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Review Article

Radiation-induced testicular damage in rats: Potential mechanisms and current testicular protective strategies

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ABSTRACT

Testicular function is essential for male reproductive health, and its impairment due to radiation exposure can result in significant physical, emotional, and psychological consequences, including infertility, hormonal imbalances, and reduced quality of life. Radiation-induced damage to the testes, particularly during spermatogenesis, is influenced by factors such as radiation type, dose, fractionation, age, and genetic predisposition. Radiation can damage testicular tissue through direct mechanisms, like DNA strand breaks, and indirect mechanisms, such as oxidative stress, inflammation, apoptosis, and autophagy. Key signaling pathways such as NF-κB and JAK/STAT, along with inflammatory mediators like interleukin (IL)-6 and IL-1β, are critically involved in driving the radiation-induced inflammatory response and associated cellular damage. To protect testicular function, both non-pharmacological and pharmacological strategies have been explored. Non-pharmacological approaches, such as testicular shielding, transposition, and cryopreservation, aim to minimize exposure and preserve fertility. Pharmacological interventions, including antioxidants and hormonal therapies, have been studied for their potential to protect against radiation-induced damage. These strategies provide hope for preserving testicular function and fertility in individuals undergoing radiation therapy, mitigating long-term damage, and improving their overall quality of life.

Keywords: *Radiation exposure; Spermatogenesis; Infertility; Oxidative stress; Inflammation; Apoptosis; Autophagy..*

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1. Introduction

Radiotherapy is a fundamental component of cancer treatment, utilized in approximately 50% of oncology cases either alone or in combination with other therapeutic modalities. While it is highly effective in targeting and eliminating malignant cells, ionizing radiation can also cause unintended damage to surrounding normal tissues **[1]**. Among the most vulnerable organs are those with high proliferative activity and radiosensitivity, such as the reproductive organs, particularly the testes. Testicular exposure to radiation, whether direct or scattered, poses a significant risk to male reproductive health, often resulting in impaired spermatogenesis, endocrine dysfunction, and potential infertility **[2]**.

Radiation-induced testicular damage is a complex pathological process mediated by

multiple molecular and cellular events. One of the principal mechanisms involved is the excessive generation of reactive oxygen species (ROS), which leads to oxidative stress and subsequent disruption of cellular homeostasis. This oxidative imbalance activates several inflammatory signaling pathways, including the nuclear factor-kappa B (NF-κB) pathway and cytokines such as interleukin-6 (IL-6), as well as the Janus kinase/signal transducer and activator of transcription (JAK-STAT) pathway. These inflammatory responses play a critical role in the progression of tissue injury and the deterioration of testicular function following irradiation **[3]**.

In addition to inflammation, radiation exposure induces apoptosis of germ cells and somatic cells within the testes, contributing to the loss of spermatogenic capacity. Moreover, recent studies have highlighted the involvement of disrupted autophagic processes in radiation-mediated testicular damage, particularly through dysregulation of key autophagy markers such as Beclin-1 and p62. The interplay between oxidative stress, inflammation, apoptosis, and autophagy forms a multifaceted network that underlies the pathophysiology of radiation-induced reproductive toxicity **[4]**.

This review article aims to comprehensively examine the molecular mechanisms implicated in radiation-induced testicular injury, with a focus on oxidative stress and the associated downstream pathways. Additionally, we discuss the current pharmacological and experimental strategies that have shown promise in mitigating testicular damage, considering critical factors such as radiation dose, exposure duration, and fractionation regimens. Through this analysis, we aim to provide a clearer understanding of potential protective interventions and their translational relevance in clinical oncology.

2. Insights on testicular anatomy and dynamics

2.1. Testicular anatomy

The scrotal septum divides the oval-shaped reproductive organs known as the testes, which are located in the scrotum. The testes are bean-shaped and measure approximately three to five centimeters in length and two to three centimeters in width **[5]**. The epididymis consists of three parts: the head, body, and tail. The head of the epididymis is located at the superior pole of the testis, where sperm are stored to mature. The body of the epididymis, a highly coiled duct connecting the head and tail, is the region where sperm continue to mature. The tail of the epididymis is continuous with the ductus deferens (vas deferens) **[6]**. The tough, fibrous covering of the testes, known as the tunica albuginea, lies deep to the tunica vaginalis. Through invagination, the tunica albuginea forms connective tissue septa between the seminiferous tubules and the rest of the testis **[5]**.

2.2. Testicular dynamics

2.2.1. Spermatogenesis

The complex chain of activities known as spermatogenesis takes place in the seminiferous tubules and results in the maturation of the male gamete. The processes include spermatogonia growth, spermatogonial differentiation into spermatocytes, spermatocyte meiosis resulting in spermatids, spermatid maturation, and the discharge of highly specialized mature spermatozoa into the lumen of the testicular tubule **[7]**.

2.2.2. Hypothalamic–pituitary–gonadal (HPG) axis

The hypothalamus secretes gonadotropin-releasing hormone (GnRH), which induces the pituitary gland to release follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The pulsatile release of GnRH, which is brought on by the disinhibition of the hypothalamic-pituitary-gonadal (HPG) axis, is the first

hormonal shift associated with puberty. The pulsatile release of LH and FSH is stimulated by the subsequent release of GnRH. In order to promote the release of androgens, estrogens, and gametogenesis, LH and FSH interact with particular gonadal cells. Testicular Leydig cells are stimulated by LH to generate testosterone. In contrast, FSH helps produce sperm by acting on the Sertoli cells found in the testes' seminiferous tubules. The creation of adult male genitalia is facilitated by increasing testosterone production, which is the result of this complex procedure **[8]**.

Since the HPG axis regulates the production of the key reproductive hormones, any disruption in this axis can result in hormonal imbalances and testicular hypogonadism. This, in turn, leads to various significant consequences, including testicular failure (TF).

3. Testicular failure (TF)

3.1. Overview

Male infertility can arise from TF due to endocrine failure, which can lead to a shortage of testosterone, or exocrine failure, which may inhibit spermatogenesis**.** These events lead to a feedback response, triggering the pituitary gland to secrete more LH and FSH in a condition termed (hypergonadotropic hypogonadism state) to force the secretion of testosterone **[9]**.

3.2. Diagnosis of TF

3.2.1. Hormone levels

Seminal fluid frequently has a limited volume when serum testosterone levels are low. Primary TF is characterized by low serum total testosterone levels, measured on two separate morning samples, along with elevated LH and FSH levels, indicating a loss of negative feedback on the hypothalamic-pituitary axis. These hormonal features are diagnostic of primary hypogonadism **[10]**.

On the other hand, low testosterone and serum FSH levels both point to hypogonadotropic hypogonadism, particularly in the case of bilateral atrophic testicles. Serum LH is frequently low in this situation as well **[11]**. In addition, testes and spermatozoa from both humans and animals contain numerous additional hormones and their receptors, including insulin-like growth factor 3, kisspeptin, leptin, and anti-Mullerian hormone, all of which are thought to play paracrine and endocrine roles in controlling testicular functions **[12]**.

3.2.2. Clinical manifestations

Primary hypogonadism is characterized by impaired testicular function resulting in insufficient testosterone production and, frequently, infertility. Clinically, affected adult males often present with symptoms of androgen deficiency, including decreased libido, erectile dysfunction, reduced spontaneous erections, fatigue, depressed mood, decreased muscle mass, increased body fat, and reduced bone mineral density. Physical findings may include gynecomastia, small or soft testes, and loss of body hair. Infertility is common due to disrupted spermatogenesis, and testicular volume is often reduced. In adolescents, delayed or incomplete puberty, lack of secondary sexual characteristics, and eunuchoid body proportions may be noted. **[13]**.

3.2.3. Imaging techniques

The male reproductive system can be studied using three primary imaging modalities: magnetic resonance imaging (MRI), ultrasound, and invasive methods like venography and vasography. Ultrasonography can be performed to assess the volume and morphology of the testes. A single testicular volume of 12–15 ml and a total volume (both testes) of >30 ml are typically considered normal. While the diagnosis of TF is primarily clinical and biochemical, imaging studies can provide valuable adjunctive

information in selected cases. Scrotal ultrasonography is the most commonly used imaging modality due to its noninvasive nature and high sensitivity in evaluating testicular size, echotexture, and the presence of focal lesions or atrophy. In patients with primary hypogonadism, ultrasound may reveal reduced testicular volume and heterogeneous echotexture, findings consistent with chronic testicular damage. Ultrasonography is particularly useful in distinguishing TF from conditions such as varicocele or obstructive azoospermia, where testicular size is usually preserved. In cases of undescended or non-palpable testes, MRI or abdominal and pelvic ultrasound may be used to localize the intra-abdominal gonads. Additionally, pituitary MRI may be indicated when secondary hypogonadism is suspected, particularly in the presence of low gonadotropins and clinical features suggesting hypothalamic or pituitary pathology. Although imaging is not routinely required in the initial assessment of TF, it is an important tool for excluding structural abnormalities and guiding further management when indicated **[14]**.

3.3. Pathophysiology of TF (Hypogonadism)

3.3.1. Primary TF

This type originates from a problem in the testes. Its common causes include a congenital abnormality of the X and Y sex chromosomes, which causes Klinefelter's syndrome, Undescended testes, Mumps orchitis, Hemochromatosis, Injury to the testes, Cancer treatment, and normal aging, which leads to sperm impairment and male infertility **[15]**.

3.3.2. Secondary TF

The testes are normal in secondary hypogonadism, but they don't work well because of a pituitary or hypothalamic issue. Secondary hypogonadism can result from a variety of situations, such as Kallmann syndrome, which is abnormal development of the hypothalamus, pituitary disorders, inflammatory disease, HIV/AIDS, medications as opiate pain medications and some hormones, and obesity. Besides, stress-induced hypogonadism occurs with the extensive use of radiation for childhood cancers. Radiotherapy is one of the most prevalent reasons for decreased testicular reserve and thus, an increased prevalence of TF **[16]**.

4. Radiotherapy and the testicular function

4.1. Background

High-energy beams or radioactive substances are used in radiotherapy, an effective therapeutic technique, to treat a variety of cancers and prevent their growth and spread. In particular, gamma rays are commonly utilized in cancer treatment. Globally, radiation therapy is involved in over half of all cancer treatments **[1]**. Radiation therapy benefits cancer patients both when used alone and in combination with other treatments. Additionally, it improves the quality of life for many patients, functioning as a palliative measure that reduces symptoms caused by the disease **[17]**.

4.2. Vulnerability of testicular tissue to ionizing radiation

Although radiation therapy can damage DNA and inhibit further cell growth and replication, it is frequently utilized in cancer treatment. However, radiation may also harm healthy tissues near the tumor **[18]**. Radiation primarily affects cells that divide rapidly and frequently synthesize new DNA, while slower-dividing cells are generally less impacted. The testes are particularly susceptible to radiation damage because they contain rapidly proliferating spermatogenic cells. Radiation has long been recognized as a causal agent of male reproductive toxicity. Spermatogenic cells are especially vulnerable, and infertility is a common side effect

of radiation exposure. Ionizing radiation (IR) disrupts normal metabolism, proliferation, and differentiation, leading to mutagenesis, apoptosis, and necrosis in radiosensitive cells, ultimately causing impaired testicular function. Therefore, it is critical to develop effective strategies for protecting testicular tissue against radiation-induced damage.

4.3. Importance of protecting the testicular function

Losing testicular function at a young age can be emotionally distressing, especially for individuals who wish to have children. Moreover, TF is a significant concern because it can substantially lower the long-term quality of life. Young men affected by TF may experience severe and abrupt developmental changes, including reduced height, gynecomastia (enlarged breasts), infertility, loss of muscle mass, decreased libido, hair loss in the pubic and underarm areas, and delayed or absent development of secondary male sex characteristics. In addition, lower testosterone levels can contribute to more serious health issues such as osteoporosis, cardiovascular problems, and cognitive decline. These physical changes significantly affect mental well-being, potentially leading to anxiety, depression, and a reduced quality of life **[19]**. In some cases, radiation can have even more severe consequences. It may cause mutations in the genetic material of testicular cells, leading to the uncontrolled proliferation of abnormal cells. As a result, the risk of developing tumors increases following exposure to gamma radiation **[20]**.

4.4. Targets of radiation inside the testes

Spermatogenesis is a vital physiological process for reproductive function. Spermatogonial stem cells undergo a complex and dynamic differentiation process within the seminiferous tubules, taking approximately 60–70 days in humans and around 30 days in mice. During this process, spermatogonia differentiate into primary spermatocytes, then into secondary spermatocytes, spermatids, and ultimately spermatozoa, which mature in the epididymis. Radiation-induced effects during spermatogenesis can be detrimental to reproductive health. Clinical evidence from high-dose radiation exposure indicates that fertility significantly declines in cancer survivors, particularly among pediatric and adolescent/young adult patients following radiation therapy **[21]**.

4.5. Factors affecting radiotoxicity inside the testes

4.5.1. Radiation type

Gamma rays can cause diffuse damage throughout the body due to their strong ability to penetrate tissues and cells. However, they are less ionizing than alpha or beta particles. Research indicates that γ-irradiation is a primary contributor to radiotoxic damage **[22]**.

4.5.2. Radiation dose

Indeed, the testis is among the tissues that are most vulnerable to radiation; even low doses of radiation can cause a major reduction in its functionality **[23]**. In terms of single-dose radiation, doses more than 2 Gy and 4 Gy impact spermatocytes and spermatids, respectively, whereas the lowest dose necessary to harm spermatogonia is as low as 0.1 Gy **[24]**.

4.5.3. Fractionation of radiation

Compared to low fractionated doses, a single large radiation dose causes more damage. Even with higher overall radiation doses, the incidence of TF was reduced when the entire body was exposed to IR in fractionated doses. In fact, because Leydig (interstitial) cells are relatively radioresistant compared to the sensitive spermatogonial cells, fractionated doses of up to

20 Gy administered to the testicles do not cause hormonal failure **[25]**. Excessive doses often cause cell death, which accelerates the body's response. On the other hand, smaller doses are more likely to result in mutations within these cells, meaning that the effects might not become apparent for years **[26]**.

4.5.4. Irradiation field

Although the testicles are often outside the primary radiation fields, their proximity to these fields and high sensitivity to radiation make them susceptible to scattered radiation. If the surviving testicle is properly shielded, only temporary azoospermia is expected to result from the internally scattered dose associated with conventional abdominal radiation therapy fields **[25]**.

4.5.5. Patient age

It has been shown that the degree of radiation-induced damage to the testes varies with age. Younger patients are generally more likely to tolerate radiation exposure **[27]**. Compared to the prepubertal testis, the germinal epithelium of the adult testis is more susceptible to injury. The estimated radiation dose threshold for children is approximately 2,400 cGy, whereas for adults it is likely between 400 and 600 cGy.

4.5.6. Target cells properties

Radiation exposure increases the vulnerability of rapidly dividing cells compared to slower-dividing ones. Testicular tissue is considered highly radiosensitive due to its rapid rate of proliferation and development. Among these, spermatogonial stem cells are more radiosensitive than mature germ cells **[28]**.

4.5.7. Growth phase of spermatocytes

The radiosensitivity of spermatocytes is also affected by the meiotic divisions during the prenatal stage. With each division, radiation resistance increases from the most sensitive spermatogonia via primary and secondary spermatocytes and spermatids, to sperm, which are highly radioresistant **[29]**.

4.5.8. Genetic factors

Certain patients may be more susceptible to testicular injury than others due to genetic abnormalities affecting DNA repair or cell signaling pathways. As an example, spermatogonial apoptosis may result in males deficient in the Ataxia-telangiectasia mutated gene, which is responsible for the DNA damage response **[30]**.

4.6. Mechanisms of radiation damage to the testes

Biological molecules are subject to the cytotoxic effects of IR either directly or indirectly:

4.6.1. Direct DNA Damage

Targeted cells may produce electrons when exposed to IR. Due to their high kinetic energy, these electrons can directly interact with biological macromolecules such as DNA, resulting in single- and double-strand breaks. Gene mutations and subsequent cell death are caused by these abnormalities. Until they run out of kinetic energy, the liberated electrons can interact with other molecules like lipids and proteins **[31]**.

4.6.2. Indirect DNA Damage

Since water is the most prevalent molecule in cells, it can be radioactively analyzed by lower energy beams, such as gamma radiation, to produce ROS or disrupt neighboring cells **[32]**. Because they are unstable, ROS such as hydrogen peroxide (H_2O_2), superoxide (O^{2-}), and hydroxyl radical ($OH^{\bullet}$) damage biological macromolecules and trigger intracellular signaling cascades that cause inflammation, apoptosis, and oxidative stress. Radiation from γ-irradiation frequently results in this type of

damage **[33]**.

4.6.2.1. Radiation-induced-oxidative stress

As shown in **Fig. 1**, DNA and proteins can be harmed by ROS that are created when exposed to IR **[34]**. Oxidative stress results from an imbalance in the redox system caused by the body's endogenous antioxidant defenses being overwhelmed by the overproduction of ROS **[35]**.

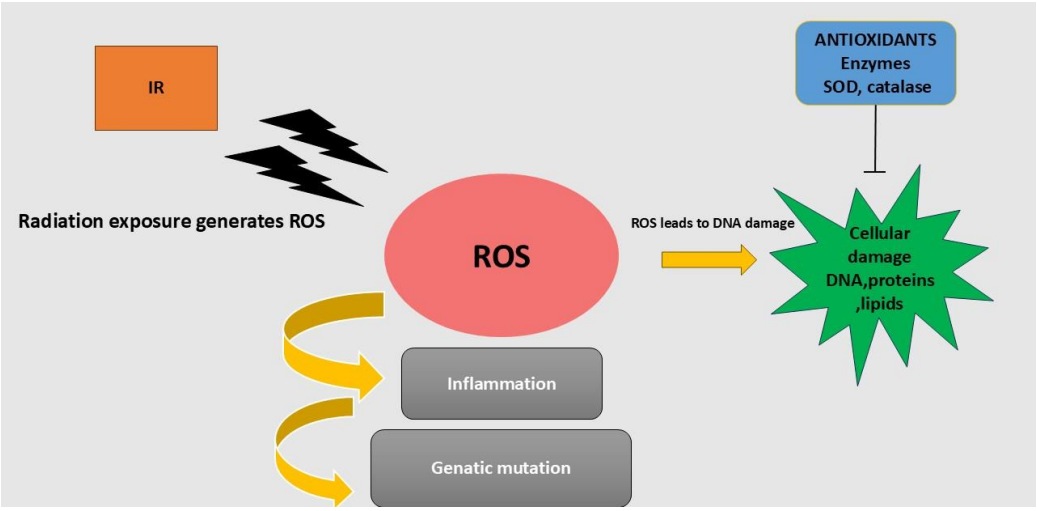

Fig.1. Radiation-induced oxidative stress

This figure illustrates the key events in the radiation-induced oxidative stress pathway. Ionizing radiation generates reactive oxygen species (ROS) within the cell, leading to oxidative damage to lipids, proteins, and DNA. In response, antioxidant defenses, such as superoxide dismutase (SOD) and catalase, are activated to neutralize ROS. However, excessive ROS can overwhelm these defenses, triggering cellular damage and activating stress response pathways, including DNA repair mechanisms and inflammatory signaling.

Free radical-induced oxidative stress is a major factor in the development and growth of abnormal sperm, as well as in sperm count reduction, morphological transformation, and DNA fragmentation. This damages sperm DNA and causes lipid peroxidation of the sperm plasma membrane, thereby reducing sperm-oocyte fusion **[36]**.

Unfortunately, because spermatozoa lack the cytoplasmic enzyme systems necessary for repair, they are unable to reverse the damage caused by high ROS levels. Therefore, rather than the mere presence or absence of disease, the effect of ROS on male fertility depends on the degree of impact.

Furthermore, elevated ROS generation creates an environment that threatens normal physiological processes in men. This can lead to several reproductive problems, including TF, prostate cancer, infertility, and erectile dysfunction **[37]**.

To maintain radical scavenging at a threshold that supports proper cell signaling, an adequate amount of antioxidant enzymes must be present. **[38]**. By eliminating ROS, these enzymes can prevent oxidative stress responses. Catalase and glutathione peroxidase (GPx) are examples of enzymes that reduce tissue damage caused by oxidative stress by converting H_2O_2 into water. **[39]**.

In a rat model of radiation-induced TF, reduced glutathione and GPx activities dramatically declined four days after testicular tissues were exposed to radiation **[40]**.

4.6.2.2. Radiation-induced inflammation

Intimate connections between oxidative stress and inflammation in the male reproductive tract create vicious cycles that physically and functionally damage male reproductive tissues. Numerous studies have provided convincing evidence for the critical role of cytokines in radiation-induced non-targeted cellular damage. **[41]**.

Transforming growth factor (TGF)-β, tumor necrosis factor (TNF)-α, and IL-6 have been reported as key cytokines mediating radiation damage. Testicular cells, especially testicular macrophages, produce large amounts of anti-inflammatory cytokines, including IL-10 **[42]**. Indeed, following radiation exposure, there may be a temporary shift in the balance between pro- and anti-inflammatory mediators. This imbalance can exacerbate chronic illnesses and cellular damage, including disturbances in male reproductive organs that impair fertility **[43]**.

Additionally, testicular torsion—a common reproductive condition caused by a deficiency in supporting tissue that leads to twisting of the testes within the scrotum and results in severe edema—may act as a mediator of inflammation. Torsion damages the testes by rupturing the blood vessels that supply them **[44]**. Mounting evidence confirms the link between misdirected inflammation and abnormal testicular function, which ultimately leads to infertility. Radiation and other external stressors disrupt testicular homeostasis, triggering a cascade of inflammatory events and an excess of pro-inflammatory mediators **[3]**.

Excessive inflammation not only reduces sperm count and quality but also increases the risk of TF **[45]**. Most data from studies on acute testicular inflammation show elevated levels of IL-1β, IL-1α, IL-6, and TNF-α, which have detrimental effects on germ cells despite the bidirectional immunoregulatory functions of cytokines. The following inflammatory signaling pathways are associated with testicular injury following IR exposure:

4.6.2.2.1. Nuclear factor kappa B

Radiation exposure stimulates several signaling pathways within the testes. As shown in **Fig. 2**, the transcription factor NF-κB regulates inflammatory processes. It plays a role in the production of numerous pro-inflammatory mediator genes as well as other genes required for apoptosis, DNA repair, and cell survival **[46]**.

The B-cell-specific protein NF-κB can bind to a specific site in the immunoglobulin kappa light chain gene enhancers. Overactivation of NF-κB is a potent inducer of inflammatory genes such as TNF-α, IL-6, and IL-8, which are involved in inflammation. Under normal physiological conditions, NF-κB is sequestered by the kappa B (IκB) protein family to prevent further tissue injury **[47]**. Radiation exposure primarily activates the classical NF-κB pathway **[46]**. The regulatory step in this cascade involves the activation of a kinase complex that includes IκB kinase (IKKα and/or IKKβ) and the regulatory non-enzymatic scaffold protein NEMO (NF-κB essential modulator, also known as IKKγ) **[48]**.

ROS can activate inflammatory signaling pathways such as NF-κB, leading to persistent inflammation after radiation exposure **[49]**. Excessive ROS binds to integral membrane receptors, leading to phosphorylation and degradation of IκB by IKK. Upon release, NF-κB initiates the production of additional inflammatory mediators **[50]**. Certain cell surface receptors, such as TNF-α receptors, can be bound by inflammatory mediators acting as signals. This triggers NF-κB activation, which in turn stimulates the production of inflammatory cytokines that exacerbate tissue damage **[51]**. In

parallel, TGF-β, TNF-α, and IL-6 are among the cytokines associated with radiation toxicity **[52]**. Rats with TF following radiation exposure showed significantly higher expressions of NF-κB and other pro-inflammatory cytokines such as IL-6 and IL-1β **[53]**.

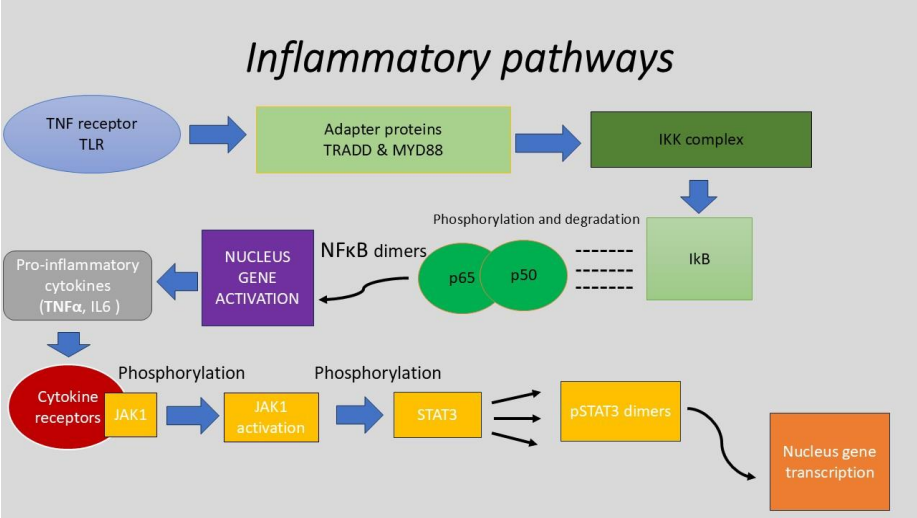

Fig.2. Radiation-induced inflammatory pathways

This figure shows the key inflammation pathways activated by radiation. Ionizing radiation triggers the activation of NF-κB and JAK/STAT signaling pathways, leading to the production of pro-inflammatory cytokines. NF-κB is activated in response to radiation-induced stress, promoting inflammation and cell survival. Simultaneously, radiation activates the JAK/STAT pathway, further enhancing the inflammatory response. These pathways contribute to tissue damage and can lead to chronic inflammation following radiation exposure.

IκB kinase; **IKK**, Interleukin 6; **IL6**, Inhibitor of kappa B; **IκB,** Janus kinase 1; **JAK1,** Myeloid differentiation primary response 88; **MYD88,** Nuclear factor kappa-light-chain-enhancer of activated B cells; **NFκB,** Phosphorylated Signal Transducer and Activator of Transcription 3; **pSTAT3,** Signal Transducer and Activator of Transcription 3; **STAT3,** Toll-like receptor; **TLR**, Tumor Necrosis Factor; **TNF,** Tumor Necrosis Factor alpha; **TNFα**, – TNF receptor-associated death domain; **TRADD.**

4.6.2.2.2. JAK/STAT pathway

The JAK/STAT pathway is a key intracellular signaling cascade triggered by cytokines, growth factors, and hormones, playing a role in maintaining the environment necessary for germ cell survival **[54]**. The binding of extracellular ligands induces receptor modifications that allow intracellular JAKs to phosphorylate each other, activating the pathway. Activated JAKs then phosphorylate the receptor and STAT proteins. Phosphorylated STATs translocate to specific enhancer regions in target genes, binding as dimers or oligomers to regulate transcription. Aberrant activation of JAK/STAT signaling has been implicated in the etiology of several oxidative stress-related disorders **[55]**.

STAT3, originally identified as an acute-phase response factor triggered by IL-6 released through NF-κB activation (see **Fig. 2**), can be stimulated by various cytokines and growth factors. NF-κB and STAT3 are the two primary transcription factors linking inflammation and carcinogenesis, interacting functionally at multiple levels. NF-κB activates STAT3 through cytokines such as IL-17, IL-21, and IL-23 **[56]**. Beyond promoting inflammation, NF-κB interacts complexly with other cellular processes and signaling pathways, including JAK/STAT **[57]**.

Additionally, JAK kinase activates the PI3K/Akt pathway; Akt phosphorylation then activates IκB kinase, which promotes NF-κB

activation and target gene transcription **[58]**. The JAK/STAT system mediates critical signals from somatic gonad cells that regulate male germ cell formation during early testicular development. JAK/STAT activity is also linked to germ cell survival in the presence of reproductive toxins **[59]**. JAK1 is found in the sperm midpiece and equatorial region **[60]**.

The JAK/STAT pathway is regulated by protein tyrosine phosphatases, protein inhibitors of activated STATs, and suppressors of cytokine signaling (SOCSs). SOCSs reduce JAK/STAT activity through various mechanisms, such as inhibiting JAK phosphorylation and preventing STAT-receptor binding. This regulation is essential to maintain cellular homeostasis and prevent overactivation of the pathway **[61]**.

4.6.2.3. Radiation-induced apoptosis

Mammalian growth depends on the physiological process of apoptosis, which is the main method for eliminating undesirable cells. Inside these cells, it is defined by morphological alterations such as pyknosis (chromatin condensation) and DNA fragmentation. The two primary processes that cause apoptosis are the intrinsic and extrinsic pathways. DNA damage and oxidative stress are cellular stressors that trigger the intrinsic pathway, activating pro-apoptotic proteins such as tumor protein (p53). **[62]**.

In the intrinsic pathway, caspase-9 activation triggers downstream effector caspase activation, which in turn causes cell death **[63]**. Conversely, external cues such as ligands attaching to death receptors trigger extrinsic apoptosis. This route is essential for controlling the immune response because it frequently helps the immune system eliminate diseased or defective cells **[64]**. According to reports, both processes take place after IR exposure **[65]**.

Furthermore, in some circumstances, radiation-induced inflammatory signaling cascades, such as NF-κB, raise the expression of several cytokines and chemical agents that cause apoptotic cell death. Additionally, this research shows how TNF-α can activate caspase to initiate apoptotic signaling, resulting in DNA breakage and cell death **[66]**. Rats exposed to IR also demonstrated a significant decrease in the expression of proliferating cell nuclear antigen, a crucial regulator of DNA repair required to maintain tissue homeostasis during growth, and a marked increase in apoptotic markers in another rat model of radiation-induced TF **[67]**.

4.6.2.4. Radiation-induced autophagy

Autophagy is an evolutionarily conserved intracellular catabolic process that enables cells to degrade and recycle damaged organelles and long-lived proteins, including the mitochondria, Golgi apparatus, and endoplasmic reticulum, through lysosomal pathways. This process is triggered by various cellular stressors, such as nutrient or growth factor deprivation, hypoxia, ROS, IR, and chemotherapy drugs **[68]**.

IR has been shown to induce autophagy both *in vitro* and *in vivo*. For example, after exposure to 5 Gy of radiation, normal mouse embryonic cells demonstrate a marked increase in autophagic activity. One of the key mediators in radiation-induced autophagy is Beclin 1, which functions at the intersection of autophagy and the cell cycle. Beclin 1 facilitates the formation of autophagosomes by enhancing the development of the isolation membrane that engulfs cytoplasmic components **[69]**. Another important marker of autophagic flux is p62 (sequestosome-1), an autophagic substrate whose intracellular levels are regulated by both transcriptional mechanisms and autophagic degradation. Upon activation of autophagy, p62 levels typically decrease, indicating enhanced autophagic activity **[70]**.

While autophagy is often associated with cell death, particularly in the context of anticancer therapies, it does not universally result in cytotoxic outcomes. Instead, it may act as a cytoprotective mechanism, enabling cancer cells to survive therapeutic stress by eliminating damaged proteins and organelles. Thus, autophagy plays a dual role in cancer biology, balancing cell survival and cell death depending on the context and intensity of the stimulus.

The role of ROS in regulating autophagy is significant. It has been observed that ROS accumulation can induce autophagy in cells through the activation of NF-κB, which in turn increases Beclin 1 expression. This suggests a feedback loop in which ROS and NF-κB signaling promote autophagy as a stress adaptation mechanism. Moreover, starvation-induced autophagy has been linked to the activation of the signal transducer and activator of transcription 3 (STAT3) pathway. STAT3 activation during autophagy contributes to the production of IL-6, further amplifying the NF-κB signaling pathway, and potentially influencing inflammatory responses and cell fate decisions **[71]**.

Table 1. Experimental models investigating the effects of radiotherapy on testicular tissue and associated cellular mechanisms

Experimental Model of Ionizing Radiation (Animal Species, Dose)	Mechanism	References
Rhesus monkeys; total-body gamma irradiation (3.5-8.5 Gy)	- Oxidative stress-induced testicular atrophy and apoptosis. - Significant increase in ROS levels, DNA fragmentation, and activation of apoptotic markers (e.g., caspase-3).	**[72]**
Wistar rats; whole-body X-ray irradiation at 0.02 Gy, 0.1 Gy, and 5 Gy	- Increased oxidative stress via elevated ROS and MDA levels. - Decreased SOD activity and increased apoptotic markers. - Inflammation is linked to activation of the NF-kB pathway.	**[73]**
Mice; total-body X-ray radiation (2.0 Gy single fraction)	- Inhibition of autophagy and apoptosis pathways, with protective effects from oxidative stress via chlorogenic acid. - Reduced caspase activation and DNA fragmentation.	**[74]**
Human ovarian and testicular cells; exposure to X-ray irradiation of 0.1 Gy	- Activation of oxidative stress and inflammatory pathways. - Induction of apoptosis and autophagy. - Altered gene expression related to cell survival and damage repair.	**[75]**
Mice; whole-body irradiation (WBI) using X-rays of 4 Gy- 7 Gy	- Inhibition of radiation-induced apoptosis through modulation of immune responses. - Downregulation of pro-apoptotic markers and prevention of autophagy induction.	**[76]**

Table 1 summarizes preclinical and cellular studies exploring the impact of ionizing radiation on testicular or gonadal systems across different species. Each model highlights the radiation dose used, observed biological responses, and the molecular pathways involved. Common mechanisms include oxidative stress, DNA fragmentation, apoptosis, inflammation, and modulation of autophagy. Several studies also explore potential radioprotective strategies or therapeutic modifiers influencing these pathways.

4.7. Approaches to protect the testes

Techniques for testicular protection can be divided into two categories: pharmacological and non-pharmacological (**Fig. 3.)**.

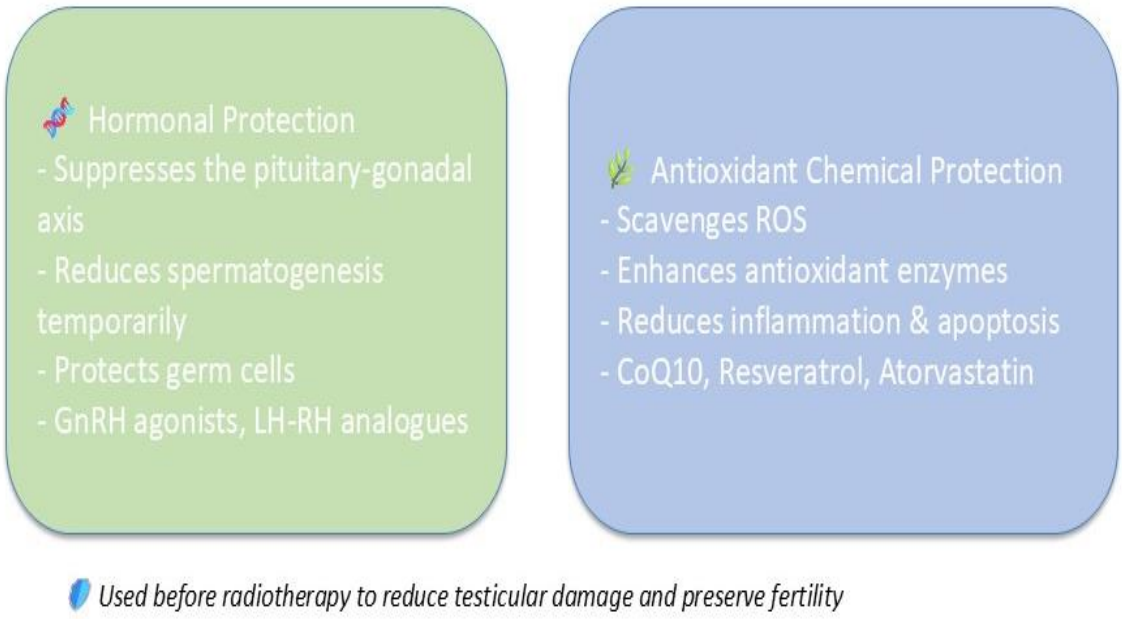

Fig. 3. Pharmacological testicular protection strategies

A schematic overview of pharmacological strategies employed to protect testicular function during radiotherapy. The diagram illustrates two major approaches: **hormonal protection**, which involves the use of agents such as GnRH agonists to suppress the pituitary-gonadal axis and reduce spermatogenesis, and **antioxidant chemical protection**, which uses compounds like Coenzyme Q10, resveratrol, and atorvastatin to scavenge free radicals, reduce inflammation, and limit radiation-induced apoptosis. These strategies aim to preserve fertility and minimize long-term testicular damage in male cancer patients undergoing radiation therapy.

4.7.1. Non-pharmacological testicular protective approaches

4.7.1.1. Testicular shielding

For males undergoing total-body irradiation treatments, testicular shielding is essential to maintaining testicular endocrine function and future fertility **[77]**. Doses as low as 0.28% of the recommended dose can be obtained with appropriate testicular shielding. It is thought that this low dosage will keep the patient fertile. Even though several studies advise local shielding of the gonads, many radiation centers do not employ these particular devices **[78]**.

4.7.1.2. Testicular transposition

There are a few reports of testicular transposition (TT) before scrotal external radiation therapy in children with cancer. In the healthy testicle, TT permits a significant reduction in radiation therapy dose and has minimal morbidity. It aims to protect the testes

from damage by radiation and preserve their function **[79]**. The TT might be a viable option for testicular preservation, but it may also result in surgical complications, such as the possibility of vascular impairment, which could cause the testis to disappear. The testis could be surgically moved out of the irradiated volume using this novel procedure without increasing morbidity or compromising the effectiveness of treatment.

4.7.1.3. Testicular tissue cryopreservation and grafting

For male patients with cancer, medical conditions, or societal concerns, testicular tissue cryopreservation is frequently the only viable alternative for preserving fertility. The cryopreservation of mature spermatozoa is the preferred method for preserving male fertility. Various methods for performing cryopreservation include vitrification or gradual freezing **[80]**. Although it has been explored for more than 20 years, testicular tissue cryopreservation in males presents ethical and legal complications **[81]**.

4.7.1.4. Sperm cryopreservation

Among the most widely acknowledged measures for preserving male fertility is sperm cryopreservation. For more than 40 years, men receiving cancer treatment have turned to sperm cryopreservation as a sperm preservation solution for their infertility problems. There are two methods for preserving sperm: vitrification and conventional freezing. The conventional approach involves a drawn-out freezing process and is mostly used in assisted reproductive technologies. However, because vitrification doesn't require water removal, it's a faster method of sperm cryopreservation **[82]**.

4.7.2. Pharmacological testicular protective approaches

These drugs are used as preventative treatments before radiation exposure. The goal is to postpone the commencement. These medications are further divided into antioxidant and testicular suppression categories based on their mode of action **[83]**.

4.7.2.1. Hormonal protection

Hormonal regulation, however, has been the focus of most research and almost all therapeutic studies to stop or repair germ-line damage caused by radiation and chemotherapy. Initially, it was thought that spermatogenesis could be protected by blocking the pituitary-gonadal axis, which would lower spermatogenesis rates and make the resting testis more susceptible to radiotherapy's effects **[84]**.

According to recent research, gonadal damage may be reduced and normal reproductive function may be restored more quickly in cancer patients receiving radiation therapy if hormonal therapeutic manipulations based on analogues of LH-releasing hormone are attempted. It was once thought that medications such as GnRH agonists could shield the testes before radiation treatments **[85]**.

4.7.2.2. Chemical protection

Antioxidants are seen as typical instances of this condition. Either directly scavenging free radicals or by boosting the activity of endogenous antioxidant enzymes is how these compounds function. Free radicals interact with biomolecules rapidly and have a short lifespan. Antioxidants must be present sufficiently in the cellular systems during IR exposure to provide suitable radioprotection against radiation damage **[86]**. It is expected that antioxidants can reduce the effects of radiation since they promote tissue healing and repair by reducing inflammation and accelerating apoptosis.

Several drugs have shown protective or therapeutic effects against radiation-induced testicular damage in experimental animals.

Antioxidants like melatonin, vitamin E, and N-acetylcysteine have been widely reported to reduce oxidative stress and improve testicular function **[87]**. Anti-inflammatory agents such as curcumin and resveratrol have also demonstrated protective effects by modulating inflammatory pathways and reducing apoptosis. Additionally, natural compounds like quercetin and genistein, as well as stem cell therapy, have shown promise in promoting tissue repair and preserving fertility **[88]**. These interventions primarily work by minimizing oxidative damage, inhibiting cell death, and supporting tissue regeneration **[89]**.

Coenzyme Q10 is a natural antioxidant that protects testicular cells by reducing oxidative stress caused by radiation and supports cellular energy production, helping to improve sperm quality. Resveratrol, a plant-derived compound, also functions as an antioxidant and anti-inflammatory agent, scavenging harmful free radicals and protecting sperm-producing cells from radiation-induced damage. Both compounds have shown promising radioprotective effects in testicular tissue **[90]**.

Low doses of atorvastatin, commonly used to lower cholesterol, have also shown protective effects against radiation-induced testicular damage. It works by reducing oxidative stress and inflammation in testicular tissue, helping to maintain normal testicular structure and function during cancer radiotherapy.

Conclusions / future directions

In conclusion, radiation therapy poses significant risks to testicular function, leading to various reproductive and hormonal complications. The testicular tissue is highly sensitive to radiation, and the effects can range from infertility to severe developmental issues in men, especially in younger individuals. Understanding the mechanisms of radiation-induced damage, including oxidative stress, inflammation, and apoptosis, is crucial for developing effective protective measures. Non-pharmacological approaches, such as testicular shielding and transposition, and pharmacological strategies, including antioxidants and hormonal treatments, offer promising solutions to protect against these harmful effects. Ultimately, preserving testicular function and fertility through preventive and protective methods remains essential for improving the long-term quality of life for male cancer patients undergoing radiation therapy.

Declarations

Ethics Approval and Consent to Participate

Not applicable.

Consent to Participate

Not applicable.

Consent for publication

Not applicable.

Availability of the data and Material

Data will be made available on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

The first draft of the manuscript was written by Nada Mehrez Mostafa, and all authors commented on previous versions of the manuscript. All authors have read and approved the final manuscript.

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