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NARRATIVE REVIEW

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Sperm mitochondria dysfunction in response to testicular cancer

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Abstract

Testicular cancer is the most common form of cancer in young men of reproductive age and its incidence is increasing globally. With the currently successful treatment and 95% survival rate, there is a need for deeper understanding of testicular cancer-related infertility. Most patients with testicular cancer experience semen abnormalities prior to cancer therapy. However, the exact mechanism of the effect of testicular cancer on sperm anomalies is not known. Mitochondria are organelles that play a crucial role in both tumorigenesis and spermatogenesis and their malfunction may be an important factor resulting in sperm abnormalities in testicular cancer patients. Within the scope of this review, we will discuss current knowledge of testicular cancer-related alterations in the ATP production pathway, a possible pathophysiological switch from oxidative phosphorylation (OXPHOS) to glycolysis, as well as the role of oxidative stress promoting sperm dysfunction. In this regard, the review provides a summary of the impact of testicular cancer on sperm quality as a possible consequence of impaired mitochondrial function including the energy metabolic pathways that are known to be altered in the sperm of testicular cancer patients.

Maryam Qasemi and Vishma Pratap Sur contributed equally to this study.

KEYWORDS
glycolysis, mitochondrial dysfunction, OXPHOS, sperm pathology, testicular cancer

1 | INTRODUCTION

Testicular cancer is the most common cancer in young men with half of the cases diagnosed between 20 and 34 years of age. Its incidence worldwide has been steadily increasing in recent years.[1] The current advances in treatment have led to relatively high survival and the global 5-year survival rate is ~95%.[2] Testicular germ cell tumour (TGCT) originates in the germ cells, which are responsible for the production of sperm, with a considerably negative impact on male reproductive health.[3] There are many risk factors for TGCT development, such as cryptorchidism, hypospadias, perinatal factors, exposure to hormone-disrupting chemicals and familial risk of testicular cancer, especially among siblings or a history of germ cell tumours in the other testis.[4] The reported increased incidence of testicular cancer is accompanied by an increase in the number of men with infertility and abnormal results of semen analysis. Men diagnosed with infertility are three times more likely to develop testicular cancer compared to healthy normozoospermic men, suggesting common etiological factors, with TGCT being a likely purveyor of low fertility/infertility.[5,6] According to a retrospective cohort study, the risk of being diagnosed with testicular cancer is increased in men with poor semen quality, which is likely due to common genetic/epigenetic abnormalities that play a role in both gamete abnormalities and aberrant cell proliferation in cancer cells.[7] It should be noted that testicular cancer has a particularly deleterious effect on semen parameters which can be improved after unilateral orchiectomy.[8] Infertility is a major reproductive health problem that affects 17.5% of adults, which means one in six couples worldwide (WHO, 1 April 2023), and it is caused by a combination of factors distributed equally between females and males.[9] A possible association between testicular cancer and infertility has drawn considerable attention in recent years. It is noteworthy that semen abnormalities are frequently observed in patients with testicular cancer even before any initiation of anticancer treatment.[10] Nevertheless, the underlying molecular mechanisms responsible for these sperm abnormalities have yet to be fully explained.

A critical consequence of testicular cancer is the pathological effect on sperm quality, including motility, which is decreased or absent due to dysregulation in mitochondria-related proteins.[11] Mitochondria are the organelles within cells that play a critical role in energy production.[12] In sperm, intact and physiologically functional mitochondria are important for the maintenance of sperm motility and viability, and they are also involved in the capacitation process.[13] The function of sperm depends on the generation of ATP, which is produced by means of glycolysis and oxidative phosphorylation (OXPHOS).[14] Decline in sperm quality is reported to be due to the effect of mitochondrial dysfunction leading to decreased ATP production and to oxidative damage due to elevated generation of reactive oxygen species (ROS), and to increased DNA fragmentation.[8,15] Given the pivotal role of mitochondria in tumorigenesis as well as spermatogenesis as a process of sperm development from germ stem cells, mitochondrial involvement in triggering sperm abnormalities and infertility in the context of testicular cancer is an intriguing avenue for investigation. Hence, this review will discuss the impact of testicular cancer on sperm quality as a consequence of impaired mitochondrial function via metabolic/bioenergetic pathways involving glycolysis and OXPHOS (Figure 1).

2 | MITOCHONDRIA IN SPERM

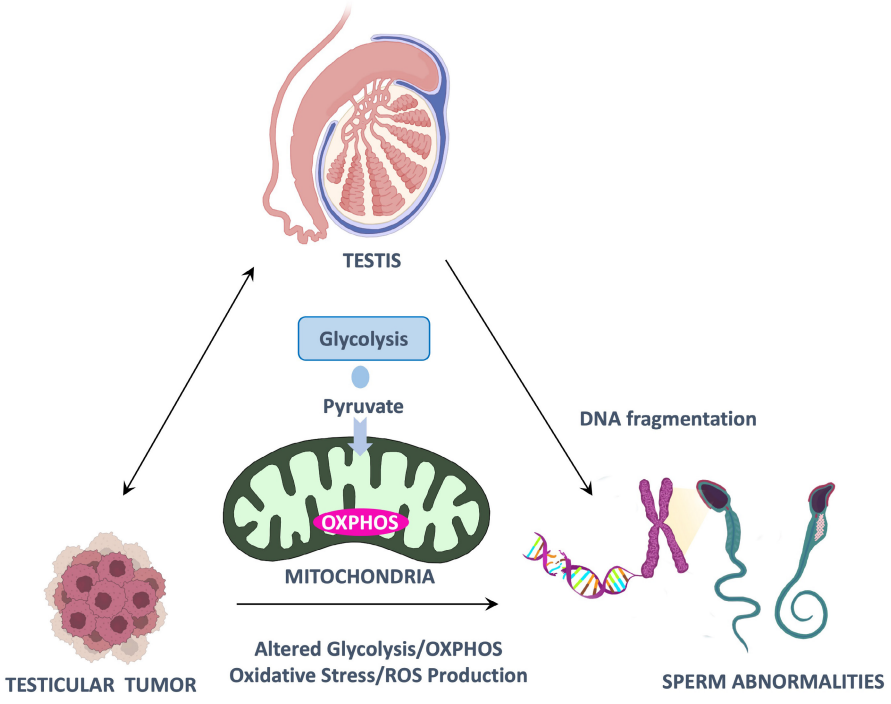
To understand the effect of testicular cancer on sperm abnormalities related to mitochondrial dysfunction, we will first look in brief at the role of mitochondria in the course of spermatogenesis.

Mitochondria provide the energy power to cells, ensuring sufficient energy for live cells.[16]

However, in the male reproductive system, mitochondria are responsible not only for the energy supply of cells, but also for steroidogenesis.[17] Cytochrome P450, an enzyme that is located on the inner membrane of mitochondria, converts cholesterol to pregnenolone, which is the precursor of steroid hormones.[17] Mitochondria are also vital for Leydig cells, which are essential somatic cells in the testis as they produce a high level of testosterone,[18] which is vital for physiological processes in men, including spermatogenesis.

During spermiogenesis, in which the haploid round spermatids are differentiated into morphologically functional spermatozoa, mitochondria undergo several gradual changes[17] (Figure 2). The shape of mitochondria is altered from the spheroid to rod-like and a high number of mitochondria are lost by reduction of the cytoplasm in spermatids.[19] In sperm, the remaining mitochondria are concentrated in the midpiece, which is the most proximal region of the human sperm tail.[20] Mitochondria in human

FIGURE 1 A schematic diagram depicting the scope of the presented review article.



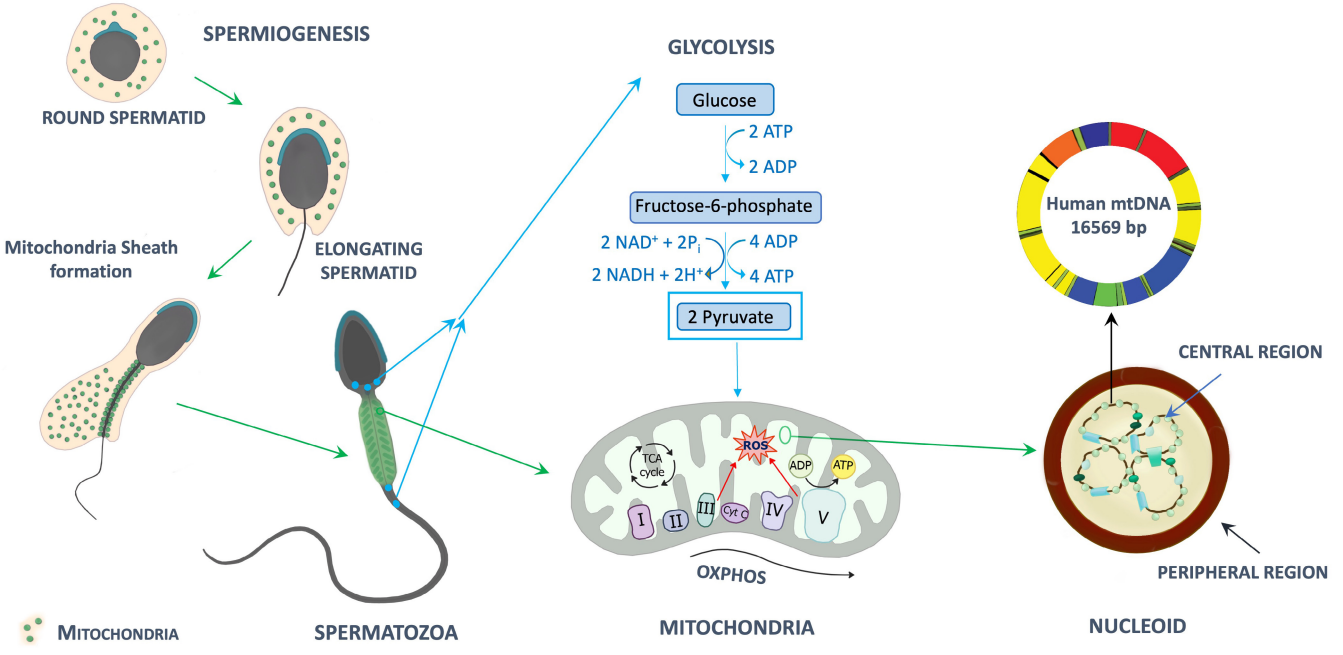


FIGURE 2 A scheme showing the mitochondrial sheath during spermiogenesis and mitochondrial function in mature spermatozoa.

sperm are arranged in an end-to-end manner forming the structure known as the 'mitochondrial sheath' that wraps along the outer dense fibres of the sperm tail.[21] These mitochondria, which are similar in ultrastructure and function to those found in other cells, assume a shape known as the 'mitochondrial capsule'.[22] This capsule, formed by disulfide bonds between cysteine- and proline-rich proteins, provides stability and resistance to sperm within the hypo-osmotic environment.[23] In sperm, mitochondria are responsible for the regulation of apoptosis, for Ca^{2+} homeostasis required for flagellum motility, for capacitation and for acrosomal reaction.[16] Furthermore, ROS, which has a detrimental effect on DNA in case of elevation, is required for the physiological function of sperm at normal levels. It is essential for cholesterol exclusion, tyrosine phosphorylation and sperm-oocyte interaction.[24]

In addition to the above-mentioned, mitochondria are, particularly, essential for energy production by means

of cellular respiration, making them crucial for sperm motility.[14,16]

Sperm are highly specialized cells that require a continuous and efficient supply of energy to carry out their various functions during different stages of the male reproductive process.[25] Energy production in sperm involves, as in any somatic cell, a combination of two main pathways, viz. OXPHOS and glycolysis.[26] Interestingly, the preferred pathway in sperm and the extent of its utilization vary among species, in the developmental stage of the germ cell during spermatogenesis and the physiological status of sperm.[13] During its final stage, referred to as spermiogenesis, particularly at the stage of elongated spermatids, OXPHOS is the main energy-generating pathway.[21] This stage is characterized by extensive remodelling of the cell structure to form the sperm tail and the acrosome, both of which require a significant amount of ATP. Consequently, OXPHOS is particularly crucial during this phase, providing the majority of ATP required for the energy-intensive processes involved in sperm maturation.[27] As sperm develop towards maturity, the reliance on OXPHOS continues to play a dominant role in supporting future motility. Mature sperm, also known as spermatozoa, utilize OXPHOS as their main energy source for maintaining their motility.[27] In addition, glycolysis comes into play to support specific sperm functions and processes that require rapid 'bursts' of energy. Glycolysis is particularly important during sperm capacitation, a process that occurs after sperm is released into the female reproductive tract.[21,27] However, it has been confirmed that spermatozoa exposed to a carbohydrate substrates-free medium or inhibitors of glycolysis[27,28] showed no significant effect on motility. This evidence showed that there must be another indirect energetic pathway which can sustain their function. It has been confirmed in a study where *Slc22a14* ablation in mice caused total infertility which can be by passed ICSI. This fact showed that the riboflavin transporter SLC22A14 as part of fatty acid oxidation plays an important role in sperm physiology.[29]

Capacitation is a prerequisite for fertilization and involves modification of sperm membrane properties and ion fluxes, which are both energy-demanding processes.[30] Glycolysis provides a quick and readily available source of ATP, facilitating these dynamic changes in sperm membrane function during capacitation.[31] Furthermore, glycolysis is involved in hyperactivation, a vigorous and asymmetric beat pattern of the sperm tail that enhances motility, enabling sperm to move through the female reproductive tract and reach the egg for fertilization.[27] Additionally, glycolysis supports the acrosomal reaction, an essential step in fertilization, where the sperm lytic vesicle, referred to as the acrosome, releases enzymes that facilitate penetration across the oocyte protective layers. These processes require rapid and brief bursts of energy, making glycolysis a relevant pathway.[27]

3 | MITOCHONDRIA IN TESTICULAR CANCER

TGCT has been linked to sperm abnormalities. There are studies pointing to the notion that TGCT can lead to a reduction in sperm motility, altered ATP production and changes in glycolysis pathway activity.[3,32,33] The exact mechanism by which TGCT affects sperm function is still being researched, but it is believed that disrupted mitochondrial function as well as hormonal and metabolic alterations associated with the presence of cancer may contribute to the observed sperm pathologies.[15] Mitochondrion is an organelle with a crucial impact not only on spermatogenesis but also on carcinogenesis,[17,34] which is affected by mitochondrial function. Various mitochondria-related mechanisms, including mtDNA mutation, ROS formation, apoptosis control, metabolic reprogramming, etc., promote the onset and progression of cancer.[35] Unfortunately, studies on mitochondrial abnormalities in testicular cancer are limited.

Notwithstanding the above, glycolysis is dynamically correlated with mitochondrial function and has been investigated in testicular cancer. It has been found that almost all types of cancer,[36] such as ovarian, colorectal, lung and liver cancer,[37–40] as well as testicular cancer,[41,42] display the so-called Warburg effect. This phenomenon is characterized by a preference for ATP generation by glycolysis, thus neglecting the need for ATP generation by OXPHOS.[37,38,41,43,44] Explaining in greater detail, the supply of sufficient ATP is crucial for tumour growth, therefore the tumour cells form blood vessels and so-called angiogenesis occurs.[45] Even in the presence of oxygen, tumour cells preferentially select glycolysis. NADH is consumed during glycolysis to form NAD, thus, to regenerate NAD for further ATP production, this coenzyme must be regenerated. The tumour cells undergo the so-called Warburg effect, therefore they use lactate dehydrogenase to regenerate NAD in order to produce lactate and NADH. This process is efficient for the tumour cells and allows them to grow rapidly. However, for the patient, this mechanism represents a poorer prognosis.[40,46] Furthermore, glycolysis creates an acidic environment which has a deleterious effect on normal cells, while it is not harmful for tumour cells. This phenomenon provides a defence mechanism for the proliferation of cancer cells.[36] Specifically, increased glycolysis in testicular cancer has been shown to contribute to increased cell division and tumour growth.[41,47]

Linked to the role of glycolysis in testicular cancer, several studies have shown that drugs such as imatinib, cetuximab, 3-bromopyruvate (3BrPA), lonidamine and FX11 that target glucose uptake and glycolysis have been shown to possess anti-tumour effects in testicular cancer.[41,48,49,50] There are different factors that can lead to a switch to the Warburg effect,[42] such as mitochondrial defects and increased expression or amplification of relevant genes[51] that, when overexpressed, contribute to increased glycolysis observed in cancer cells, including testicular cancer, promoting tumour growth and aggressiveness.[41,52] In cancer, some of the genes that are overexpressed in glycolysis include hexokinase-1, hexokinase-2, glucose phosphate isomerase, aldolase A and glyceraldehyde-3-phosphate dehydrogenase. Hexokinase-1 and -2 are enzymes that catalyse conversion of glucose to glucose-6-phosphate, a first step in glycolysis.[52–54] They are the main regulated enzyme of glycolysis. The upregulation of genes encoding these enzymes leads to increased glycolysis activity, thus promoting the Warburg effect.[55] Overexpression of hexokinase-2 has been observed in various types of cancer, including glioblastoma multiforme[41,56] and TGTCs. Thus, the notion that mitochondria are significantly dysregulated in testicular cancer and that they play a crucial role in spermatogenesis[15,57] may explain the sperm abnormalities in testicular cancer prior to any treatment,[11,15,58] although this relation needs deeper investigation to be confirmed and better understood.

Relevant to this topic, cancer/testis antigens (CTAs) are a group of genes that are typically physiologically expressed exclusively in the testes during spermatogenesis and are also expressed in certain types of cancer such as breast, lung, melanoma and prostate cancers, according to the pathological condition.[59] During spermatogenesis, distinct CTAs are expressed in individual differentiation stages. Although the exact function of CTAs is poorly understood, their expression is essential for spermatogenesis. The lack of these CTAs was reported to compromise physiological spermatogenesis, and result in infertility or subfertility,[59,60] supported by the evidence, that mice depleted for particular CTAs, such as tudor domain contain 6 (Tdrd6), testis expressed 15, meiosis and synapsis associated (Tex15), and maelstrom spermatogenic transposon silencer (Mael) were infertile.[59–63] CTAs present unique expression patterns in different cancer environments, which makes them potential targets for cancer immunotherapy and diagnostic markers for cancer detection and monitoring.[60,64–66] Studies have suggested that the molecular processes involved in these two apparently distinct biological events may share common metabolic adaptations related to energy supply.[40,59,65] Specifically, CTAs such as spermatogenesis-associated-protein 19 (SPATA19), glycerol-3-phosphate acyltransferase 2 (GPAT2), foetal and adult testis expressed 1 (FATE1), cytochrome C oxidase subunit 6B2 (COX6B2), and KIAA0100 are related to mitochondrial function.[59] It has been shown that these CTAs are involved in a variety of cancers such as breast, prostate, melanoma and lung neoplasia.[59,67–69] So far, the information is missing on the abovementioned CTAs in testicular cancer. Their aberrant expression needs to be studied, particularly in mitochondria-related CTAs, with relevance to testicular tumours and sperm anomalies.

4 | EFFECT OF TESTICULAR CANCER ON SPERM QUALITY AND THE ROLE OF MITOCHONDRIAL ABERRATIONS

Recent studies have suggested a correlation between the increasing incidence of testicular cancer and declining semen quality leading to male infertility.[70,71] According to the current data, there is a relationship between the increased risk of testicular cancer and changes in specific semen parameters such as low semen concentration, poor sperm motility and the high proportion of morphologically abnormal spermatozoa. Generally, it is estimated that 6%–24% of testicular cancer patients face azoospermia and 50% oligozoospermia at the time of cancer diagnosis.[11,32,58,72] Although the exact mechanism is not well understood, mitochondria are likely an important organelle lined to testicular cancer initiation and progression that has been investigated by several studies.[8,11,15,57,73] Dias et al.[15] reported that the level of ubiquinol-cytochrome C reductase core protein 2 (UQCRC2) and NADH:ubiquinone oxidoreductase core subunit S1 (NDUFS1) proteins, which are related to the sperm mitochondrial function, as well as testis-specific sodium/potassium-transporting ATPase subunit alpha-4 (ATP1A4), are downregulated in non-seminoma testicular cancer patients when compared to healthy men. More specifically, NDUFS1 is a subunit of mitochondrial complex I that is an entry point of electrons into the electron respiratory chain (ETC).[74] UQCRC2 is a subunit of complex III that accepts electrons for complex III and moves them on to complex IV, processes essential for the function of OXPHOS.[75] Downregulation of these proteins suggests mitochondrial dysfunction with ensuing reduced activity of OXPHOS.[15] Furthermore, dysregulation of proteins in the OXPHOS system may result in ETC dysfunction and the consequent induction of oxidative stress.[76] As a result of oxidative stress, increased sperm abnormalities, such as DNA fragmentation,[8] occur in addition to aberrant function of proteins that are essential in sperm physiological processes due to their oxidative modification.[77]

Mitochondrial dysfunction and inhibition of the OXPHOS process have been suggested to explain the impaired sperm motility observed in testicular cancer patients, as OXPHOS plays an important role in energy supply to support sperm movement.[11,27,57] Furthermore, NDUFS1 and UQCRC2 downregulated in non-seminoma testicular cancer patients were proposed to be involved in the rapamycin-insensitive companion of the mammalian target of rapamycin (RICTOR) signalling pathway.[11,57,73] This pathway is important for spermatogenesis, helping to maintain the blood-testis barrier and proper regulation of spermatogenesis.[78,79] Thus, downregulation of NDUFS1 and UQCRC2 not only impairs sperm quality by means of dysfunctional OXPHOS but also represents the deregulation of an important signalling pathway playing an important role in spermatogenesis.

ATP1A4, reported to be downregulated in testicular cancer patients,[15] is a protein localized in the sperm flagellum, with an important role in sperm maturation, motility and fertilization involving potassium and sodium exchange across the plasma membrane.[15,80,81,82] ATP1A4 is a subunit of a Na^+/K^+-ATPase pump, which is responsible for maintaining the ion gradients across the plasma membrane of sperm cells.[81,82] The Na^+/K^+-ATPase pump is essential for male fertility, making ATP1A4 an attractive target for male contraception.[81] Furthermore, dysregulation of ATP1A4 has been correlated with mitochondrial dysfunction and abnormal ROS production, which results in oxidative stress.[83] These aberrations could explain semen abnormalities such as reduction in sperm motility, concentration and total count of sperm in testicular cancer patients.[15] Similarly, ATP5A1 which is essential for ATP production via OXPHOS is downregulated in testicular cancer patients with asthenozoospermia,[11] further pointing to a role of mitochondrial dysfunction in sperm abnormalities in testicular cancer.[11]

An interesting study compared mitochondrial activity and DNA fragmentation in the sperm of TGCT patients before and after orchiectomy.[8] Although no significant difference was observed in semen parameters such as motility, there was a significant increase in mitochondrial activity and decrease in DNA fragmentation in sperm 30 days after unilateral orchiectomy.[8] Consequently, the authors recommended preservation of TGCT patient semen to post-orchiectomy surgery to keep sperm with fewer abnormalities. These data also revealed that TGCT, even in cases with unchanged semen parameters, causes sperm abnormalities such as mitochondrial dysfunctionality and DNA fragmentation, which should be considered for fertility management of the patients.[8] Imbalance in ROS production is related to lipid peroxidation, further linked to increased DNA fragmentation, which is mainly a consequence of mitochondrial dysfunction resulting in oxidative stress,[84] which negatively impacts fertility.[85,86] It was demonstrated by Calamai et al.[87] that although the levels of oxidative stress and DNA fragmentation were increased in sperm of testicular cancer patients, there was no significant association between oxidative stress and sperm DNA fragmentation, which could be ascribed to several other unknown factors.[87] For instance, mitochondrial priming, an event that promotes intrinsic apoptosis pathway,[88] may induce DNA fragmentation and should be investigated in the context of sperm anomalies in testicular cancer patients. Studies demonstrated that mitochondria priming is high for testicular cancer, particularly for TGCT, and is associated with a positive response to cancer therapy.[89–91] Since DNA fragmentation is reported to be also a consequence of activation of apoptosis-inducing factors in mitochondria,[92] this phenomenon in testis, especially in TGCT, may present yet another cause of sperm DNA fragmentation that should be investigated.

The abovementioned studies highlight the importance of mitochondria and the relevant pathways in testicular cancer that could directly affect spermatogenesis with or without sperm parameter alterations, which ought to be considered in the field of testicular cancer.

5 | CONCLUSIONS

Among men suffering from various types of cancers, testicular cancer patients are diagnosed with low semen quality prior to anti-cancer therapies,[93,94] and the extremely poor recovery rate of thawed cryopreserved sperm.[95,96] The association between testicular cancer and sperm abnormalities further highlights the intricate relationship between mitochondrial function, energy metabolism and sperm quality, underscoring the importance of understanding these mechanisms for potential therapeutic interventions in male reproductive health. Energy metabolism in sperm is a finely tuned interplay between OXPHOS and glycolysis, with one dominant pathway that varies among sperm developmental stages.[23,30] OXPHOS predominantly supports energy demands during spermiogenesis, epididymal maturation and motility, while glycolysis provides rapid bursts of ATP required for processes such as capacitation, hyperactivation and the acrosomal reaction,[27] and moreover, fatty acid oxidation contributes to the sperm energy metabolism and fertilizing ability.[29]

Continued research into mitochondrial function in sperm is essential to further unravel the molecular basis of these associations and to develop targeted strategies to address sperm abnormalities associated with testicular cancer. Based on the current knowledge, impairment of ATP

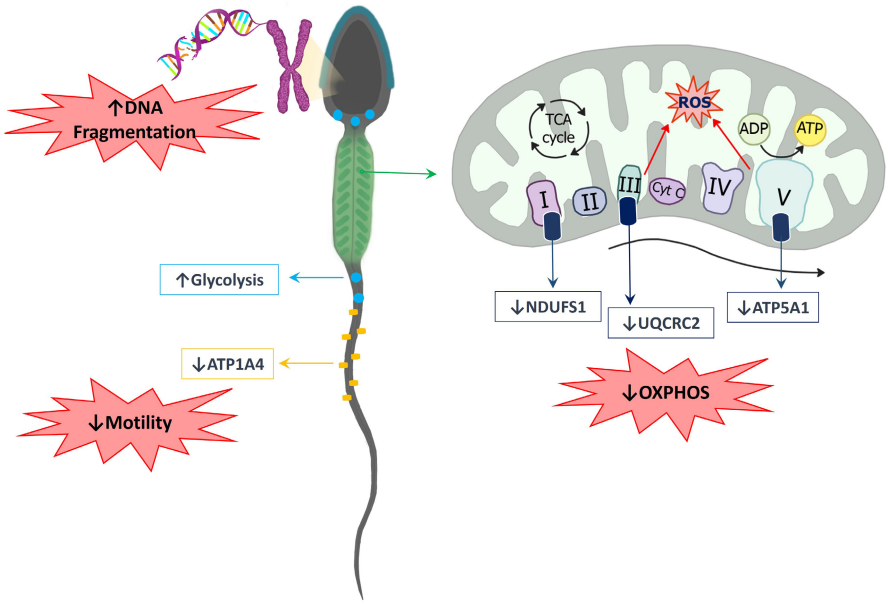


FIGURE 3 A scheme summarizing the influence of testicular cancer on sperm mitochondrial function. The arrows pointing up or down indicate elevated or decreased levels of individual aspects.

TABLE 1 Summary of mitochondria-related dysregulations in sperm of testicular cancer patients.

Dysregulated factor	Type of dysregulation	Assessed in sample(s)	References
NDUFS1 (subunit of complex I in ETC)	↓	Sperm of non-seminoma testicular cancer patients	[15,73]
UQCRC2 (subunit of complex III in ETC)	↓	Sperm of non-seminoma testicular cancer patients	[11,15]
ATP5A1 (subunit of complex V in ETC)	↓	Asthenozoospermic sperm of testicular cancer patients	[11]
ATP1A4 (Na^+/K^+-ATPase pump)	↓	Sperm of non-seminoma testicular cancer patients	[11,15]
Proteins associated with OXPHOS	↓	Sperm of cancer patients including testicular cancer, Hodgkin's disease, etc.	[57]
Mitochondrial activity	↓	Sperm of TGCT patients before unilateral orchiectomy	[8]
DNA fragmentation	↑	Sperm of testicular cancer patients before unilateral orchiectomy	[8]
Oxidative stress	↑	Sperm of testicular cancer patients	[87]

production by OXPHOS negatively affects sperm quality, especially their motility (summarized in Figure 3 and Table 1).[11] Sperm are cells with high demands for energy, and OXPHOS is the main pathway for providing ATP for spermatogenesis, maturation as well as sperm motility,[27] while energy supply in cancer is more dependent on glycolysis.[8] Several proteins required for OXPHOS, including NDUFS1, UQCRC2 and ATP5A1 are downregulated in testicular cancer, which can explain sperm abnormalities in testicular cancer patients.[8,11,15,57,73]

Sperm motility is not the only factor that is affected by mitochondria in testicular cancer, as even patients with normal semen parameters are reported to show mitochondrial dysfunction prior to orchiectomy.[73] Mitochondrial function and DNA fragmentation, being a consequence of oxidative stress due to dysfunctional mitochondria, show improvement after orchiectomy.[8] Since sperm cryopreservation prior to orchiectomy is one of the approaches to fertility management of testicular cancer patients,[94] mitochondrial abnormalities should be considered. Reaching specific biomarkers reflecting sperm mitochondrial functionality in testicular cancer patients may help improve fertility management strategies. Thus, uncovering the common mechanisms that are involved in testicular tumours and infertility occurrence in testicular cancer patients could be applied not only for diagnostic approaches but also for targeted therapy. Although several studies have pointed to the important role of mitochondria in testicular cancer-related infertility,[8,11,15,57,73] broader investigations, particularly in energy metabolism of sperm as well as mtDNA abnormalities under burden of testicular cancer are required. mtDNA aberrations, such as deletions,

mutations and polymorphisms are reported to be associated with different types of cancer,[97] while mtDNA genetic abnormalities in testicular cancer have not been identified to date. Since paternal mtDNA inheritance was detected in some cases,[98,99] it is crucial to investigate possible mtDNA abnormalities in testicular cancer as well as in cryopreserved sperm of cancer patients to avoid their potential inheritance. Furthermore, new research targeting CTA and mitochondrial priming is likely to provide new insights into sperm pathogenesis as a consequence of testicular cancer.

AUTHOR CONTRIBUTIONS

All the authors contributed to this review article. MQ, VPS, OS, JN and KK writing the main text; MQ and KK designing Figures 1–3; KK funding acquisition. PP, PS, TH, LB, LZ, TB contributed to the manuscript outline. LB, LZ, TB and KK funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflict of interest.

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