Section A-Research paper

Brief overview about Busulfan induced toxicity on reproductive system

Eman M. Askar[1], Amira M. Abdelmegid, Laila M. Elshal& Mohamed A. Shaheen[1]

[1] Medical histology and cell biology department, faculty of medicine, Zagazig university, Egypt

Email: dr.amira111213@gmail.com, amirams@medicine.zu.edu.eg

Article History: Received 10th June, Accepted 5th July, published online 10th July 2023

Abstract

Background: The testis is largely surrounded by an intrascrotal extension of peritoneal cavity (the processus vaginalis), which becomes the tunica vaginalis. Its visceral layer is opposed to the fibrous capsule of the testis, the tunica albuginea, and its parietal layer lines the most internal aspect of the scrotal wall. A small amount of fluid separates the visceral from the parietal layer. Each testis is covered by a dense irregular collagenous connective tissue capsule known as tunica albuginea that is rich in lymphatic endothelial cells. Deep to this layer is a highly vascularized loose connective tissue called tunica vasculosa which forms the vascular capsule of the testes. Tunica albuginea is thickened posteriorly to form mediastinum testis from which connective tissue septa subdivide testis into compartments called testicular lobules. Each lobule is occupied by one to four seminiferous tubules. Each seminiferous tubule is surrounded by loose connective tissue (interstitium) containing interstitial cells such as Leydig cells, fibroblasts, lymphocytes and macrophages. The seminiferous epithelium and the interstitium are separated by the basal lamina. Infertility is one of the important issues in medical science. A couple being unable to achieve pregnancy after one year of intercourse without the use of contraceptives can be considered infertile. Animal models are commonly used to study human infertility. The methods of creating infertility models include chemical, physical and endocrine factors. Chemical inducers include formaldehyde, gossypol**)** and BUS, The advantage of the BUS-induced sterile mouse model is the similarity between humans and animals regarding the dysfunction of reproductive system and infertility. BUS gives excellent results in cancer treatment, but it also produces numerous side effects, among which its toxicity on the reproductive system. It has inhibitory effects on cells with a high proliferation rate like spermatogonia, increases sperm abnormalities and oligo-azoospermia rate, decreases testicular weight and sperm motility, destroys testicular germ cells, and finally it causes temporary or permanent sterility.

Keywords: Busulfan, toxicity, reproductive system

DOI: 10.53555/ecb/2023.12.Si12.286

Testes, commonly known as testicles, are a pair of ovoid glandular organs located in the hollow sac of the scrotum. In rats, each testis is about 1.5 to 2 inches along its long axis and around 1 inch in diameter**.** They are wrapped by the tunica vaginalis, an extension of the peritoneum and the tunica albuginea which is a tough protective sheath of dense irregular connective tissue. They are responsible for the production of sperms and male sex hormones mainly testosterone **(1).**

The adult human testis is 40–50 mm long, 30 mm high, 25 mm wide and weighs 16–20 g. The testes are housed within the scrotum (scrotal sac) below the penis, the left is hanging a little lower than the right one.

The testis is oval shaped and slightly flat sided and has two smooth surfaces (internal and external), two poles (anterosuperior and posteroinferior), and two margins (anteroinferior and posterosuperior) **(2).**
The testis is largely surrounded by an intrascrotal extension of peritoneal cavity (the processus vaginalis), which becomes the tunica vaginalis. Its visceral layer is opposed to the fibrous capsule of the testis, the tunica albuginea, and its parietal layer lines the most internal aspect of the scrotal wall. A small amount of fluid separates the visceral from the parietal layer **(3).**
The paired testicular arteries arise directly from the abdominal aorta and descend through the inguinal canal to supply testes. The scrotum and the rest of the external genitalia are supplied by the internal pudendal artery which is a branch of the internal iliac artery. The testis has collateral blood supply from the cremasteric artery and the artery to the ductus deferens. Therefore, if the testicular artery is ligated the testis will usually survive on these other blood supplies. Lymphatic drainage of the testes follows the testicular arteries back to the para-aortic lymph nodes at the level of the Lumbar (L2) vertebra while lymph from the scrotum drains to the inguinal lymph nodes **(4).**
The sympathetic nerve fibers that innervate the testes originate from the thorathic (T) 10 spinal segment. They travel by way of the lesser splanchnic nerves and relay at the celiac ganglion. Sensory root fibers also take a similar course and pass information via the dorsal root ganglion cells of the T10 segment **(5)**.

Histological Structure

Each testis is covered by a dense irregular collagenous connective tissue capsule known as tunica albuginea that is rich in lymphatic endothelial cells**.** Deep to this layer is a highly vascularized loose connective tissue called tunica vasculosa which forms the vascular capsule of the testes. Tunica albuginea is thickened posteriorly to form mediastinum testis from which connective tissue septa subdivide testis into compartments called testicular lobules. Each lobule is occupied by one to four seminiferous tubules. Each seminiferous tubule is surrounded by loose connective tissue (interstitium) containing interstitial cells such as Leydig cells, fibroblasts, lymphocytes and macrophages. The seminiferous epithelium and the interstitium are separated by the basal lamina **(6).**
Each seminiferous tubule is approximately 50 centimeters (cm) long (range, 30 to 80 cm) and 150 to 250 micrometers (μm) in diameter. The seminiferous epithelium is an unusual complex stratified epithelium composed of two basic cell populations: spermatogenic cells and Sertoli cells (**7**).
By electron microscope, the seminiferous tubule has a well-developed basement membrane which is formed of basal lamina and reticular lamina. Outside it, there is a clear zone has type I collagen fibrils in varying orientation. Peripheral to this collagen zone, there is a layer of flattened cells called peritubular myoid cells followed by a layer of lymphatic endothelium and fibroblast cells **(8).**

Spermatogenic cells

Spermatogonia are small diploid cells (46XY 2n, 2C Deoxyribonuceic acid (DNA)) which lie on the basal lamina of seminiferous epithelium. At puberty and under the effect of testosterone, they undergo mitosis and give rise to type A dark (Ad) spermatogonia which have ovoid nuclei with intensely basophilic, finely granular chromatin. These spermatogonia are thought to be the stem cells of the seminiferous epithelium **(9).**

Type Ad spermatogonia divide at irregular intervals to give rise to either a pair of type Ad spermatogonia that remain as reserve stem cells or to a pair of type A pale (Ap) spermatogonia which have pale nucleus with a fine "dusty" distribution of heterochromatin throughout the nucleus. They are committed to the differentiation process that produces the sperm and are called renewing stem cells. They undergo several successive mitotic divisions and give rise to type Ap spermatogonia or type B cells. Type B spermatogonia are usually identical to type A pale cells, but usually have rounded nuclei. Through miosis these cells divide to give primary spermatocytes (**7**). The synaptonemal complex, in the nuclei of primary spermatocyte, is a meiosis-specific multiprotein complex that forms between homologous chromosomes during prophase of meiosis I. Upon assembly, it mediates the synapses of the homologous chromosomes, leading to the formation of bivalents, and physically supports the formation of programmed double-strand breaks and their subsequent repair and maturation into crossovers, which are essential for genome haploidization. Defects in the assembly of the synaptonemal complex or in the function of the associated meiotic recombination machinery can lead

to meiotic arrest and human infertility **(10)**. It is seen as a tripartite structure at the EM level. Two lateral elements (LEs) are kept together by fine transverse filaments (TFs) crossing perpendicularly from one LE to the other, forming a central region (CR) of around 100 nm in width. In the center, the amino terminal regions of antiparallel TFs interact with other meiosis-specific proteins to form the central element (CE) **(11).**

The diploid (2N) primary spermatocytes enter meiosis I and divide to become haploid (1N) secondary spermatocytes. Secondary spermatocytes have the shortest life span of all types of spermatogonia. They are rarely seen among the germinal cells. These cells are about 8-9 µm in diameter and have spherical nuclei with centrally located clumps of chromatin substance. Their mitochondria show great similarity to those of the spermatids. These cells do not replicate their chromosomes. They quickly enter the second meiotic division and divide into two smaller cells containing haploid number of chromosomes known as spermatids (**7**).

The early spermatids are rounded cells contain large spherical nuclei which contain chromatin clumps. Their cytoplasm has endoplasmic reticulum and mitochondria which tend to aggregate at the periphery of the plasma membrane. They contain haploid number of chromosomes and haploid amount of DNA. The round spermatids do not divide but undergo a complex metamorphosis, called spermiogenesis to become spermatozoa. This process involves: Condensation of DNA and nucleus acrosomal development, flagellum development and elimination of excess cytoplasm **(12)**.

Morphologically, the mature human spermatozoon is about 45– 50 µm in length and consists of a head, neck, and tail. The normal head is smooth and symmetrically oval with a broad base and tapering apex. The sperm head measures between 4.0–5.5 µm in length and 2.5–3.5 µm in width. The head is the most important part as it contains a nucleus, which contain paternal genetic material (23 chromosomes)**.** The head also contains a well-defined acrosome region, a cap-like covering of the anterior two thirds of the head, which contains several hydrolytic enzymes, such as hyaluronidase and acrosin, that are required for fertilization **(13).**

The neck is short about 1 µm and attached to the basal plate. A transversely oriented centriole is located immediately behind the basal plate. The neck also contains nine columns of fibrous material which continue as the outer dense fibers into the tail **(14).**

The tail of sperm is divided into middle, principal, and end pieces. The axoneme arrangement of microtubules is like that in cilia (9 doublet microtubules with 2 central singlets) it begins in the middle piece and is surrounded by nine outer dense fibers. In the middle piece, the axoneme and dense fibers are surrounded by a sheath of mitochondria **(15)**.

The middle piece is terminated by a dense ring called annulus. The principal piece is about 45 µm in length and contains a fibrous sheath, which consists of dorsal and ventral longitudinal columns interconnected by regularly spaced and seven dense fibers. Lastly, in the end piece, the axoneme is only surrounded by the cell membrane **(16)**.

Mature spermatids are released from Sertoli cells into the seminiferous tubule lumen through a process called spermiation. This process takes place over several days at the apical edge of the seminiferous epithelium. It includes several discrete steps starting from remodeling of the spermatid head and cytoplasm, removal of specialized adhesion structures and the final liberation of the spermatid from Sertoli cell **(14)**.

The non-motile spermatozoa are transported to the epididymis in the testicular fluid secreted by the Sertoli cells with the aid of peristaltic contraction of the myoid cells in the tubular wall. Human spermatozoa must migrate through the epididymis and undergo a specific maturation process to become a functional gamete. The epididymis is a dynamic organ that promotes sperm maturation under the influence of androgens. It also provides a place for sperm storage and plays a role in the transport of the spermatozoa from the testis to the ejaculatory duct. In addition, the epididymis protects the male gametes from harmful substances and reabsorbs both fluids and products of sperm breakdown, thus enabling the sperm to fertilize the ovum and to contribute to the formation of a healthy embryo **(17).**

Sertoli cells are specialized epithelial cell type that surround the male germ cells, regulate the organization of testicular structures and differentiation of other somatic cell linages in the testis, Leydig cells and peritubular myoid cells. These cells must continually alter their shape to accommodate the structural transformation and mobilization of germ cells from the base to the free surface of the seminiferous epithelium .Sertoli cells are pyramidal or columnar cells located directly on the basal membrane of the seminiferous tubules. These cells

are the only ones to reach from the basal membrane to the tubular lumen (**7**). They have prominent nucleoli and two satellite nucleosomes outside the nucleus **(18).** It was reported that Sertoli cells go through only two developmental stages, i.e., from immature to mature. They remain immature until the peak of testosterone production during puberty **(19).**

Sertoli cells have several parts: the basal foot, trunk regions, lateral cell processes and apical cell surfaces. The basal foot rests on the basal lamina and the trunk region extends toward the lumen of tubules. There is an elaborate system of thin processes radiate laterally from the trunk region to surround the spermatocytes and round spermatids and occupy all of the spaces among them. The apical Sertoli cell surface is indented by shallow or deep recesses and houses elongated spermatids and residual bodies **(20)**.

Sertoli cells have mitochondria that exhibit a great diversity in shape, they may be round, oval, spherical or even S shape. The Golgi apparatus are frequently located near the nucleus but some are also found in the apical cytoplasm. They also contain abundant smooth endoplasmic reticulum (SER), little rough endoplasmic reticulum, free ribosomes, microtubules, actin, and vimentin filaments. There are variable amounts of dense bodies usually are collections of lysosomes or multivesicular bodies or may be heterophagic vacuoles **(21).**

Sertoli cells have unique tight junctions between them, making anatomical and functional subdivision of the seminiferous epithelium into basal and adluminal components. The basal component contains spermatogonia, preleptotene and leptotene primary spermatocytes. The adluminal component is beyond the level of tight junctions and has advanced spermatocytes and spermatids **(22).**

The tight junction is composed of up to 50 parallel fusion lines in the adjacent membranes and two cytoplasmic components characterize this unique junctional complex: flattened cisternae of SER and actin filament bundles interposed between SER cisternae and plasma membranes (**7**).

Blood testis barrier (BTB) consists of tight junctions, basal ectoplasmic specialization, basal tubulobulbar complexes, gap junctions, and desmosomes between Sertoli cells **(23).** Tight junctions between the basal portions of adjacent sertoli cells form the BTB which controls the passage of molecules into the germinal epithelium **(17)** and generates an immune-privileged microenvironment. In rodents, the myoid cell layer in the tunica propria shares significantly to the barrier function of BTB. **Smith and Walker, (24)** reported the role of testosterone in the maintenance of the BTB dynamic ultrastructure. It supports assembly and disassembly of Sertoli-germ cell junction, and its deficiency will result in detachment of advanced germs cell from the Sertoli cells. Blood testis barrier (BTB) is dissolved above the preleptotene spermatocyte and reformed below as the germ cell begins its movement toward the lumen of the tubule **(23).**

Sertoli cells are so important that their mere absence in testes can lead to infertility in adult males even though the spermatogenesis is normal**.** Sertoli cells have many nutritive, protective, and supportive roles for spermatogenic cells. They phagocytose the degenerating spermatogenic cells and detached residual bodies of spermatids. They release the sperms into the lumen of the seminiferous tubules by the process of spermiation. Additionally, they participate in the organization of spermatogenic events through the action of follicle stimulating hormone (FSH) and testosterone on the germ cells. Moreover, they produce androgen binding protein (ABG) and secrete other constituents of the intratubular fluid such as transferrin and inhibin (**7**). They secrete inhibin protein under the effect of FSH by negative feedback mechanism**.** Also, they secrete AMH which suppresses the formation of Mullerian duct thus establishes the maleness of the developing embryo **(25).**

Sertoli cells secrete numerous growth factors such as stem cell factor (SCF), bone morphogenetic protein 4 (BMP4), retinoic acid (RA) and glial cell line-derived neurotrophic factor (GDNF)**.** SCF, BMPs, and RA induce spermatogonia cell differentiation; GNDF has been demonstrated to stimulate the self-renewal of spermatogonia cells**.** The SCF is a cytokine that activates the tyrosine kinase receptor c-KIT, which regulates both differentiation and proliferation of spermatogonia and mediates the effects of both BMP4 and RA on spermatogonia cell differentiation **(26).**

In humans, the interstitial compartment represents about 12–15% of the testicular volume**.** In laboratory rodents, presenting rather small testes, the interstitial compartment is comparably small and comprises groups of Leydig cells gathering around blood capillaries **(27).**

The Leydig cells are widely recognized cell type in mature testis. They are relatively large, polymorphoic cells with spherical eccentrically located nuclei containing a small amount of peripherally disposed heterochromatin and prominent nucleoli. Their cytoplasm is acidophilic and contains lipid droplets. As other steroid secreting endocrine cells, the most ultrastructural feature of Leydig cell is extensive SER containing the enzymes necessary for the biosynthesis of androgenic steroids as well as the mitochondria that possess tubular cristae and are involved in the first step of steroid hormones production **(28).**

Nicholson and Ricke, (29) mentioned the role of testosterone secreted by Leydig cells in the preservation of the Wolffian duct and its differentiation into efferent ductules and epididymis. Also, it triggers the growth of male accessory glands, secondary sex characters, promotion of normal sexual behavior (libido) and control of spermatogenesis. **Smith and Walker, (24)** also reported the importance of testosterone in meiosis, Sertoli-spermatid adhesion, spermiogenesis, spermiation and maintenance of BTB. Additionally, Leydig cells produce proteins having endogenous and xenotoxic metabolic functions, which also reduce oxidative stress to protect the testis from toxins**. Adamczewska et al. (30)** stated that Leydig cells play a crucial role in male reproductive tract development, overall male reproductive function, and maintenance of appropriate spermatogenesis.

Beside the Leydig cells, the interstitial compartment is composed of loose connective tissue with many blood vessels, nerve fibers, lymph vessels as well as cells of the lymphatic system. The interstitial compartment also contains cells belonging to the immune system as macrophages and lymphocytes. One macrophage can be seen for every 10–50 Leydig cells which influences their function by secreting stimulators and inhibitors of steroidogenesis**.** Basal gonadotropins and Leydig cell hormones are normally very low till the onset of puberty. So, direct biomarkers of Sertoli cells which are serum AMH and inhibin B are important tools in infancy and childhood **(31).**

Intratesticular ducts

At the end of each seminiferous tubule, there is abrupt transition into straight tubules or tubuli recti that are lined by Sertoli cells only. They empty into rete testis, a complex system of interconnected channels within the highly vascular connective tissue of the mediastinum testis. Rete testis is lined by simple cuboidal or low columnar epithelium with single apical cilium and few short microvilli (**7**).

Hormonal Regulation of Spermatogenesis

Spermatogenesis is controlled by the hypothalamic-hypophyseal-gonadal axis (HHG). The hypothalamus secretes the gonadotropin releasing hormone (GnRH) which stimulates pituitary secretion of luteinizing hormone (LH), which in male is sometimes referred to as interstitial cell-stimulating hormone (ICSH) and FSH. Both gonadotropins are secreted into the peripheral blood and reach the testis where they provoke several responses. Stimulation of steroidogenesis by testicular Leydig cells is the main function of LH in males, whereas FSH stimulates spermatogenesis via Sertoli cell function stimulation**.** Under the influence of the FSH, Sertoli cells secrete ABG, tissue plasminogen activator (T-PA) and inhibin. ABG is necessary to maintain the high level of androgen locally and is important for spermatogenesis (**7**). T-PA has critical functions during spermatogenesis and inhibin has a negative feedback effect on FSH secretion by the anterior pituitary gland **(32).**

Testosterone and estrogen are important hormones in both sexes. Although testosterone is the primary sex hormone in males, estrogen is locally produced in the testis and plays a vital role in male reproductive system as well, such as in the processes of spermatogenesis and spermiogenesis**.** The estrogen 17β-estradiol (E_2) is synthesized in the presence of the P450 aromatase enzyme by irreversible conversion of testosterone**.** The action of E_2 is mediated by two intracellular estrogen receptors (ERs), namely, ERα and ERβ**.** ERs are present in the testis, epididymis and efferent ductules of most species. Although, ERα is absent in the testis of a few species including man. ERs are abundant in the efferent ductular epithelium where they function in regulation the expression of proteins involved in fluid reabsorption. The disruption of ERα either by treatment with a pure anti-estrogen or by the gene specific knockout results in disruption of sperm morphology, dilution of cauda epididymal sperm, increased secretion of chloride, inhibition of sodium transport and subsequent water reabsorption and finally decreased fertility **(33).**

Testosterone is the principal androgen produced by the Leydig cells in the testis under the influence of LH. The main functions of testosterone are development and maturation of internal and external reproductive organs in male, stimulation of spermatogenesis, regulation of accessory sex gland functions, development of the secondary sex characters and regulation of gonadotrophin secretion by negative feedback mechanism**.** Testosterone, estrogens and inhibin as well as other hormones are secreted from the testis into the bloodstream and once, they reach the hypothalamus and pituitary gland exert negative feedback on the release of GnRH, LH and FSH. The activin secreted from the testes also acts at two different levels, first on the hypothalamus stimulating the release of GnRH and second on the pituitary gland stimulating FSH secretion by the gonadotroph cells **(34).**

Busulfan

Infertility is one of the important issues in medical science. A couple being unable to achieve pregnancy after one year of intercourse without the use of contraceptives can be considered infertile**.** It is a worldwide serious health problem, and its incidence is increasing. It is approximately 24% in humans, with 18% in men**.** Male factors contribute to at least 50% of the infertility cases. About 30%-50% of male infertility cases are idiopathic**.** Its impact on social functioning, existential aspects of well-being, personal quality of life, and in larger scale on social health is dramatic **(35).**

Several factors, such as obesity, genetics, sex problems, psychological stress, hormonal disorders, and medications, in addition to a variety of unknown etiologies, lead to male infertility. The failure to produce sperms is manifested as severe oligospermia and azoospermia which displays the male infertility phenotypes **(36).**

Animal models are commonly used to study human infertility. The methods of creating infertility models include chemical, physical and endocrine factors. Chemical inducers include formaldehyde**,** gossypol and BUS**.** The advantage of the BUS-induced sterile mouse model is the similarity between humans and animals regarding the dysfunction of reproductive system and infertility **(37).**

For many reasons, the incidence of malignancies continues to increase worldwide and resulted in the increased use of chemotherapy drugs**.** BUS is a chemotherapy drug belonging to the class of alkyl sulfonates, chemically known as 1, 4-butanediol dimethanesulfonate**.** It is used in two forms of doses; low doses for long-term treatment of ovarian cancers and chronic myelogenous leukemia, and high doses to induce bone marrow suppression in patients undergoing bone marrow transplantation**.** It is also used to treat blood disorders including thalassemia, polycythemia vera, primary thrombocythaemia, sickle cell disease and mucopolysaccharide disorder**.** It is one of the very few chemotherapy drugs used in children under the age of three **(38).**

Zhang et al. (39) mentioned that in bone marrow transplantation and cancer treatment, preservation of fertility gains an important priority. Before starting gonadotoxic treatments, cryopreservation of mature sperm has been recommended worldwide to preserve male fertility and for allowing the conception of a healthy baby with assisted reproductive technology; however, these technologies are not achievable for men with spermatogenic failure and prepubertal boys**.** Other approaches for preserving future fertility in patients receiving gonadotoxic treatments include gonadotropin suppression, which failed to improve sperm count in all clinical trials; and spermatogonial stem cell transplantation, at a risk of reintroducing malignant cells into the patient's body following a cancer cure **(40).**

BUS gives excellent results in cancer treatment, but it also produces numerous side effects, among which its toxicity on the reproductive system**.** It has inhibitory effects on cells with a high proliferation rate like spermatogonia, increases sperm abnormalities and oligo-azoospermia rate, decreases testicular weight and sperm motility, destroys testicular germ cells, and finally it causes temporary or permanent sterility**.** It also causes chromosomal anomalies and lethal mutations, mostly in sperms **(41).**

Pisoschi et al. (42) stated that oxidative stress may result from any imbalance between the generation of ROS and the antioxidant system. The overproduction of ROS may result in many diseases including infertility**.** Oxidative stress is thought to be responsible for some 30% to 80% of idiopathic male infertility**. Agarwal et al. (43)** mentioned that testes are considered a perfect environment to react with ROS due to the high levels of germ cell proliferation and metabolism in the testes and the high amounts of unsaturated fatty acids. The

enhancement of ROS levels can also lead to peroxidation of the spermatozoa membrane and apoptosis of testicular germ cells, and subsequently affect the sperm parameters**.** Spermatozoa are sensitive to over-production of ROS because of loss of a large volume of cytoplasm during spermatogenesis **(44).**
There are several factors that increase the levels of ROS in the male reproductive system including life style, smoking obesity, radiation, infections, aging **(43)**, drugs, irradiation, non-alcoholic fatty liver disease, diabetes, testicular torsion/detorsion and chemotherapy**.** However, endogenous ROS have been identified as critical factors that contribute to self-renewal of spermatogonia stem cells by activating mitogen-activated protein kinase (MAPK14/MAPK7/BCL6B pathway) **(45).**

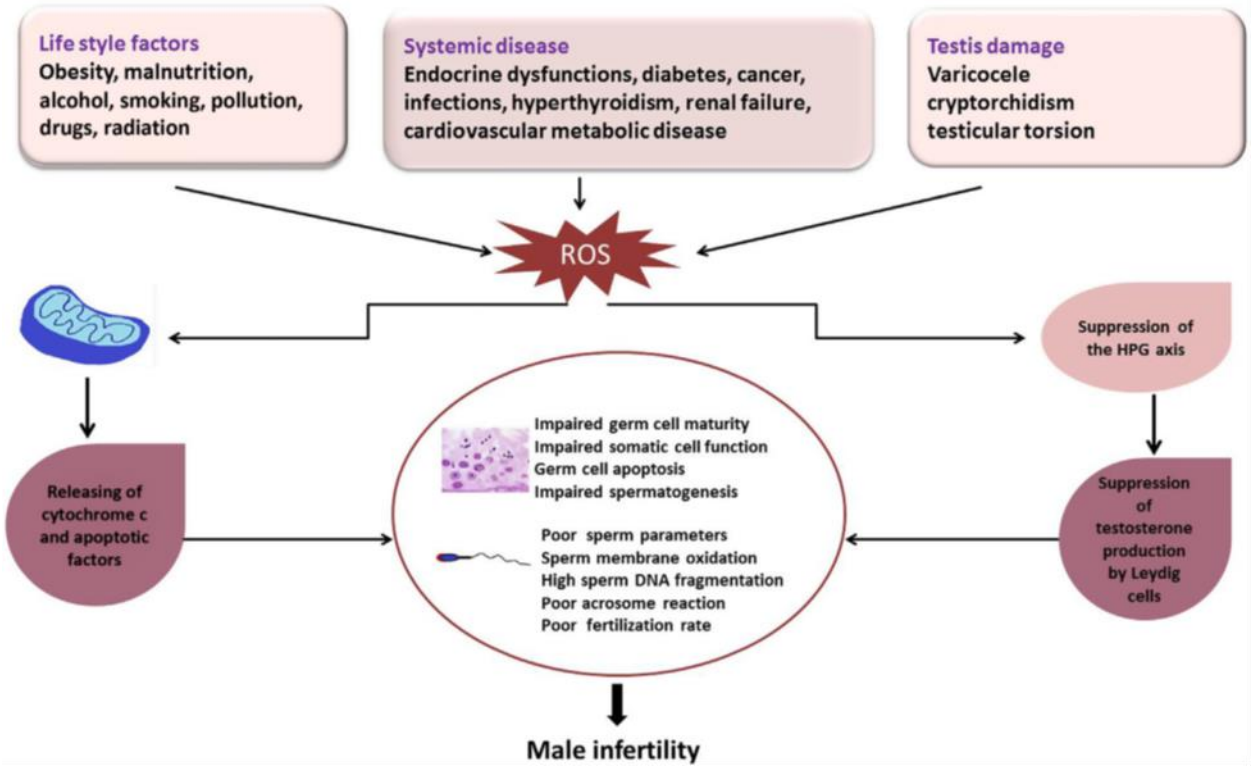

Diagram I: Diagram showing side effects of ROS on male fertility. Adapted from **Mohammadghasemi (46).**
BUS exerts its cytotoxic effects not only on spermatogonial stem cells but also on Leydig, Sertoli and peritubular cells as well**.** It exerts its cytotoxic effects via the formation of DNA–protein and DNA–DNA cross-links and single strand breaks of G1 phase of cell cycle**.** Based on free radical production, it can induce cell death through impairment in the synthesis of lipids, proteins, and nucleic acids of cells**.** The accumulation of free radicals in the cell increases ROS level, decreases activity of antioxidant enzymes and induces lipid oxidation**.** This phenomenon, in turn, causes inactivation of specific proteins, DNA breakdown and thus loss of biologic cell membranes **(47).**

BUS gives excellent results in cancer treatment, but it also produces numerous side effects, among which its toxicity on the reproductive system. It has inhibitory effects on cells with a high proliferation rate like spermatogonia, increases sperm abnormalities and oligo-azoospermia rate, decreases testicular weight and sperm motility, destroys testicular germ cells, and finally it causes temporary or permanent sterility

References

1. Popoola, O. B.; Adefule, A. K.; Ajayi, R. T.; Akinyemi, R. A.; Otulana, O. J. and Akpan, H. B. (2014): Effects of tetracycline on testis and testosterone level in adult male wistar rats. Scholarly Journal of Medicine, 4: 4-10.
2. Vaamonde, D.; du Plessis, S.S. and Agarwal, A. (2016): Erratum to: Exercise and Human Reproduction: Induced Fertility Disorders and Possible Therapies. In Exercise and Human Reproduction. Springer New York. pp. E1-E1.
3. Giannarini, G.; Dieckmann, K.P.; Albers, P.; Heidenreich, A. and Pizzocaro, G. (2010): Organ-sparing surgery for adult testicular tumours: a systematic review of the literature. European urology, 57(5), 780-790.
4. Gracia-Calvo, L. A.; Duque, J.; Balao da Silva, C.; Ezquerra, J. and Ortega-Ferrusola, C. (2015): Testicular perfusion after standing laparoscopic peritoneal flap hernioplasty installions. Theriogenology, 84(5): 797-804.
5. Sinnatamby C. (2013): last's anatomy: regional and applied. 12th edition. Churchill Livingstone, Elsevier. Pp: 357- 360.
6. Itoh, M. (2017): Testicular Autoimmunity: A Cause of Male Infertility.1st edition. Tokyo, Japan, Springer. pp. 1-232.
7. Pawlina W. (2020): Histology: A text and Atlas, 8th edition, Lippincott Williams & Wilkins. Philadelphia, Pp: 794-800.
8. Mescher A. L. (2018): Junqueira's Basic Histology: Text and Atlas. 15th edition. New York, McGraw-Hill. Pp: 413-437, 439-457.
9. Ramm, S.A.; Schärer, L.; Ehmcke, J. and Wistuba, J. (2014): Sperm competition and the evolution of spermatogenesis. Molecular Human Reproduction, 20(12): 1169-1179.
10. Llano, E. and Pendás, A. M. (2023): Synaptonemal Complex in Human Biology and Disease. Cells, 12(13): 1718.
11. Pyatnitskaya, A.; Borde, V. and De Muyt, A. (2019): Crossing and zipping: molecular duties of the ZMM proteins in meiosis. Chromosoma, 128(3): 181-198.
12. Ni, F. D.; Hao, S. L. and Yang, W. X. (2020): Molecular insights into hormone regulation via signaling pathways in Sertoli cells: With discussion on infertility and testicular tumor. Gene, 753: 144812.
13. Esteves, S.C. and Miyaoka, R. (2015): Sperm physiology and assessment of spermatogenesis kinetics in vivo. Handbook of Fertility: Nutrition, Diet, Lifestyle and Reproductive Health. Amsterdam: Elsevier. Pp. 383-396.
14. O'Donnell, L.; Smith, L. B. and Rebourcet, D. (2022): Sertoli cells as key drivers of testis function. In Seminars in Cell & Developmental Biology. Academic Press, 121: 2-9.
15. Mohri, H.; Inaba, K.; Ishijima, S. and Baba, S.A. (2012): Tubulin-dynein system in flagellar and ciliary movement. Proceedings of the Japan Academy, Series B, 88(8): 397-415.
16. Borg, C.L.; Wolski, K.M.; Gibbs, G.M. and O'Bryan, M.K. (2010): Phenotyping male infertility in the mouse: how to get the most out of a 'non-performer. Human Reproduction Update, 16(2): 205-224.
17. França, L. R.; Hess, R. A.; Dufour, J. M.; Hofmann, M. C. and Griswold, M. D. (2016): The Sertoli cell: one hundred fifty years of beauty and plasticity. Andrology, 4(2): 189-212.
18. Ma, C.; Song, H.; Guan, K.; Zhou, J.; Xia, X. and Li, F. (2016): Characterization of swine testicular cell line as immature porcine Sertoli cell line. In Vitro Cellular & Developmental Biology-Animal, 52(4): 427-433.
19. Tan, K. A.; De Gendt, K.; Atanassova, N.; Walker, M.; Sharpe, R. M.; Saunders, P. T. and Verhoeven, G. (2005): The role of androgens in Sertoli cell proliferation and functional maturation: studies in mice with total or Sertoli cell-selective ablation of the androgen receptor. Endocrinology, 146(6): 2674-2683.
20. Abd-Elmaksoud, A. (2005): Morphological, glycohistochemical and immunohistochemical studies on the embryonic and adult bovine testis. PhD thesis, Ludwig-Maximilians University., Munich, Germany.
21. Sarma, K. and Devi, J. (2012): Changes in the seminiferous epithelium of the testes during postnatal development in Assam goat. Anatomy Research International, 2012: 620924.
22. Heininger, K. (2013): The mutagenesis-selection-cascade theory of sexual reproduction. Webmed Central Reproduction 4(9): WMC004367.
23. O'Donnell, L.; Nicholls, P. K.; O'Bryan, M. K.; McLachlan, R. I. and Stanton, P. G. (2011): Spermiation: the process of sperm release. Spermatogenesis, 1(1): 14-35.
24. Smith, L. B. and Walker, W. H. (2014): The regulation of spermatogenesis by androgens. In Seminars in cell & developmental biology. Academic Press, 30: 2-13.
25. Barbotin, A. L.; Peigné, M.; Malone, S. A. and Giacobini, P. (2019): Emerging roles of anti-müllerian hormone in hypothalamic-pituitary function. Neuroendocrinology, 109(3): 218-229.
26. Cardoso, H. J.; Figueira, M. I. and Socorro, S. (2017): The stem cell factor (SCF)/c-KIT signalling in testis and prostate cancer. Journal of cell communication and signaling, 11(4): 297-307.
27. Setchell, B.P. and Breed, W.G. (2006): Anatomy, vasculature, and innervation of the male reproductive tract. In: J.D. Neill ed. Knobil and Neill's Physiology of Reproduction. 3rd ed. Elsevier, pp. 771–825.
28. Gartner L, and Hiaat J. (2015): Color Text book of Histology. 3rd ed. WB Saunders Company. Philadelphia, London, New York. Pp. 680-720.
29. Nicholson, T. M. and Ricke, W. A. (2011): Androgens and estrogens in benign prostatic hyperplasia: past, present and future. Differentiation, 82(4-5): 184-199.

30. Adamczewska, D.; Słowikowska-Hilczer, J. and Walczak-Jędrzejowska, R. (2022): The Fate of Leydig Cells in Men with Spermatogenic Failure. Life, 12(4): 570.
31. Valeri, C.; Schteingart, H. F. and Rey, R. A. (2013): The prepubertal testis: biomarkers and functions. Current Opinion in Endocrinology, Diabetes and Obesity, 20(3): 224-233.
32. Hall, J. E. and Hall, M. E. (2020): Guyton and Hall textbook of medical physiology e-Book. In: John FK ed. 14th edition. Philadelphia, Elsevier Health Sciences.
33. Hess, R. A. and Carnes, K. (2018): The role of estrogen in testis and the male reproductive tract: a review and species comparison. Animal Reproduction (AR), 1(1): 5-30.
34. Damián, J. P.; Bausero, M. and Bielli, A. (2015): Acute stress, hypothalamic-hypophyseal- testicular axis and testicular function – A review. Annals of Animal Science, 15(1): 31-50.
35. Parviz, R.; Solomianyi, R. and Zasieda, Y. (2020): complex treatment of non-obstructive forms of male infertility with platelet-rich plasma, lowintensity pulsed ultrasound and human placenta hydrolysate. Men's Health, Gender and Psychosomatic Medicine, (1-2): 79-85.
36. Cooper, T. G.; Noonan, E.; Von Eckardstein, S.; Auger, J.; Baker, H. W.; Behre, H. M. and Vogelsong, K. M. (2010): World Health Organization reference values for human semen characteristics. Human reproduction update, 16(3): 231-245.
37. Panahi, S.; Abdollahifar, M. A.; Aliaghaei, A.; Nazarian, H.; Paktinat, S.; Abdi, S.and Farahani, R. M. (2017): Application of stereological methods for unbiased estimation of sperm morphology in the mice induced by busulfan. Anatomy & cell biology, 50(4): 301-305.
38. Faraci, M.; Bekassy, A. N.; De Fazio, V.; Tichelli, A. and Dini, G. (2008): Non-endocrine late complications in children after allogeneic haematopoietic SCT. Bone marrow transplantation, 41(2): S49-S57.
39. Zhang, X.; Xia, Q.; Wei, R.; Song, H.; Mi, J.; Lin, Z. and Zou, K. (2019): Melatonin protects spermatogonia from the stress of chemotherapy and oxidation via eliminating reactive oxidative species. Free Radical Biology and Medicine, 137: 74-86.
40. Benavides-Garcia, R.; Joachim, R.; Pina, N. A.; Mutoji, K. N.; Reilly, M. A. and Hermann, B. P. (2015): Granulocyte colony-stimulating factor prevents loss of spermatogenesis after sterilizing busulfan chemotherapy. Fertility and sterility, 103(1): 270-280.
41. Abofoul-Azab, M.; Lunenfeld, E.; Levitas, E.; Zeadna, A.; Younis, J. S.; Bar-Ami, S. and Huleihel, M. (2019): Identification of premeiotic, meiotic, and postmeiotic cells in testicular biopsies without sperm from sertoli cell-only syndrome patients. International Journal of Molecular Sciences, 20(3): 470.
42. Pisoschi, A. M; Pop, A.; Iordache, F.; Stanca, L.; Predoi, G. and Serban, A. I. (2021): Oxidative stress mitigation by antioxidants-an overview on their chemistry and influences on health status. European Journal of Medicinal Chemistry, 209: 112891.
43. Agarwal, A.; Virk, G.; Ong, C. and Du Plessis, S. S. (2014): Effect of oxidative stress on male reproduction. The world journal of men's health, 32(1): 1-17.
44. Asgari, R.; Bakhtiari, M.; Rezazadeh, D.; Yarani, R.; Esmaeili, F. and Mansouri, K. (2021): TSGA10 as a potential key factor in the process of spermatid differentiation/maturation: deciphering its association with autophagy pathway. Reproductive Sciences, 28(11): 3228-3240.
45. Morimoto, H.; Kanastu-Shinohara, M.; Ogonuki, N.; Kamimura, S.; Ogura, A.; Yabe-Nishimura, C. and Shinohara, T. (2019): ROS amplification drives mouse spermatogonial stem cell self-renewal. Life science alliance, 2(2): e201900374.
46. Mohammadghasemi F. 2020: Melatonin, antioxidant capacity, and male reproductive function. In: Preedy VR, ed.Pathology. London, Elsevier. pp. 265–75.
47. Salahshoor, M. R.; Haghjoo, M.; Roshankhah, S.; Makalani, F. and Jalili, C. (2018): Effect of thymoquinone on reproductive parameter in morphine-treated male mice. Advanced biomedical research, 7: 18.