

Role of Endoplasmic Reticulum Stress in The Male Reproductive System

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Abstract

Testicular dysfunction, whether linked to varicocele, obesity, diabetes, aging, inflammation, or lifestyle or environmental issues, is frequently accompanied by an accumulation of unfolded or misfolded proteins, indicating impaired endoplasmic reticulum (ER) function. In this review, we examined the Google Scholar, Scopus and PubMed databases (from 2011 to 2022) to support the association of ER stress with defective spermatogenesis in animal models and humans. ER stress, whether in its pro-survival or pro-apoptotic aspect, appears to be closely linked to each studied situation. Several studies have demonstrated a significant increase in oxidative stress (OS) levels in infertile men compared to fertile individuals, which is associated with poor spermatogenesis quality. OS is likely the result of the interplay between ER stress and spermatogenesis defects. These findings suggest that therapeutic strategies aimed at mitigating both ER stress and OS could be of interest in restoring male reproductive function.

Keywords: Apoptosis, Endoplasmic Reticulum Stress, Spermatogenesis, Varicocele

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Introduction

Male infertility exhibits a wide range of origins, encompassing individual genetics and environmentally associated factors (1). Recent evidence suggests a frequent association of male infertility with other conditions such as cancer, cardiovascular disease, and autoimmune responses (2). This implies that male infertility and/or impaired sperm quality could potentially serve as early indicators of underlying dysfunctions, serving as essential biomarkers for an individual's overall health (3).

The specific nature of these associations remains uncertain, but they likely stem from shared factors such as genetic predisposition and/or lifestyle and environmental influences. There is a general consensus indicating that oxidative stress (OS), whether originating systemically and/or locally due to environmental interactions or inherited imbalances in reactive oxygen species (ROS) generation/recycling inherent to aerobic cellular metabolism, may serve as a common underlying mechanism contributing to

these pathological associations (3). At the subcellular level, OS closely correlates with a fundamental cellular dysfunction known as endoplasmic reticulum stress (ER-stress) (4), which has received limited investigation in relation to male infertility. This review aims to enhance our comprehension of ER-stress and male infertility, examining various scenarios, both pathological and non-pathological, where ER-stress has been linked to male fertility. These scenarios encompass varicocele, obesity, diabetes, aging, inflammasome activation, and environmental impacts.

The review study was conducted in the PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), Google Scholar (<https://scholar.google.com/>), and Scopus (<https://scopus.com/>) databases from 2017 to 2022. The screening process was performed by two independent investigators to identify eligible publications, focusing on keywords such as male fertility, reproduction, varicocele, spermatogenesis, apoptosis, and ER stress. Excluded from the study were publications without complete data, poster studies, and repeated review papers that shared identical

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scientific content. Based on our initial pool of 898 papers, we have carefully reviewed and selected 79 published papers that align with the main objectives of the current study.

The endoplasmic reticulum organelle

The ER is a vast intracellular membrane network where translated nascent proteins mature with the assistance of chaperones. It is within this organelle that protein structures are formed and acquire their three-dimensional organization through complex post-translational modifications. ER protein maturation is influenced by various pathophysiological conditions, leading to an ER-stress response characterized by the accumulation of misfolded or unfolded proteins, also known as the unfolded protein response (UPR). Depending on the intensity of ER-stress, this response can result in either a pro-survival

or pro-apoptotic outcome. To promote survival, the ER response operates at multiple levels, including the temporary reduction of translation to decrease the influx of nascent proteins into the ER. Additionally, it enhances ER protein trafficking, such as by increasing chaperone production and expanding the ER organelle network. Ultimately, to handle misfolded proteins, the ER response augments their degradation via the ubiquitin-proteasome system and/or autophagic-lysosomal processes in a pathway known as the ER-associated degradation pathway (ERAD) (5). If these responses fail to restore ER homeostasis, apoptosis is initiated. The BIP/GPR78/HSPA5 protein, a member of the heat shock protein family (6), mediates the orchestration of this pro-survival/pro-apoptotic balance. As depicted in Figure 1, BIP activates the ER response by interacting with ER membrane sensor proteins, including PERK, IRE1, and ATF6 (5).

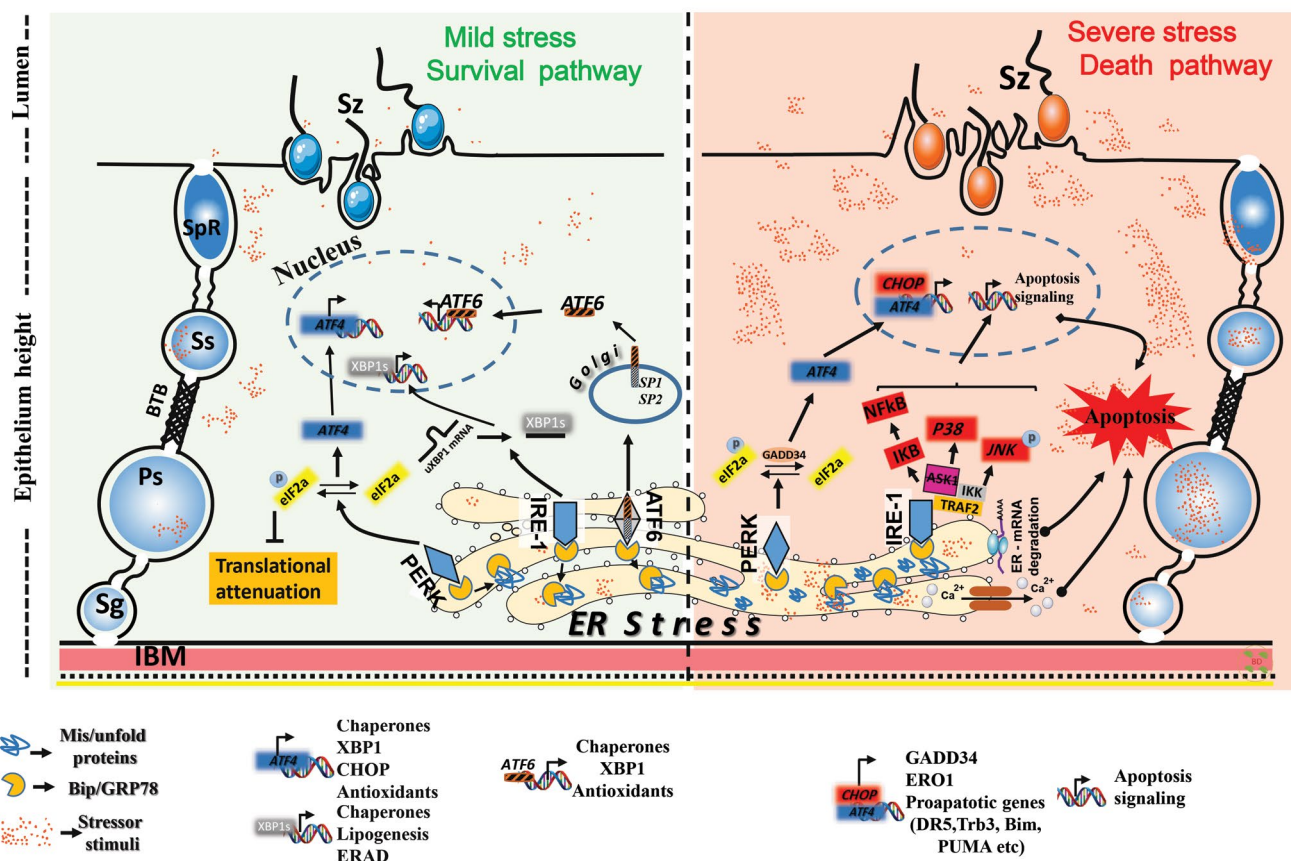


Fig.1: Survival and death pathways associated with ER stress. The UPR pathway is modulated by three ER sensors under all circumstances. During survival conditions, PERK phosphorylates eIF2a, resulting in reduced translational activity. PERK also promotes the expression of ATF4, which further triggers the expression of genes involved in ER quality control chaperones, XBP-1, CHOP, and antioxidants. Additionally, activated IRE1 leads to the specific splicing of XBP1 messenger RNA, generating the XBP1s variant that regulates the transcription of ER-resident chaperones, lipogenesis, and the ER-associated degradation pathway (ERAD). Simultaneously, ATF6 translocates to the nucleus, stimulating the expression of ER chaperones (Grp78, Grp94, etc.), XBP1, and antioxidants. Under conditions of chronic stress, these same sensors initiate a death signal. In particular, PERK induces the transcription of CHOP, which leads to the activation of numerous genes involved in cell apoptosis. Activated IRE1 also triggers the apoptotic pathway by recruiting TRAF2 and ASK1 to the ER membrane, activating the IKB/NFkB pathway, MAPK signaling, and RNA degradation within the ER. Furthermore, calcium flux dysregulation contributes to apoptosis. ER; Endoplasmic reticulum and UPR; Unfolded protein response.

Endoplasmic reticulum-stress and spermatogenesis

Spermatogenesis is a complex differentiation pathway that occurs in the testis and is regulated by Sertoli and Leydig cells. These two cells tightly control germ-cell mitotic proliferation, meiotic reduction, and spermatozoa cyto-differentiation through their endocrine functions (7). Receptor tyrosine kinases (RTKs) play a crucial role in multiple steps of this pathway, with their activities being negatively modulated by phospho-tyrosine phosphatases (PTPs) (8). Maintaining a balance between RTK and PTP activities is crucial for optimal spermatogenesis and cellular homeostasis. However, under stress, particularly OS in aerobic metabolism, proteins in the ER undergo oxidative modifications, such as defective

disulphide bridging processes, protein carbonylation, protein sulfoxidation, and the generation of advanced glycation end-products (AGEs), which impairs their maturation and function (9) (Fig.2A, B). Both PTP and RTK are susceptible to these events, as PTP can be oxidized, leading to inactivation, and RTK may lose its ability to bind ligands due to defective sulfoxidation. It is not surprising that oxidative stress-associated conditions, such as Varicocele, are accompanied by ER stress when defective spermatogenesis occurs. For instance, Tisp40, a testis-specific transcript induced during spermiogenesis, has been found to play a critical role in the testis' ER-stress survival response, thereby participating in sperm chromatin packaging processes (10).

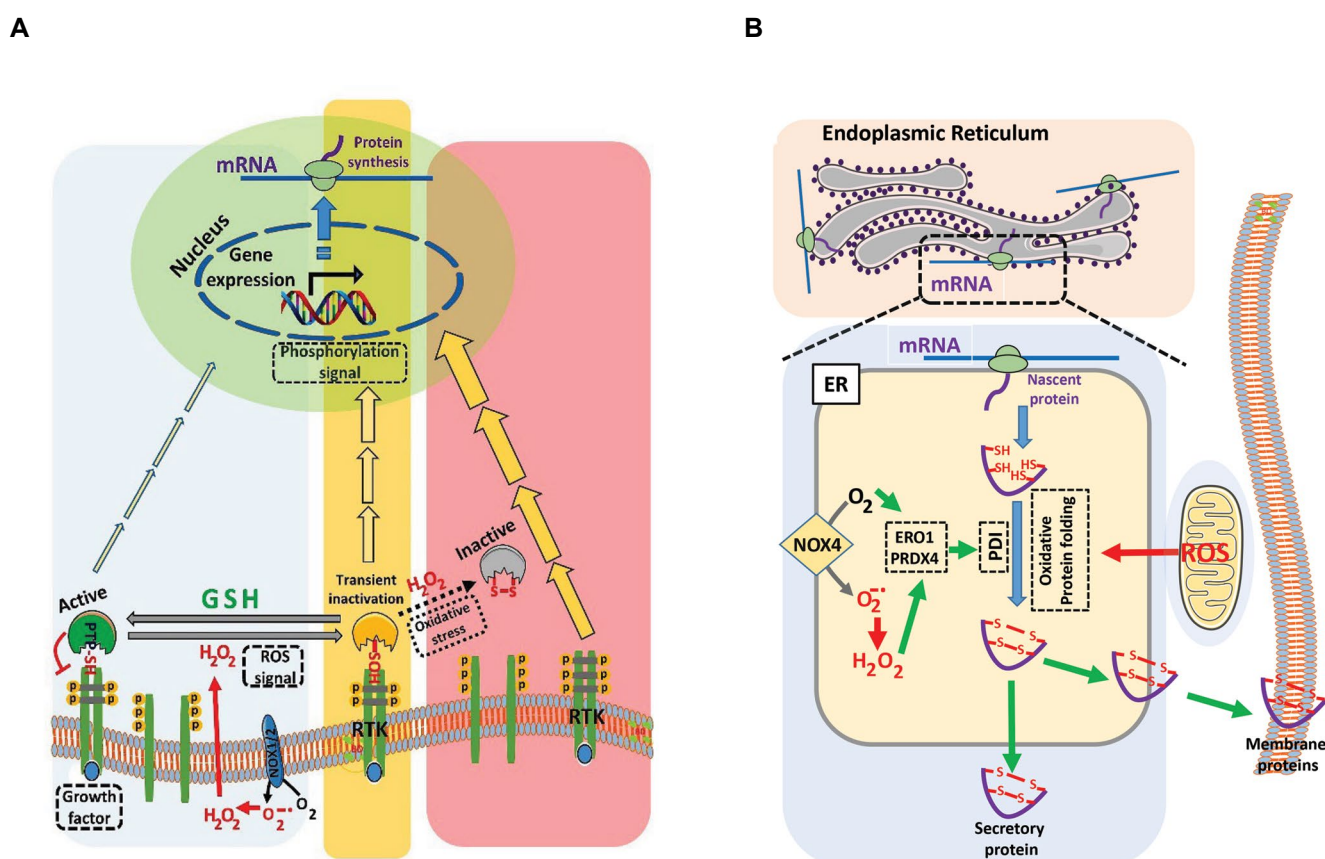


Fig.2: Phosphorylation signals, ROS, and the endoplasmic reticulum roles. **A.** Phosphorylation signals play a crucial role in initiating protein synthesis by ribosomes, whether they are free or associated with the rough ER. The RTK receptor, existing in inactive non-oligomerized and active oligomerized forms through binding to a growth factor, transmits a significant phosphorylation signal. Depending on the strength of this signal - weak, moderate, or severe - different pathways are activated. In the case of a weak signal (left pathway), the active form of PTP, responsible for preventing excessive responses to stimuli, dephosphorylates the RTK, allowing only weak signals to be transmitted to the nucleus. Under oxidative stress, particularly after NOX activation, PTP undergoes oxidation of a thiol group (Cys-SH to Cys-SOH) at its catalytic site, resulting in temporary inactivation of PTP. This inactivation enables the middle pathway for RTK signaling. In instances of high ROS production and accumulation, intramolecular disulfide bonding occurs within PTP, leading to its inactivation and subsequent transmission of intense RTK phosphorylation signals to the nucleus. **B.** In addition to the RTK/PTK pathway, ROS also influence the ER-mediated folding of nascent proteins. ROS contribute to the formation of disulfide bonds in maturing proteins within the ER via two main pathways. First, hydrogen peroxide generated by NOX4, ERO1, and PRDX4 facilitates the formation of disulfide bridges in PDI, which then exerts its bridging activity on thiol-containing proteins transiting through the ER. ROS originating from mitochondrial activity directly impact PDI activity, thereby affecting the disulfide bridging processes of nascent proteins transiting the ER. ROS; Reactive oxygen species, ER; Endoplasmic reticulum, NOX; NADPH oxidase, P; Phosphate group, RTK; Receptor tyrosine kinase, PTP; Phosphotyrosine phosphatase, NOX; NADPH-dependent oxidase, PDI; Protein disulfide isomerase, SH; Sulfhydryl, SOH; Sulfenic acid, Cys-SH; Cysteine sulfhydryl, and GSH; Glutathione.

Varicocele and endoplasmic reticulum-stress

Varicocele is characterized as an abnormal dilation of the pampiniform venous plexus of the spermatic cord in the scrotum. Clinically, it is the most well-known cause of male infertility, affecting about 15% of the general population and between 19–41% of the infertile population. Varicocele leads to progressive testicular insufficiency due to the malfunctioning of the spermatic vein valves, resulting in slowed blood flow and/or reflux (11). This compromised blood supply to the testis impairs its function by depriving it of systemic factors, including gonadotropic and sex hormones. Moreover, varicocele induces testicular hypoxia and hyperthermia, causing damage to the organ and its sperm-producing function (12). Nutrient deprivation, along with a redox imbalance due to hypoxia and hyperthermia, gradually leads to the development of an inflammatory state, ultimately resulting in the apoptosis of germ cells once the countermeasures of testicular cells are overwhelmed. Increased ROS, accumulation of misfolded/unfolded proteins and AGEs, as well as evidence of ER-stress and UPR activation, have been extensively reported in the testis affected by varicocele (13). Soni et al. (14) were the first to demonstrate that ROS-mediated ER stress is triggered in the human testis affected by varicocele. In an animal model of varicocele, Karna et al. (15) also established the presence of testicular oxidative stress, ER stress, and mitochondria-mediated apoptosis. Specifically, they observed elevated testicular expression of ER-stress markers, including BIP, as well as activated phosphorylated forms of IRE1 α (p-IRE1 α) and JNK (p-JNK), alongside upregulation of apoptotic factors, namely cleaved caspase-3 and Bax/Bcl2 ratio. More recently, studies conducted by us and others have reported that the IRE1 α pathway, associated with UPR, acts as the primary mediator of germ cell death in a rat model of varicocele, triggering the pro-apoptotic JNK response (16). Interestingly, the early marker of ER-stress response, BIP, was not found to be elevated in the human testis affected by varicocele. However, this discrepancy may be attributed to the different kinetics observed between acute varicocele situations in animal models and the chronic varicocele situation commonly observed in humans (17).

Animal models with experimentally induced varicocele can be considered acute scenarios, while in human varicocele patients, it is typically a chronic condition that may require different management approaches. This difference could explain the contrasting findings when evaluating early markers of ER-stress in the human testis compared to rodent models. Nonetheless, both animal models and human varicocele patients exhibit evidence of ER-stress in the testis, in addition to the early activation of the ER-stress chaperone BIP.

Efforts to mitigate OS in varicocele and alleviate ER stress have been pursued due to the suggested role of excessive ROS. Various approaches have been taken, including supplementation with herbal concoctions possessing antioxidant properties or a single antioxidant like alpha-lipoic acid (ALA), as well as the utilization of iron chelators such as Deferasirox, all of which have been evaluated in animal models of varicocele. These interventions consistently demonstrate a reduction in ROS generation and ER-stress response, along with improvements in sperm production and quality (18, 19). Recently, the translation of these findings into clinical practice has been explored in patients undergoing Varicocelectomy, revealing significant enhancements in sperm functional parameters when accompanied by ALA supplementation (20).

Alongside hypoxia, hyperthermia is recognized as another significant factor contributing to testicular dysfunction in varicocele (12). Given the feasibility of investigating this parameter, extensive research has been conducted to examine the effects of acute and/or chronic heat stress on testicular dysfunction and the induction of ER/UPR stress responses. Furthermore, this line of research has shed light on the impact of repeated exposure to hot baths or saunas on male reproductive capacity. Notably, studies have demonstrated that subjecting mouse testes to a single 15-minute heat stress at 42°C triggers ER/UPR stress as an adaptive pro-survival signal (21). Conversely, repeated heat stress has been found to induce ER-stress-mediated apoptosis in the testis. In the same study, it was observed that BIP, the early initiator of ER stress, exhibited high expression in the mouse testis compared to other tissues, suggesting a specific adaptation of the testis to heat stress-induced cell death—a response expected due to the temperature sensitivity of spermatogenesis in mammals. Interestingly, it was observed that testicular BIP levels decreased after repeated heat stress, leading to ER-stress-mediated apoptosis facilitated by caspase-3 activation (21, 22). Additionally, among the three ER membrane sensors involved in integrating the ER/UPR response, only PERK and ATF6 were found to be activated, while IRE1 α was down-regulated through autophagic processes (22). In a separate study, Li et al. (22) proposed that testicular hyperthermia was associated with an increase in serum testosterone levels, suggesting an anticipated response to Leydig cell dysfunction that triggers central compensation through activation of the hypothalamus/pituitary/gonadal (HPG) axis. Leydig cell dysfunction and reduced testosterone synthesis during heat stress, accompanied by the induction of ER stress, have been confirmed in other studies (23). It has been

suggested that the increased testosterone supply facilitated by HPG axis adjustment may safeguard the testis from oxidative damage by activating Nrf2-dependent antioxidant mechanisms (24). Obesity is another condition in which OS and chronic low-grade inflammation have been linked to ER stress and male infertility, alongside varicocele.

Obesity, endoplasmic reticulum-stress, and male fertility

Lipid disorders, including hypercholesterolemia leading to metabolic syndrome and obesity, have been clearly associated with male infertility, yet this connection has not received the attention and public awareness it deserves (25). Particularly in developed and developing countries with unbalanced diets, this issue remains overlooked (26). In both human and animal models of dyslipidemia, elevated levels of saturated free fatty acids (FFA), such as palmitic acid (PA), have been observed in plasma (27). These FFAs have demonstrated a time- and dose-dependent ability to suppress Leydig cell survival and drive them towards apoptosis (28). Consequently, FFA-mediated impairment of Leydig cells has been proposed as a contributing factor to reduced reproductive performance and hypogonadism in obese individuals (29). Moreover, FFAs have been found to induce increased expression of BIP, activation of the ER membrane sensor PERK, and heightened production of the ER-mediated apoptotic signal enhancer, the homologous C/EBP (CHOP) protein (30). Correspondingly, mice fed high-fat diets exhibit elevated expression of BIP and CHOP, as well as phosphorylation of IRE1 and PERK in the testis, indicating that high-fat consumption may induce ER stress in the testis, a condition that can potentially be mitigated by antioxidant supplementation (31). Similarly, in the study conducted by Mu et al. (32), it was demonstrated that a high-fat diet not only increases the expression of BIP and CHOP but also activates other ER-stress sensors, including ATF6 and XBP-1. The detrimental effects of this activation could be alleviated through the administration of sulforaphane, an antioxidant, anti-inflammatory, and anticancer compound found in cruciferous vegetables. Furthermore, ATF6, the ER-stress adaptation factor, has been shown in other research to act as a positive regulator for testis-specific serine/threonine protein kinase 4 (TSSK4), which is involved in sperm maturation and serves as a link between ER-stress responses and spermatogenesis (33). In addition to the direct impact of FFAs on ER stress, lipid disorders are closely linked to chronic systemic oxidative stress, which contributes to pro-inflammatory conditions known to exacerbate the ER/UPR response in

various cell types (34). Therefore, the combination of systemic lipid imbalance and OS plays a critical role in the testicular ER-stress response and the development of subfertility/infertility associated with dyslipidemia.

The dysregulation of adipokines, such as leptin, commonly observed in dyslipidemic conditions, represents another mechanism by which lipid disorders can affect testosterone production and, consequently, testicular function (35). This disruption leads to hypogonadism and oligoasthenozoospermia. Additionally, studies have demonstrated that the adipokine Vaspin binds to and activates the ER-stress sensor BIP in the adult rat testis, where it is suspected to regulate steroidogenesis and, incidentally, spermatogenesis (36). Further investigations have unveiled the potential involvement of other endocrine factors, such as irisin, a myokine induced by exercise, in the regulation of obesity-related impairments in spermatogenesis. In both obese men and obese mice, irisin levels were found to be correlated with insulin resistance, severity of NAFLD (non-alcoholic fatty liver disease), and the decline in sperm quality associated with OS (37). Recently, it was reported that supplementing obese mice with irisin could alleviate high-fat diet-induced oxidative stress, ER stress, and testicular apoptosis through the activation of the AMPK α signaling pathway (38).

Diabetes, endoplasmic reticulum-stress and male fertility

Diabetes is a disease known to be associated with elevated systemic oxidative stress, inflammation, and ER stress, which contribute to male hypogonadism and impaired testicular function (39). Shi et al. (40) also demonstrated that diabetes reduces testicular autophagy, a cellular process involved in managing the accumulation of misfolded/unfolded proteins while increasing OS and ER stress. Increased expression of insulin receptor substrate-1 (IRS-1) in the testes has been observed in association with diabetes and is considered a compensatory response to tissue dysfunction (41). Specifically, the upregulation of IRS-1 in the diabetic testes has been linked to reduced expression of CHOP and NF-KB, both of which play roles in ER/UPR stress and inflammatory responses (40). Moreover, similar to dyslipidemia, the reduction of systemic OS through antioxidant supplementation in a rat model of diabetes has been shown to decrease NF-KB and P38 MAPK PMK-3 expression, as well as inhibit P-JNK activation, all of which are active components in the IRE-1-mediated ER-stress signaling cascade (42). Consistent with these findings, hyperglycemia has been demonstrated to induce Leydig cell apoptosis through mitochondrial

pathways (Bax/Bcl2/caspase-12) and BIP/CHOP-mediated ER stress, and these effects can be alleviated by antioxidant supplementation, such as melatonin, resveratrol, and fucoidan (43, 44).

Inflammasome, endoplasmic reticulum-stress and male fertility

Testicular inflammatory conditions, whether originating from immune responses or other causes, have a significant impact on spermatogenesis and male fertility (45). In a recent review, we highlighted the crucial role of the NLRP3 (nucleotide-binding oligomerization domain-like receptor family Pirin domain containing 3) inflammasome pathway, a Pattern Recognition Receptor (PRR), in testicular dysfunction (46). It has been observed that uncontrolled UPR/ER stress in the testis consistently triggers the activation of the NLRP3 inflammasome, leading to the release of pro-inflammatory cytokines that adversely affect sperm structure and function (46, 47). Furthermore, bacterial or viral unilateral/bilateral orchitis has been shown to activate both the NLRP3 inflammasome and the UPR/ER stress responses (48, 49). OS has emerged as a common underlying factor, as antioxidant supplementation has proven effective in protecting the testis from UPR/ER stress and inflammasome activation (46).

Aging, endoplasmic reticulum stress, and male fertility

Human aging is characterized by a decline in testicular and sperm function, which can result in reduced sperm nuclear integrity and have consequences for reproductive success, embryonic development, live birth rates, and the quality of life of offspring (50). In animal models of aging, it has been observed that BIP expression decreases, potentially leading to disengagement from ER/UPR membrane sensors and subsequent activation of the ER-stress response (51). Interestingly, in aging human testes, the three ER sensors (IRE-1, PERK, and ATF6) are also downregulated, limiting the ability to initiate the ER/UPR stress response. This downregulation is considered an adaptive process in aging, preventing excessive activation of the ER-stress response in a less efficient ER metabolic context (51, 52). However, despite the reduced ER capacity to respond, downstream effectors of the ER-stress response, such as CHOP, P-JNK, and Caspase-12, are significantly increased in the aging testes (53), indicating the presence of an active ER-stress response. This observation is somewhat contradictory and requires further investigation. While the activation of p-JNK and caspase-12 in the aging testes may be influenced by other signaling pathways, the activation of CHOP is particularly perplexing and

necessitates additional testing for a comprehensive understanding.

Lifestyle/environmental impacts, endoplasmic reticulum stress, and male fertility

Testicular function and reproductive performance are increasingly recognized as important indicators of individual fitness and the ability to cope with various chronic and acute stresses. Regardless of the type of stress—whether chemical, physical, or psychological—reports suggest that testicular function can be impaired, and ER stress is observed as a consistent response in the testis (54). Researchers have explored different approaches to mitigate the detrimental effects of chemical components on spermatogenesis and the induction of ER stress. For example, Yin et al. (55) demonstrated that Bisphenol A (BPA) disrupts cell and testis functions, inhibits cell proliferation, increases apoptosis rates, and accumulates testicular ROS. They found that knocking down the PERK/eIF2 α /CHOP pathway and using the ROS scavenger NAC (Acetylcysteine) can help restore cellular survival. Ji et al. (56) showed that Melatonin alleviates cadmium-induced cellular stress and germ cell apoptosis in male CD-1 mice by effectively inhibiting ER stress and the UPR in the testes. Zou et al. (57), working with Leydig cells from rat testes, demonstrated that NAC prevents Nickel-induced ROS generation and inhibits apoptosis through mitochondrial and ER stress pathways (GADD153/Caspase 12) in rat Leydig cells. Additionally, Soni et al. (58) investigated the effects of DA-9401 (a commercial antioxidant) in Sprague Dawley rats and found that it reduced ER stress and apoptotic markers while improving fertility, genital organ weight, sperm parameters, and sex hormone levels. To summarize the available information in the literature, Table 1 provides a compilation of environmental toxins/pollutants, specific drugs, and a limited number of bacterial toxins that have been tested for their ability to induce ER/UPR-stress responses either in cultured testicular cells or in animal models. Where available, Table 1 also includes the potential corrective effects obtained with various strategies. Overall, it is evident that ER stress is a common response of the testis to these different exposures, and antioxidant treatment frequently proves effective in reducing the extent of the testicular ER stress response.

Conclusion

It is noteworthy that ER stress is a prevalent characteristic in various cases of testicular dysfunction linked to male subfertility/infertility. OS stands out as a key factor in this association, implying that therapeutic interventions targeting antioxidants may hold promise for enhancing reproductive performance.

Table 1: Compilation of environmental toxins, drugs, and bacterial toxins inducing ER/UPR-stress responses in testicular cells and animal models

| Agent | ER/UPR components monitored | Corrective treatment used | Main findings, model and reference |
|--|--|---|---|
| Environment | | | |
| Dibutyl phthalate (DBP) | p-PERK/NRF2/ | Melatonin Specific inhibitor p-PERK (GSK2606414) | Melatonin attenuates DBP-induced damage (ROS generation/ER stress and mitochondrial-related damage/decrease in mitochondrial mass, mtDNA copy number, COX IV protein level, and ATP level) and mitochondrial-dependent apoptosis by regulating PERK/Nrf2/ARE the signal antioxidant path in mouse spermatocyte-derived GC-2spd(ts) cell line model (59) |
| Di-(2-ethylhexyl) phthalate (DEHP) | p-IRE-1 α / p-JNK/ CHOP/GRP78 | - | Pubertal exposure to high doses of DEHP induces germ cell apoptosis and distorted seminiferous tubules by initiating ER stress in testes (five-week-old male mice) (60) |
| Lead diacetate | GRP-78 and BAX | Turmeric and vitamin C | Turmeric and vitamin C mitigate testicular atrophy induced by lead diacetate via regulation of GRP-78/17 β -HSD pathways and subsequent thr reduction of oxidative injury in male Wistar albino rats model (61) |
| Particulate matter (PM2.5) | IRE1, p-JNK, beclin-, LC3/LC31 | STF083010 (an IRE1 inhibitor) | STF083010 exerted specific protective effects on reproductive injury-related effects in male rats (male Sprague–Dawley rat model) exposed to PM2.5 such as improved sperm quality and serum testosterone level, with effects mediated via ER (IRE1/ JNK)/autophagy signaling (62) |
| ZnO Nanoparticles | p-IRE1 α /XBP1s/BIP/ CHOP/p-JNK | Salubrinal, a specific inhibitor of eIF2 α phosphatase enzymes and ER stress inhibitor | Salubrinal inhibit or preserve ER stress and apoptotic marker, and also improve sperm parameters, and testis function, molecular and enzymatic testosterone hormone in male Kunming mice model (63) |
| 1-Nitropyrene | GRP78/p-IRE1 α /XBP1s/ CHOP/p-eIF2 α /p-PERK | - | Long-term 1-NP activated oxidative stress and ER stress and downregulate steroidogenic genes and enzymes, and disrupted T biosynthesis without disturbing testicular spermatogenesis in 6-week-old male ICR mice model testes (64) |
| Nonylphenol (Np) | JNK, MKK4, p53, and p38, | - | NP led to testicular structure disruption and a reduction in testicular size and testosterone levels and induces apoptosis through ROS/ JNK signaling in GC-1 spg cell model (65) |
| X-Irradiation | GRP78, IRE1 α , CHOP and Caspase-12, and Caspase-3 | Human amniotic membrane-derived mesenchymal stem cells (hAMSCs) and conditioned medium (hAMSCs-CM) | Transplantation of hAMSCs and hAMSCs-CM into testis mice led to reducing ER stress by suppressing the UPR response as well as a decrease in FSH and LH and an increase in testosterone level which have contributed to the improvement of spermatogenesis (66) |
| Dinitramine (DN), a synthetic herbicide | GRP78/IRE-1/CHOP/p-eIF2 α /Erk/p-P38/p-Jnk | 2-APB, inhibitors of IP- receptors and TRP channels BAPTA, a chelate Ca ²⁺ | 2-ABP and BAPTA prevent anti-proliferative DN effects on testicular cell lines (Leydig and Sertoli cells model) mediated via activating Pi3k/Akt pathway and inhibiting ER stress-induced calcium dysregulation in the cytosol and mitochondria (67) |
| Zearalenone (ZEA), a non-steroidal mycotoxin | CHOP/BIP | BAPTA-AM, chelator of Ca ²⁺ & Mg ²⁺ 2-APB, inhibitors of IP- receptors and TRP channels NAC (N-acetylcysteine) AICAR, an activator of AMP-activated protein kinase | NAC decreases activating of AMPK and autophagy-related protein by scavenging ROS and also 2-APB and BAPTA-AM prevent ER stress markers by activating CaMKK β and AMPK and decreasing the concentration of Ca ²⁺ and autophagy in TM4 cells (mouse Sertoli cell line model) (68) |
| PbSe nanoparticles (PbSe-NPs) | GRP78/Caspase-12 | - | PbSe-NP administration led to a reduction in the quantity and quality of sperm, which caused a great fertility decrease by endoplasmic reticulum and mitochondria-mediated cell apoptosis in specific-pathogen-free (SPF) Sprague–Dawley (SD) rats (6 to 7 weeks old, 170-200 g) (69) |

Table 1: Continued

| Agent | ER/UPR components monitored | Corrective treatment used | Main findings, model and reference |
|--|--|---|---|
| Chlorocholine chloride (CCC) | GPR78, CHOP | 4-phenyl butyric acid (4-PBA), an ER stress inhibitor | 4-PBA rescued the testosterone secretion disorders and alleviated CCC-induced increase in the ER stress-related protein levels in Sprague-Dawley rats Leydig cells model (70) |
| Aluminum (AlCl ₃) | CHOP, Bcl-2, Bax, and XBP1 | Taurine | Taurine leads to increased gene expression of vimentin, Bcl-2, and PNCA accompanied by decreased CHOP, Bax, and XBP1 gene expression. In other words, Taurine amends both ER stress and mitochondrial impairment in the testes and epididymis induced by AlCl ₃ in forty-eight adult male albino rats model (71) |
| Drug toxicant | | | |
| Cisplatin | Bad, Cyt c, caspase-9, caspase-3, caspase-12, GRP78, CHOP, IRE1 α , p-IRE1 α , XBP-1S, PERK, p-PERK, eIF2 α , and p-eIF2 α | Grape seed proanthocyanidin (GSPE) | GSPE relieved endoplasmic reticulum stress-mediated apoptosis via PREK/eIF2 α and IRE1 α /XBP-1S/caspase-12 pathways as well as PI3K/Akt/mTOR and Bad/CytC/caspase-9/caspase-3 pathways in Forty-eight male Wistar rats model (72) |
| Cyclophosphamide | CHOP/NRF2/PERK/p-PERK/IRE1/p-IRE1 (Ser724)/ | LCZ696 (sacubitril/valsartan), a receptor neprilysin inhibitor and a Ca ²⁺ sequestration inside the ER | LCZ696 reduce apoptotic, oxidative, and ER stress markers LCZ696 and improve Testicular atrophy, sperm characteristics, hormonal profile, testicular function, and antioxidant defenses, and cause increased lncRNA TUG1 in adult male 8-week-old Wistar rats model (200 \pm 20 g) (73) |
| Sodium fluoride (NaF) | GRP78/PERK/p-eIF2 α /CHOP/eIF2 α /NRF2 | N-acetylcysteine (NAC) CHOP knockdown | NAC effectively blocked the damage of Sertoli cells through the activation of ER stress, suggesting that NaF-induced ROS is an early event that triggers ER stress in Sertoli cells from male Sprague-Dawley rats (18-day-old) (<i>in vitro</i>) model (74) |
| Busulfan | Caspase-12/CHOP/GRP78/ATF6/p-IRE1/XBP1 | Melatonin | Melatonin blocks or decreases ER stress markers and related apoptosis proteins, therefore, reducing the extent of damage to mouse testes and improving the survival rates of busulfan-treated mice in Mouse testes (<i>in vivo</i>) and the C18-4 cell line (type A spermatogonia stem cell, <i>in vitro</i>) model (75) |
| Midazolam (MDZ) | p-eIF2 α /ATF4/ATF3/CHOP/JNK/ERK1,2/p38 | - | Midazolam could activate caspase, MAPKs, and ER stress pathways and impede Akt pathway and cell cycle to induce apoptosis in TM3 mouse Leydig progenitor cells model (76) |
| Triptolide (TP), a diterpenoid epoxide | PERK/CHOP/JNK/NRF2/p-JNK/ATF4/p-eIF2 α /eIF2 α | Aucubin (AU) Nrf2siRNA | AU prevented apoptosis through an effective inhibition of PERK/CHOP and JNK-dependent apoptosis pathway, as well as improved testicular weight, and sperm morphology and protected the integrity of BTB by corresponding up-regulating genes in male adult mice (25-27 g) (<i>in vivo</i>) (77) |
| High hCG, Human chorionic gonadotropin | Grp78/Chop/ATF4/Xbp1/p-IRE1 | Knockdown of melatonin receptors (MT1 and MT2) | Inhibition of melatonin receptors increased hCG-induced expression of Grp78, Chop, and ATF4, but not Xbp1 and IRE1, suggesting that hCG may modulate IRE1 signaling pathways in a melatonin receptor-dependent manner in male Kunming White outbred strain mice (<i>in vivo</i>) and the murine Leydig tumor cell line (<i>in vitro</i>) model (78) |
| Bacterial toxin | | | |
| Microcystins-LR (MCLR) | GRP78/caspase-12/CHOP/PERK/p-eIF2 α /eIF2 α /XBP1/p-p38/p-JNK/JNK/p38/p-Erk1&2 | Bapta-AM, a Ca ²⁺ chelator | Bapta-AM pretreatment attenuated partially MCLR-stimulated such as elevated intracellular Ca ²⁺ , p-CaMKII, and mitochondrial dysfunction in mouse TM4 Sertoli cells model (79) |

HCG; Human chorionic gonadotropin, ER; Endoplasmic reticulum, ROS; Reactive oxygen species, and mtDNA; Mitochondrial DNA.

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Authors' Contributions

M.R.; Methodology, Software, and Validation. M.T., M.R.; Investigation. M.R., J.R.D., M.H.N.-E.; Writing - Original Draft. M.R., J.R.D., M.T., M.H.N.-E.; Writing - Review and Editing. J.R.D., M.H.N.-E.; Conceptualization. M.H.N.-E., M.T.; Project administration. M.H.N.-E.; Supervision.

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