ cells constantly undergo mitosis, meiosis, and morphological changes during spermatogenesis, and the smooth progress of this process depends on the essential functions of Sertoli cells, such as nutrition, support, and secretion.[3] The shape and function of mitochondria at each stage of spermatogenic cell development are markedly different.[4] The number of mitochondria increases rapidly from meiosis of spermatocytes to acrosome formation of spermatids. In the final stage of

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000003126

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Chinese Medical Journal 2025;138(4)

Received: 17-01-2024; **Online:** 07-06-2024 **Edited by:** Yanjie Yin

spermatogenesis, mitochondria aggregate to form mitochondrial sheaths, while excess mitochondria are shed with the cytoplasm to form residual bodies, which are swallowed by Sertoli cells.[5] In addition, mitochondria differ in undifferentiated spermatogonia and differentiated spermatogonia. Mitochondria of differentiated spermatogonia require a high level of fusion for further development.[6] Varuzhanyan *et al*[6] found that spermatocytes cannot undergo meiosis and spermatogenesis in the absence of mitotic proteins. However, the proliferation of undifferentiated spermatogonia was unaffected. OXPHOS is the key to maintaining the energy demand and nutrient supply of Sertoli and germ cells during spermatogenesis.[3] Studies have shown that male infertility factors account for 20–70% of infertile couples, and male sperm quality is gradually declining.[7,8] The interruption of OXPHOS may induce energy metabolism disorders, increased oxidative stress, and the occurrence of apoptosis and ferroptosis in Sertoli cells or germ cells, thus interfering with the normal progression of spermatogenesis and resulting in male infertility issues such as decreased sperm motility and asthenospermia.[9,10]

OXPHOS

During OXPHOS, ATP is produced by F1Fo-ATP synthase driven by proton motive force (PMF), a transmembrane proton electrochemical gradient generated by the respiratory chain.[11]

PMF is composed of a pH gradient (ΔpH) and potential gradient (Ψ), and its production depends on four enzyme complexes in the respiratory chain and two electron transmitters, namely panquinone (Q) and cytochrome c (cyt c). In complex I, flavin mononucleotide (FMN) accepts 2 H^+ and 2 e^- in NADH to form $FMNH_2$, and transmits electrons to Q to form QH_2 by Fe-S. Each transfer of two electrons moves four protons to the mitochondrial membrane gap.[12] The mechanism by which this proton-coupled electron transfer (PCET) proceeds remains unclear. Studies have shown that the process of electron transfer from NADH to Q occurs at three sites: NADH / FMN site, eight FeS center chains, and the Q redox site. The Q in site 1 is reduced to QH_2, changes its conformation, and moves to site 2 to initiate the proton transfer reaction.[13,14] Mühlbauer *et al*[15] believe that PCET is determined by the hydration state of the proton channels. Complex II accepts electrons from succinic acid and transfers them to Q by reduced flavin adenine dinucleotide and Fe-S but does not function as a proton pump.[16] Q accepts electrons from the above two pathways to be reduced to QH_2 and then passes them to cyt c via complex III. The electron transfer of complex III is completed by the Q cycle. An electron in QH_2 located at the Q_P (also known as Q_o) site is transferred to cyt c via the Fe-S center and cyt c_1 to generate semiquinone (QH^-) and two protons are pumped out to the mitochondrial membrane gap. The generated QH^- transfers electrons through Cyt b_L and Cyt b_H to Q at the Q_N site, which is oxidized to Q, while Q at the Q_N (also known as Q_i) site is reduced to QH^-. After cycling, every two molecules of QH_2 can deliver two electrons to cyt c and pump four protons.[16,17] At the exit of the respiratory chain, complex IV delivers electrons from cyt c to oxygen, which consumes four protons per two molecules of water, thus pumping four protons per four electrons transferred to the mitochondrial membrane gap.[12] The F1Fo-ATP synthase (complex V) includes the F1 structural domain that phosphorylates ADP to generate ATP and the Fo structural domain that drives the rotation of the c-loop with the help of proton translocation[18] and can synthesize ATP under the drive of PMF.

OXPHOS during spermatogenesis

As spermatogenesis progresses, the morphological structure of the mitochondria continuously changes. Spermatogonia and early spermatocytes (meiotic stage I) contain small orthodox mitochondria with low OXPHOS activity. The mitochondria in pachytene spermatocytes are elongated and contain 'condensed' cristae, which is an ultrastructural conformation associated with a high utilization rate of OXPHOS. In sperm cells after meiosis, mitochondrial fragments and crests return to an intermediate state between orthodoxy and concentration.[4] Lactic acid secreted by Sertoli cells can be used as a substrate for germ cells to synthesize ATP, and the energy supply of Sertoli cells depends on mitochondrial metabolism. Therefore, energy metabolism disorders and decreased lactic acid secretion in Sertoli cells caused by the obstruction of mitochondrial OXPHOS will lead to insufficient ATP synthesis in germ cells, which in turn impairs the development of germ cells, spermatogenesis, and male fertility.[19,20]

Interruption of mitochondrial OXPHOS in Sertoli cells leads to male infertility

Sertoli cells are targets of various environmental toxins that cause male infertility.[21] Zhang *et al*[22] showed that Bisphenol A (BPA) caused Sertoli cell damage by changing the expression of cell secretory protein-related genes, thereby changing the testicular microenvironment and leading to spermatogenesis disorders. Sertoli cells participate in the formation of the BTB and interact with germ cells, which guarantees the normal progression of spermatogenesis. Damage to mitochondrial OXPHOS in Sertoli cells causes energy metabolism disorders, oxidative stress, apoptosis, and ferroptosis, which are key factors in spermatogenesis disorders and male infertility.

Disruption of OXPHOS in Sertoli cells leads to impaired energy metabolism and oxidative stress

Impaired mitochondrial OXPHOS in Sertoli cells leads to insufficient ATP production and excessive production of ROS, which are mainly derived from complexes I and III in the respiratory chain.[19,23] Excessive ROS production leads to depolarization of the mitochondrial inner membrane, further increasing ROS production.[24] High ROS levels that exceed antioxidant capacity cause oxidative stress. Oxidative stress damages the nucleus and mitochondrial DNA, leading to further damage to OXPHOS. Sperm plasma membranes are rich in polyunsaturated fatty acids, making them targets for ROS, resulting in lipid

peroxidation of the plasma membrane and production of a series of toxic substances, which includes malondialdehyde (MDA) and 4-hydroxynonenal, causing decreased sperm viability and male infertility.[25,26]

Excessive ROS levels and weak antioxidant capacity of seminal plasma are factors that contribute to decreased male fertility, such as decreased sperm motility and weak spermatozoa.[23,27] Exogenous estradiol benzoate induces oxidative stress, causing structural damage to the mitochondrial structure of Sertoli cells and OXPHOS dysfunction, leading to azoospermia in mice.[28] Studies have shown that pulp and paper mill wastewater treatment causes uncoupling of mitochondrial OXPHOS in the Sertoli cells of immature rats, interfering with the uptake of glucose by Sertoli cells for lactate synthesis.[19] Dysfunction of mitochondrial OXPHOS in Sertoli cells leads to insufficient ATP synthesis and increased ROS production, impairing cell function and preventing cells from providing sufficient energy and nutrients to germ cells.[19,28] Lactate secreted by Sertoli cells provides nutrients to germ cells and is converted to pyruvate as a substrate for ATP synthesis by germ cell OXPHOS.[3] In addition, lactate secreted by Sertoli cells can act downstream of the Fas receptor to inhibit apoptosis of male germ cells and regulate spermatogenesis.[29] Thus, Sertoli cell dysfunction ultimately impairs testicular energy metabolism and spermatogenesis, leading to a decrease in male fertility (the mechanisms by which impaired mitochondrial OXPHOS in Sertoli cells leads to male infertility are shown in Figure 1A).[19]

Disruption of OXPHOS in Sertoli cells leads to apoptosis

During OXPHOS, electron transfer occurs simultaneously with the pumping of protons from the mitochondrial matrix side to the mitochondrial membrane gap. This establishes a potential difference between the negative and positive inner and outer mitochondrial membranes, known as the mitochondrial membrane potential (MMP, ΔΨm).[30] The maintenance of ΔΨm is a marker for the normal occurrence of ATP production by mitochondrial OXPHOS. ΔΨm depolarization is thought to be an early manifestation of apoptosis.[31]

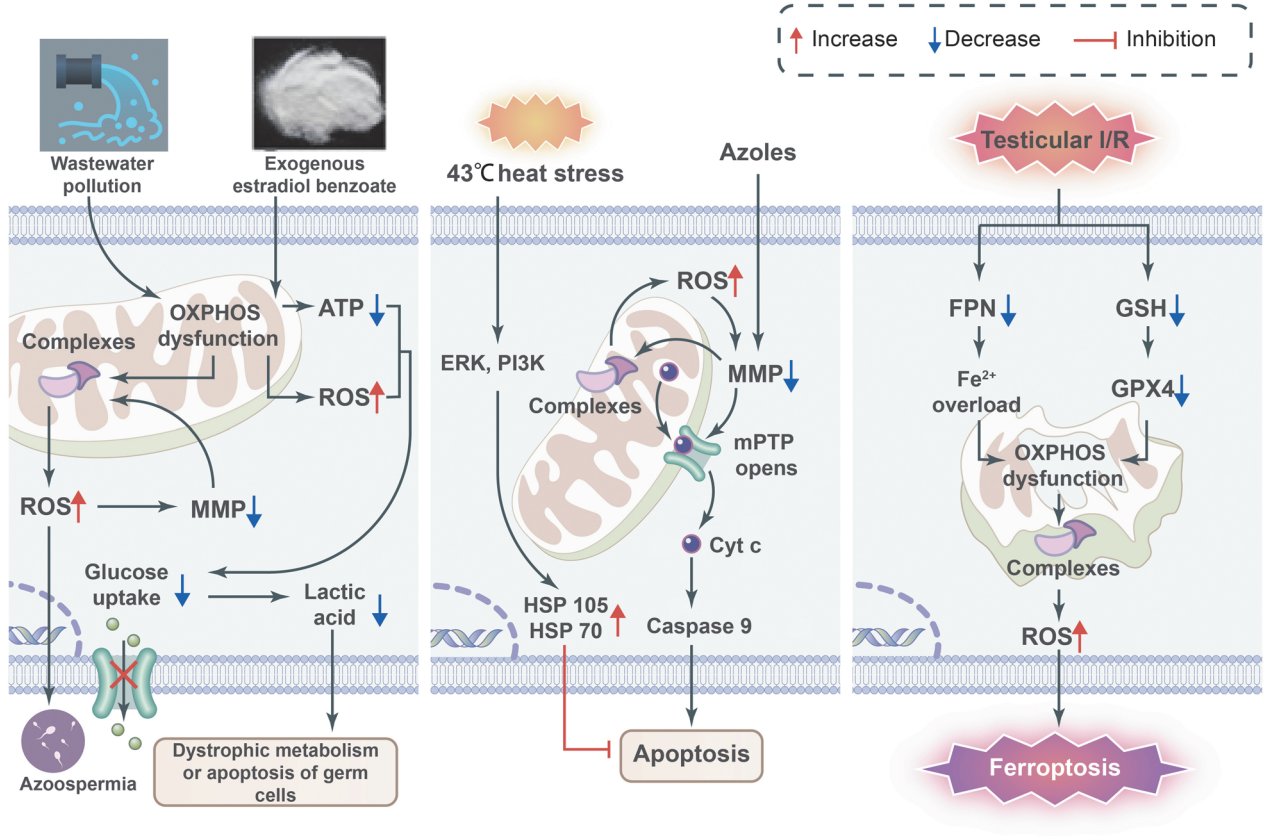

Figure 1: Sertoli cells OXPHOS dysfunction leads to decreased male fertility. (A) Wastewater or exogenous estradiol benzoate attacks Sertoli cells, resulting in OXPHOS dysfunction, insufficient ATP synthesis, and excessive production of ROS by complexes I and III, which in turn leads to a decline in the function of Sertoli cells to take up glucose and synthesize and secrete lactic acid, resulting in germ cell dystrophy or apoptosis. (B) Azoles cause a decrease in the MMP of Sertoli cells, followed by the opening of mitochondrial permeability transition pores, the release of cyt-c by mitochondria and activation of caspase 9, causing mitochondria-mediated apoptosis in Sertoli cells. The decline in MMP causes mitochondria to release more ROS, which further damages mitochondria, resulting in a decline in MMP, thus forming a vicious circle. Under heat stress conditions, ERK and PI3K signaling pathways were activated in Sertoli cells, causing upregulation of heat shock protein 105 and 70 and preventing Sertoli cell apoptosis. Sertoli cell dysfunction or death induced by the above factors can lead to decreased male fertility. (C) Testicular I/R and other factors can lead to the depletion of glutathione in Sertoli cells, which in turn causes the inactivation of glutathione peroxidase 4. Thereafter, intracellular iron overload occurs, the damaged mitochondria produce excessive lipid ROS, and Sertoli cells die as a result of ferroptosis. ATP: Adenosine triphosphate; cyt c: Cytochrome c; ERK: Extracellular signal-regulated kinase; FPN: Ferroportin; GPX4: GSH-dependent peroxidase 4; GSH: Glutathione; I/R: Ischemia reperfusion; MMP: Mitochondrial membrane potential; MPTP: 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; OXPHOS: Oxidative phosphorylation; PI3K: Phosphoinositide 3-kinase; ROS: Reactive oxygen species.

A decrease in ΔΨm in mouse spermatogonia and Sertoli cells, which leads to apoptosis, is one of the molecular mechanisms underlying the development of decreased male fertility.[32] Petricca *et al*[33] demonstrated that a decrease in ΔΨm in azole-treated mouse Sertoli cells, indicated impaired mitochondrial OXPHOS, which can lead to the production of excess ROS and apoptosis of Sertoli cells. Studies have shown that the testis is a major target organ for flurochloridone attack and that flurochloridone induces apoptosis in mouse testis-Sertoli cells.[34] Flurochloridone leads to ROS accumulation, causing depolarization of ΔΨm (or decrease of ΔΨm), which opens the mitochondrial permeability transition pore, releases pro-apoptotic proteins and cyt c, activates the cysteine cascade reaction, and initiates mitochondrial pathway-mediated apoptosis in Sertoli cells.[24,34] Apoptosis of Sertoli cells interferes with the proliferation of spermatogonia and meiosis of spermatocytes, resulting in spermatogenesis disorders and decreased male fertility.[35] (The mechanisms supporting disruption of OXPHOS in Sertoli cells leading to apoptosis are shown in Figure 1B) In addition, our previous experiments showed that 43°C heat treatment induced apoptosis in germ cells, but not in Sertoli cells. This is due to the upregulation of heat shock protein 105 and 70 in the cytoplasm of adolescent monkey Sertoli cells under heat stress condition.[36,37] Blocking ERK or PI3K signaling can inhibit the protective effect of heat shock protein 105 and 70 on Sertoli cells; thus,we believe that the ERK or PI3K signaling pathway exists upstream of heat shock protein.[37]

Disruption of OXPHOS in Sertoli cells results in ferroptosis

Ferroptosis is a novel cell death mode that differs from apoptosis. Its morphological manifestations are mainly mitochondrial changes, including mitochondrial shrinkage, mitochondrial crest reduction, and mitochondrial outer membrane rupture.[38] It has been shown that increased respiratory chain activity in mitochondrial OXPHOS leads to the production of lipid ROS and promotes ferroptosis caused by cysteine deprivation. Unlike mitochondria-mediated apoptosis, ΔΨm are hyperpolarized during this process.[2]

Li *et al*[39] demonstrated that glutathione depletion induced glutathione peroxidase 4 inactivation in mouse Sertoli cells after testicular ischemia-reperfusion treatment, and the expression of ferroportin decreased, resulting in a decrease in iron export cells and intracellular iron accumulation. Glutathione peroxidase 4 inactivation and iron accumulation can cause lipid ROS accumulation, which leads to Sertoli cell ferroptosis. The mechanisms supporting disruption of OXPHOS in Sertoli cells leading to cell ferroptosis [Figure 1C]. In addition, mitochondrial ROS-mediated ferroptosis was observed in mouse Sertoli cells exposed to PM2.5.[40] Therefore, lipid ROS induced by glutathione peroxidase 4 inactivation and iron accumulation may be due to mitochondrial OXPHOS damage. Studies have shown that di (2-ethylhexyl) phthalate induces damage to the male reproductive system, including testicular hypoplasia and decreased sperm concentration. Sertoli cells are the key targets of di (2-ethylhexyl) phthalate toxicity.[41] Di (2-ethylhexyl) phthalate interferes with glutathione metabolism in Sertoli cells and increases transferrin receptor levels, further causing intracellular iron overload, eventually inducing oxidative stress and lipid peroxide accumulation, which will lead to Sertoli cell ferroptosis, thereby disrupting Sertoli cell secretion functions and causing damage to blood-testis barrier (BTB) integrity.[41] Spermatogenesis is iron dependent, and there is autonomous circulation of iron in spermatogenic tubules, which depends on the expression and secretion of ferritin and transferrin by Sertoli cells.[5] In addition, Sertoli cells can take up iron from the interstitial tissues through transferrin receptors.[5] Iron is a vital component of iron-sulfur clusters and heme in the mitochondrial OXPHOS respiratory chain and is involved in redox and Fenton reactions. Oxidative stress and lipid peroxide accumulation caused by iron overload will induce ferroptosis.[42,43] During spermatogenesis, germ cells and mature sperm are highly sensitive to oxidative stress. Sertoli cells have high levels of superoxide dismutase and glutathione peroxidase activity, which can protect germ cells from oxidative stress and lipid peroxidation damage.[44,45] The basal membrane of spermatogenic epithelium and myoid cells constitutes the first barrier, while the BTB, in which Sertoli cells participate, constitutes the second barrier. They can prevent excessive accumulation of iron in the peripheral tissues and testicular interstitial tissue from invading germ cells.[5] Therefore, Sertoli cell dysfunction may lead to iron overload in Sertoli cells and germ cells. Intracellular iron overload leads to oxidative stress and lipid peroxidation, which in turn cause ferroptosis and interfere with spermatogenesis and male fertility.

In addition, di (2-ethylhexyl) phthalate also hinders testicular development by disrupting the Wnt/β-catenin signaling pathway.[46] Our previous studies have shown that abnormalities in the Wnt/β-catenin signaling pathway can induce mitochondrial-mediated apoptosis, ferroptosis, and oxidative stress in ischemia-reperfusion injury of organs such as the heart and brain.[47] However, few studies on the Wnt/β-catenin signaling pathway in the testis exist. Therefore, in male infertility mediated by mitochondrial oxidative phosphorylation disorder, how the Wnt/β-catenin signaling pathway performs deserves further study.

Interruption of mitochondrial OXPHOS in germ cells leads to male infertility

Disruption of OXPHOS in germ cell leads to energy metabolism disorders and oxidative stress

Mitochondrial ATP synthase catalyzes ATP synthesis to energy storage and is essential for germ cell survival and maturation during spermatogenesis. Using Drosophila flies with specific cell types with ATP synthase knock out revealed that ATP synthesis deficiency causes defects in germ cell maintenance and maturation, resulting in male sterility.[48]

A study by H. J. Shih *et al*[49] found that testicular torsion resulted in a significant decrease in semen quality. Sufficient mitochondrial OXPHOS in germ cells is essential

for the maintenance of male fertility. Testicular torsion produces deformation of mitochondrial cristae and inner mitochondria, leading to mitochondrial OXPHOS dysregulation. A mouse model of testicular torsion showed a significant reduction in key components of the mitochondrial respiratory chain complex, such as NDUFS1 (complex I), SDHC (complex II), ATP5J, and ATP5C1 (complex V) in germ cells.[49] The mechanism supporting testicular torsion, resulting in a significant decrease in semen quality, is shown in Figure 2A.

In a rat model of varicocele, blood pooling and increased scrotal temperature due to varicose veins resulted in decreased activity of various enzymes associated with OXPHOS. Decreased activity in OXPHOS enzymes, such as NADH cyt c reductase, succinate cyt c reductase, and cyt c oxidase, led to a significant decrease in the energy charge of adenosine monophosphate. Lower energy metabolism of adenosine monophosphate can lead to spermatogenic epithelial cell detachment, resulting in the development of oligospermia, azoospermia, or malformed spermatozoa, which can cause male fertility decline.[50–52] The mechanism by which varicocele cause male fertility decline is shown in Figure 2B.

Disruption of OXPHOS in germ cells leads to apoptosis

Apoptosis is an important cause of male infertility. Mitochondrial signal-mediated intrinsic apoptosis has been shown to play a key role in regulating the apoptosis of primordial germ cells.[53]

Lysiak *et al*[54] investigated the apoptotic pathway of germ cells through a rat testicular ischemia reperfusion injury (tIRI) model. After tIRI occurred in the rat testis, the increase of proinflammatory cytokines INF1α and IL-1β induced phosphorylation of the N-terminal kinase c-Jun (JNK) in testicular vascular endothelial cells, resulting in increased expression of E-selectin in endothelial cells, which further promoted leukocyte rolling, migrating, and infiltrating into the testicular subtunical venules. Endothelial cells become activated and leukocytes invade, leading to an increase in ROS levels.[54,55] On the one hand, ROS released by white blood cells can directly stimulate the mitochondrial signal-mediated intrinsic apoptosis. On the other hand, it initiates an apoptotic pathway that starting from the mitochondria by increasing the level of Bax and releasing cyt c. Activated caspase 9 and its subsequent caspase cascade lead to germ cell DNA degradation and ultimately germ cell apoptosis.[54,56] The mechanism by

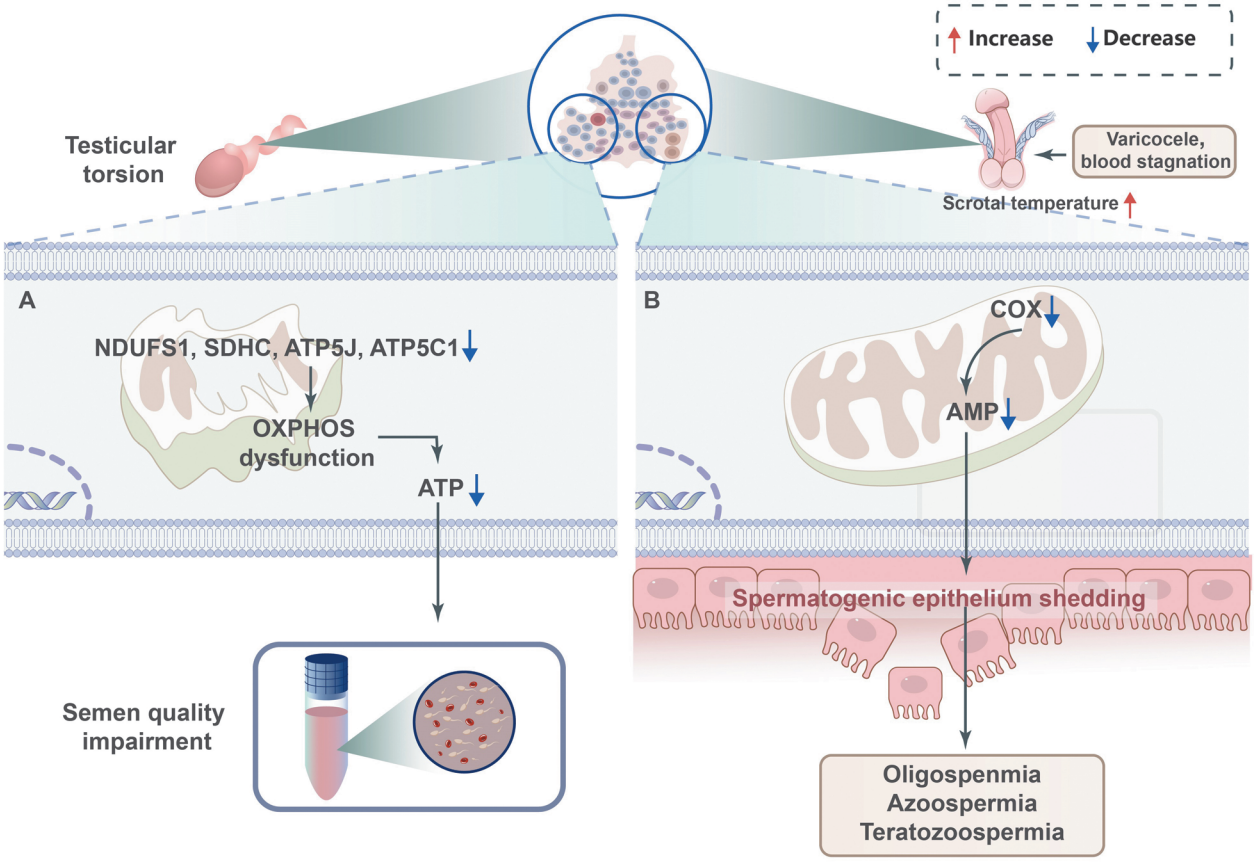

Figure 2: Impaired mitochondrial OXPHOS in germ cells leads to reduced male fertility through reduced energy synthesis. (A) Testicular torsion deforms the mitochondrial cristae and the mitochondrial inner membrane, which is associated with reduced synthesis of key components of the mitochondrial respiratory chain complex, such as NDUFS1 (complex I), SDHC (complex II), ATP5J, and ATP5C1 (complex V) in germ cells, resulting in dysregulation of mitochondrial OXPHOS, leading to reduced semen quality. (B) In patients with testicular varicose veins, scrotal temperature is increased due to blood pooling and the activity of various enzymes associated with the OXPHOS process (including NADH cyt c reductase and succinate cyt c reductase) is reduced, leading to a significant decrease in the energy charge of AMP and the shedding of spermatogenic epithelial cells, resulting in the development of oligospermia, azoospermia and teratozoospermia. AMP: Adenosine monophosphate; ATP: Adenosine triphosphate; COX: Cyclooxygenase; cyt c: Cytochrome c; NADH: Nicotinamide adenine dinucleotide; NDUFS1: NADH-ubiquinone oxidoreductase core subunit S1; OXPHOS: Oxidative phosphorylation; SDHC: Succinate dehydrogenase complex subunit C.

which high levels of ROS lead to germ cell apoptosis is shown in Figure 3.

JNK regulates hydrogen peroxide-induced germ cell apoptosis by enhancing ROS production, decreasing both superoxide dismutase and catalase activities, and increasing the formation of the oxidative stress marker MDA.[57] JNK is involved in tIRI-induced oxidative testicular damage by inhibiting ASK-1, an important kinase activated by JNK.[58] During tIRI-induced germ cell apoptosis, the JNK signaling pathway plays a significant role in regulating testicular oxidative stress (TOS), activating p53, and regulating mitochondrial function.[59] Fadel *et al*[59] treated the testis of male Sprague–Dawley rats with the JNK inhibitor SP600125, and found that JNK inhibition reduced sperm damage induced by tIRI, and prevented DNA strand breaks and DNA adduct formation caused by oxidative stress. The effect of JNK on the testicles is shown in Figure 3.

Vera *et al*[60] found that the mitochondrial-dependent pathway is involved in the mechanism of heat-induced male germ cell death using a mouse heat-induced model. In heat-induced testicular germ cells, members of the B cell lymphoma 2 (Bcl-2) protein family release cyt c from the mitochondria to the cell membrane through inserting mitochondrial membranes.[61–63] Cyt c then initiates caspase activation and the mitochondria-dependent apoptotic pathway by binding to the caspase-activating protein Apaf-1.[63–64] In an experimental cryptorchidism macaque model, germ cells undergo multiple signal pathways controlling germ cell apoptosis, including intrinsic mitochondria pathway and death receptor mediated extrinsic pathway.[65] Another study showed that testicular orphan receptors 3 and p53 may be significant regulators of germ cell apoptosis in heat-treated testis of cynomolgus monkeys.[66] TR3 is able to translocate from the nucleus to mitochondria and interact with Bcl-2 to induce apoptosis.[67]

Another mitochondrial protein, Smac, is released from the mitochondria into the cytoplasm, thus promoting testicular germ cell apoptosis by antagonizing inhibitors of apoptosis protein.[64] The mechanism of the mitochondrial-dependent pathway involved in germ cell apoptosis is shown in Figure 3.

Disruption of OXPHOS in germ cells leads to cell death other than apoptosis

Abnormal mitochondrial OXPHOS is closely associated with ferroptosis.[68] Glutathione peroxidase 4 (GPX4) plays a very important role in ferroptosis. It is not only an enzyme that oxidizes vesicular thiols, but also a structural protein that prevents the oxidation of phospholipids. The stability of the mitochondrial vesicle of mature sperm depends on Gpx4. The mitochondrial isoform of Gpx4 knockout mice can survive completely, but male mice present with subfertility.[69] Patients with weak spermatozoa had higher levels of ROS, MDA, and iron, but lower MMP, SLC7A11, and GPX4.[70]

Cryptorchidism is one of the most common causes of nonobstructive azoospermia, leading to a decline in male

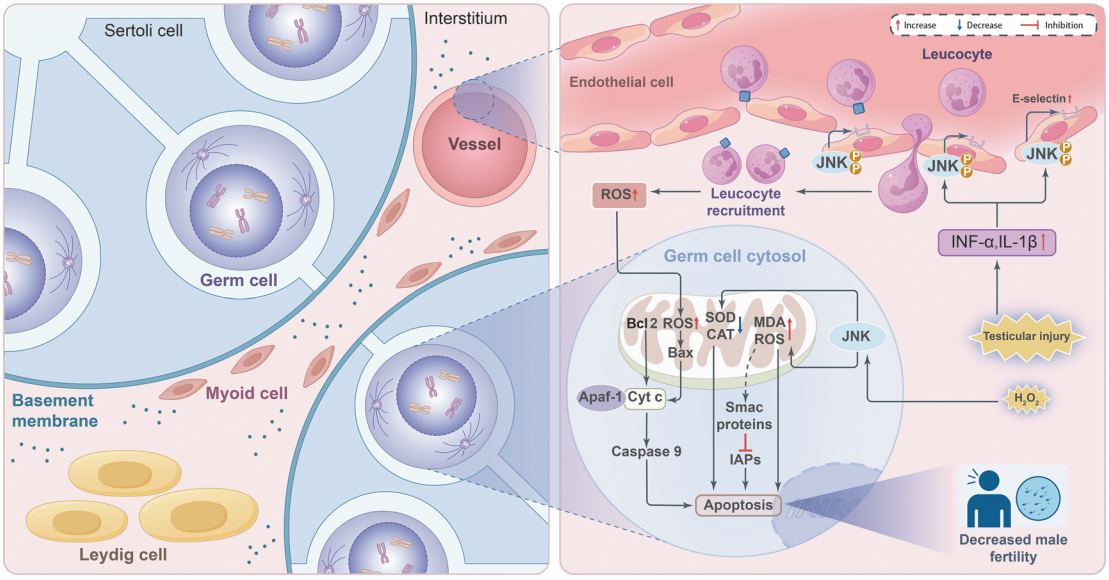

Figure 3: Impaired mitochondrial OXPHOS in germ cells leads to reduced male fertility through different modes of cell death. Testicular ischemia reperfusion injury occurs in the testis and activates endothelial cells, the increase of proinflammatory cytokines INF1α and IL-1β induced phosphorylation of JNK in testicular vascular endothelial cells, resulting in increased expression of E-selectin in endothelial cells, which further promoted leukocyte invasion. This leads to an increase in ROS levels. ROS directly stimulates the mitochondria-driven apoptotic pathway leading to germ cell apoptosis; however, by increasing the level of Bax, cyt c is released and initiates the mitochondria-driven apoptotic pathway, and activates caspase 9 and its subsequent caspase cascade, leading to germ cell DNA degradation and apoptosis. Under H_2O_2 induction, JNK in germ cells upregulated ROS and MDA levels while decreasing SOD and CAT activity, leading to germ cell apoptosis. Heat-induced insertion of Bcl-2 protein family members into the mitochondrial membrane in testicular germ cells allows the release of cyt c from the mitochondria into the cell membrane. Cyt c activates caspases by binding to Apaf-1 and initiating a mitochondria-dependent apoptotic pathway. At the same time, Smac proteins are released from the mitochondria into the cytoplasm and promote apoptosis in testicular germ cells by antagonizing IAPs. Bcl-2: B-cell lymphoma-2; CAT: Catalase; cyt c: Cytochrome c; H_2O_2: Hydrogen peroxide; IAPs: Inhibitors of apoptosis protein; JNK: The N-terminal kinase c-Jun; MDA: Malondialdehyde; OXPHOS: Oxidative phosphorylation; ROS: Reactive oxygen species; SOD: Superoxide dismutase; tIRI: Testicular ischemia reperfusion injury.

fertility. Some patients still experience nonobstructive azoospermia after undergoing cryptorchidopexy surgery.[71] Tang *et al*[72] showed that Johnson scores from HE staining were significantly reduced in cryptorchid tissues compared to normal tissues, indicating maturation arrest during spermatogenesis in post-cryptorchidopexy patients with non-obstructive azoospermia. Next-generation sequencing results showed that OXPHOS is an important KEGG pathway enriched in differentially expressed genes.

In a scrotal hyperthermia mouse model, heat stress disrupted spermatogenesis with degenerative changes in the germinal epithelium inside the seminiferous tubules.[73] In this model, significant increases in Caspase-1 (Pyroptosis), Bclin1 (autophagy), Atg7 (autophagy), MLkL (necroptosis), and Acsl4 (ferroptosis) proteins have been observed, indicating that scrotal hyperthermia can induce various types of cell death.[73] Pyroptosis is accompanied by caspase-1 activation followed by cell membrane rupture, which causes DNA damage and fragmentation. Enhanced protein expression of Bclin1 and Atg7 induces autophagy activation.[74] Ferroptosis induces ROS production by mediating lipid peroxidation and activating the Acsl4 pathway. Lineage kinase domain-like protein (MLKL) is a key component in the formation of the functional necrosome.[75] It involves a sudden release of cell contents and the destruction of mitochondria and nuclear DNA.[76] The mechanism of germ cell death in the testes of scrotal mice may be like that in cryptorchidism patients. High temperatures cause OXPHOS disorder in testicular tissue.[72]

Summary and Perspectives

This review explored how abnormal mitochondrial OXPHOS affects spermatogenesis and leads to impaired fertility. We also discussed the mechanism of mitochondrial OXPHOS dysfunction in testicular Sertoli and germ cells, leading to abnormal spermatogenesis regarding energy metabolism disturbance, oxidative stress, cell apoptosis, and ferroptosis. Past treatments for abnormal spermatogenesis caused by mitochondrial OXPHOS disorders include gene and drug therapies. Gene therapy, such as the use of tissue-specific gonad-associated viruses, can increase OXPHOS levels.[77] Drug treatment, such as intra-tumoral injection of the mitochondria-derived peptide humanin, can inhibit apoptosis of the germline mitochondria pathway and ensure normal spermatogenesis during cellular stress.[78,79] Resveratrol improves mitochondrial metabolism, increases $\Delta\Psi m$, promotes spermatogenesis, and treats asthenospermia.[80,81] Antioxidants, such as oral coenzyme Q10, reduce damage to sperm caused by excessive ROS and reduce oxidative stress, thus treating male fertility.[82] Moreover, mitochondrial transplantation *in vitro*, especially in oral mitochondria, has become a potential research direction for treating mitochondrial diseases. Xiao *et al*[83] found that through oral administration, mitochondria are absorbed by the small intestine and transported to the pulmonary artery using red blood cells as carriers, subsequently alleviating pulmonary hypertension. This type of treatment may enhance the treatment of mitochondrial OXPHOS disorders during spermatogenesis. This study explored the mechanisms of OXPHOS disorders and spermatogenesis abnormalities, providing ideas for future therapies targeting oxidative stress, apoptosis, and ferroptosis downstream of mitochondrial OXPHOS. Co-targeting the mitochondria and their downstream targets will undoubtedly play a better role.

In addition, the mechanisms by which mitochondria are involved in spermatogenesis are diverse and complex. For example, changes in mitochondrial dynamics can also damage germ cells. A study found that the expression of mitochondrial fusion-related proteins decreased in mice exposed to TiO2 NPs. In contrast, the expression of DRP1 was upregulated, and mitochondrial dynamics were disordered, resulting in reduced sperm motility and a swollen sperm count.[84] In addition, the expression of mitochondrial fusion-related proteins decreased in chickens exposed to CuSO4 and in male Wistar rats exposed to Cr (VI). In contrast, the expression of DRP1 was upregulated, which caused mitochondrial over-division, promoted cyt c release, activated Caspase-9, promoted germ cell apoptosis and testicular tissue destruction, and affected male fertility.[85–87]

Although the current exploration of the mechanism of mitochondrial OXPHOS disorders and spermatogenesis abnormalities has achieved remarkable results in animal models, the mechanism observed in animal experiments requires much research prior to application in pre-clinical and clinical trials. The lack of clinical trials has become a bottleneck restricting the transition from theoretical research to experimental application. Future research should further explore the pathogenic mechanism of abnormal spermatogenesis and the corresponding treatment plan and strive to apply it to pre-clinical and clinical trials to provide more accurate treatment for more patients with spermatogenesis disorders and improve male fertility.

Acknowledgement

The authors would like to express their gratitude to EditSprings (https://www.editsprings.com/) for the expert linguistic services.

Funding

This work was supported by fundings from the Research Start-up Fund of Jining Medical University (Nos. 600791001 and 600980001), Research Fund for Lin He's Academician Workstation of New Medicine and Clinical Translation in Jining Medical University (No. JYHL2021MS13) and the National Natural Science Foundation of China (No. 81700055).

Conflicts of Interest

None.

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How to cite this article: Meng K, Liu Q, Qin YD, Qin WJ, Zhu ZM, Sun LL, Jiang MC, Adu-Amankwaah J, Gao F, Tan RB, Yuan JX. Mechanism of mitochondrial oxidative phosphorylation disorder in male infertility. Chin Med J 2025;138:379–388. doi: 10.1097/CM9.0000000000003126