Introduction
1. Introduction

The male reproductive system encompasses the anatomical structures and physiological functions that produce mature sperm. The processes of sex determination and embryonic development produce a male child, setting the stage for the virilization and onset of fertility that begin with puberty. The study of the physiology of reproduction began as early as 300 B.C. by the gonadal exterpanation technique of Aristotle (1862; 1943) and the first microscopic examination of germ cells was done in 17th century (Van Leeuwenhoek, 1679). The discovery that the spermatozoa develop from cells residing in testis was followed by the description of the microscopic characteristics of the interstitial cells (Leydig, 1857) and Sertoli cells (Sertoli, 1865).

The basic structure of testis includes a capsule composed of three distinct layers enclosing testicular parenchyma. The parenchyma is composed of seminiferous tubules, interstitial tissue and macrophages (Fig. 1).

Source: Dr. D. B. Hales, Dept. of Biophysics, University of Illinois, Chicago

Fig. 1. The scanning electron micrograph of cross section of testis showing, (A) alignment of seminiferous tubules and (B) immature germ cells at the basal membrane and mature sperm towards the center. The interstitium between two tubules is also seen.
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The seminiferous tubules show a complex pattern of convulsions. The seminiferous epithelium consists of continually dividing germ cells and Sertoli cells (which stop dividing during puberty) and the peritubular cells that surround the Sertoli and germ cells. The interstitial tissue is composed of Leydig cells, blood vessels, extensive lymphatic channels and numerous macrophages. The brain, specifically the hypothalamus, signals the pituitary gland to secrete luteinizing hormone (LH) and follicle stimulating hormone (FSH) in a pulsatile pattern characteristic of adulthood (Fig. 2).

![Fig. 2. Hormonal regulation of reproductive system by Hypothalamus-pituitary-gonadal axis](image)

FSH stimulates male germ cells to develop into mature sperm cells, a process called spermatogenesis. LH stimulates testis accessory cells, called Leydig cells, to produce sex steroids, especially testosterone, through the process of steroidogenesis. Male sex hormones are needed for optimal sperm
production, as well as for sexual function, healthy blood and bones, and general well being.

1.1 Regulation of testicular function

The endocrine control of testicular function involves interactions between the central nervous system, particularly the hypothalamus and the anterior pituitary gland, and the testis itself. Endocrine regulation of the testis is mediated primarily by two hormones under the control of the hypothalamic gonadotropin-releasing hormone (GnRH), the luteinizing hormone (LH) which stimulates Leydig cells, and the follicle-stimulating hormone (FSH), that acts on Sertoli cells. Leydig cells produce testosterone that inhibits LH, whereas Sertoli cells secrete inhibin/follistatin, which inhibit FSH, and activin that stimulates FSH. Both FSH and testosterone are required for normal qualitative and quantitative spermatogenesis (Sharpe, 1994; Weinbaur and Nieschlag, 1997).

The existence of communication between cells within the testis was suggested by the first histological observations of the testis (Jegou et al., 1992). Sertoli (1865) himself suggested that Sertoli cells were nursing cells for germ cells. Testosterone was the first intratesticular regulatory factor to be identified, when it was shown to maintain spermatogenesis in hypophysectomised animals (Steinberger, 1971).

1.1.1 Steroidogenesis

Steroid hormones are derivatives of cholesterol that are synthesized most prominently by adrenal gland and gonads. The pituitary gonadotropin, luteinizing hormone (LH) binds to the receptors on Leydig cell and releases
cAMP, which mediates further actions (Saez, 1994). The response to LH also involves an increase in cytosolic Ca\(^{++}\) via influx through plasma membrane channels and the release of intracellular Ca\(^{++}\) stores as well as efflux of chloride ions. The precursor cholesterol for steroidogenesis comes from cholesterol synthesized within the cell from acetate or from cholesterol ester stores in intracellular lipid droplets or from uptake of cholesterol containing low-density lipoproteins. The basic cyclopentanoperhydrophenanthrene ring structure and carbon numbering system of all steroid hormones uses pregnenolone as an example. Pregnenolone is an example of a "C-21 steroid" because it has 21 carbons and testosterone is referred to as a "C-19 steroid". Biosynthesis of steroid hormones requires a battery of oxidative enzymes located in both mitochondria and endoplasmic reticulum. The rate-limiting step in this process is the transport of free cholesterol from the cytoplasm into mitochondria. Because mitochondrial matrix is highly hydrophilic and cholesterol being a hydrophobic molecule, cannot pass through the mitochondrial matrix. This function is taken up by a protein called steroidogenic acute regulatory protein (StAR) which is a 37-kDa nuclear-encoded mitochondrial target protein and is composed of matrix targeting largely cationic amino-acid sequence that interacts with the mitochondrial translocation complexes on the outer and inner mitochondrial membranes. The acidic nature of these sequences provides an ionic driving force that helps propel proteins bearing these sequences into the electronegative matrix. After StAR protein enters the matrix, it is proteolytically processed by matrix metalloproteases to the intermediate 32- and mature 30-kDa forms (Stocco,
2001; Arakane et al., 1998). Within mitochondria, cholesterol is converted to pregnenolone by an enzyme in the inner membrane called cholesterol side-chain cleavage p450 (CYP11A1). Pregnenolone diffuses out of the mitochondria to the smooth endoplasmic reticulum where it is enzymatically converted to testosterone via actions of 3β-hydroxysteroid dehydrogenase Δ4-5 isomerase (3β-HSD), 17β-hydroxylase/C17-20 lyase P450 (P450c17, encoded by the cyp 17 gene), and 17β-hydroxysteroid dehydrogenase (17β-HSD) (Payne, 1990) (Fig. 3).

**Fig. 3. Role of StAR in Leydig steroidogenesis**
1.1.2. Spermatogenesis

Spermatogenesis in mammals is a continuous process in which mitotic proliferation of spermatogonia is followed by meiosis and differentiation of haploid spermatids through a complex series of biochemical and morphological transformations leading to the formation of mature spermatozoa (Bellve, 1979) (Fig. 4). Spermatogenesis depends on hormonal stimulation as well as dynamic interactions between the Sertoli cells and the germ cells of the seminiferous epithelium. Tight junctions between adjacent Sertoli cells create two separate compartments within the seminiferous epithelium: a basal compartment below the tight junction and an adluminal...
compartment above. Sertoli cells secrete hormonal and nutritive factors into the adluminal compartment that creates a specialized microenvironment that fosters the development and viability of residing germ cells. At the start of the spermatogenesis, diploid spermatogonia proliferate producing three populations of cells with markedly different destinies - one subpopulation of spermatogonia are presumably identical to their progenitors and continue to function as stem cells, the majority of spermatogonia enter a differentiative pathway and become spermatozoa, and a sizable number of spermatogonia undergo apoptosis (Dym, 1994).

The three main phases involved in spermatogenic process are: **spermatogonial multiplication** in which the least mature germ cells, spermatogonia, located closest to the basement membrane of the seminiferous tubules of the testes, undergo a regulated proliferative activity to form more mature germ cells. **Meiosis** during which the spermatogonia differentiate into primary spermatocytes and then the first meiotic division occurs resulting in reduction of chromosome number to half the diploid number, leading to the formation of secondary spermatocytes. The meiotic prophase involves paring of homologous chromosomes and genetic exchange. The chromosome pairing is preceded by chromosomal condensation in the leptotene and zygotene stages of primary spermatocytes concomitant with the movement of the spermatocytes from the basal membrane compartment of the seminiferous tubule to the adluminal compartment of the seminiferous tubule. This process places the later stage male germ cells inside a blood testis barrier created by the somatic Sertoli cells, thereby removing these germ cells from direct effects
of circulatory factors. The secondary spermatocytes rapidly undergo a division analogous to mitosis, to produce the haploid spermatids (Clermont, 1972). Then **Spermiogenesis** follows in which the newly formed haploid spermatids undergo an extraordinary complex and successive series of changes involving nuclear transformation, chromatin condensation and formation of acrosome and flagellum, leading to the formation of highly differentiated motile cells, the spermatozoa.

**1.2. Male infertility**

One of the important human aspects is that despite the widespread desire to have children, 2-7% of couples remain childless at the end of their reproductive life (Spira, A., 1987). Infertility can have a range of causes in either partner. In males, it is frequently associated with either gross reduction in number of sperm (oligozoospermia) or their complete absence (azoospermia) in the ejaculate. A significant proportion of cases of oligo- and azoospermia are idiopathic (with no obvious cause). The infertility due to male factor problems amounts to 35% of the total human infertility causes (Fig. 5).
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The male infertility can be caused due to various factors like genetic causes, trauma, infections, anatomic defects, and endocrine disorders.

1.2.1. Role of infections in male infertility

Infertility is an increasingly common presenting problem among men with chronic medical illnesses. Infections can be defined as the active or passive invasion of microorganisms into a macro-organism where they attach, multiply and induce a local or generalized reaction. Gram-negative bacteria elicit systemic inflammatory responses by releasing a structural component of their cell wall, namely lipopolysaccharide (LPS) (Fig. 6).

![Structure of bacterial lipopolysaccharide](image)

**Fig. 6. Structure of bacterial lipopolysaccharide**

As a bacterial factor, LPS was first isolated from *Vibrio cholerae*. The bacterial LPS (endotoxin) can systemically activate endothelial cells, platelets,
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macrophage-monocytes and neutrophils to produce numerous endogenous mediators including reactive oxygen intermediates collectively known as septic cascade resulting tissue damage. The structure of LPS constitutes a lipid ‘A’ moiety and a polysaccharide moiety. Strain differences are due to difference in sugars not lipid ‘A’. LPS binds to receptors on macrophages and stimulate the acute early release of cytokines like interleukins, tumor necrosis factor-α (TNF-α) and prostaglandins, which mediate tissue damage.

Microbial infections are known to cause male infertility (Sanocka et al., 2004; Dohle, 2003; Krause et al., 2002). Localized infections of gonads and non-localized/systemic infections by *Borrelia spirochetes*, *Treponema palladium*, *Brucella canis*, and *Chlamydia trachomatis* are known to cause temporary/permanent loss of male fertility, but it is not clear how infections affect the male reproductive system (Akinci et al., 2003; Pacey and Eley, 2004). The reactions brought about by different germs in testis, epididymis and other accessory reproductive tissues as well cause damage to these tissues, which results in temporary or permanent infertility. It has long been known that infection and inflammatory disease can lead to testicular dysfunction, even though the testis is considered to be an immunologically privileged site (Hedger, 1997).

Systemic infections often influence testicular function even without causing orchitis. Many mechanisms are involved including the effects of fever (including effects of TNF-α & cytokines), weight loss and chronic catabolism and the net effects depend on the severity and duration of the infection. A characteristic example is the testicular dysfunction that is common in AIDS
reflecting the stage of clinical disease and/or its treatment (Raffi et al., 1991). Male infertility usually occurs when the sperm are abnormal and there is a problem in the number of sperm produced. Abnormal sperm occurs when the sperm has a short lifespan or they are malformed which prohibits them from swimming correctly. One or more of the following conditions may cause abnormal sperm:

- Inflammation of the testicles
- Swollen veins in the scrotum
- Abnormally developed testicles

Infections (acute: smallpox, mumps, other viral infections; chronic: TB, leprosy, prostatitis), sexually transmitted diseases, post-infective and systemic illnesses cause inflammation in reproductive tissues. *Neisseria gonorrhoea* colonizes human sperm and *Chlamydia trachomatis* is known to cause epididymitis in men. Leukocytospermia (raised WBC > 1 x10^6 ml in semen) has been associated with infections of the epididymis and accessory sex glands by *Chlamydia trachomatis* and *Ureaplasma urealytium*.

1.2.2. Rat as a model of male infertility

Numerous biochemical, physiological and morphological studies utilizing the rat as animal model system has contributed greatly to an understanding of the events and control of spermatogenesis as the organization of the testis in rats is similar to humans. Where as a few aspects of spermatogenesis are unique in the human, the process in the rat is remarkably similar to that observed in all mammalian species that have been studied, including primates.
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1.3. Role of stress response proteins and inflammatory mediators in testicular pathophysiology

Heat Shock Proteins (HSPs) are usually cytoplasmic. They play important role in proper protein folding and prevention of inappropriate protein aggregation. They are synthesized under different kinds of stress conditions (Ashburner and Bonner, 1979; Subject and Shyy, 1986). Heat shock protein-60 (HSP-60), a member of the chaperonin family, has an essential role in mediating correct folding of nuclear encoded proteins imported into mitochondria. Earlier studies show that HSP-60 was expressed in the germ cells organized into sex cords and in the developing Leydig cells of the testis. In the pubertal testis, Leydig cells showed strongly, spermatogonia and premeiotic spermatocytes showed moderately, and spermatids showed least expression of HSP-60 (Paranko et al., 1996).

The chromatin non-histone high mobility group proteins 1 and 2 (HMG-1 and HMG-2), are 27 and 25 kDa members of a family of proteins containing multiple HMG-boxes, conserved domains of ~80 amino acids which mediate DNA binding of many proteins. HMG box domains recognize DNA structures, such as prebent, supercoiled or four way junction DNA, and non-specific DNA sequences (Bustin and Reeves, 1996). Both HMG-1 and HMG-2 contain an N-terminal HMG box, a central HMG box, and an acidic carboxy terminus. The acidic tails of these proteins contain multiple serine residues which match the phosphorylation consensus sites of casein kinase II, and phosphorylation of this domain appears to be important for proper functioning of these proteins (Wisniewski et al., 1999). HMG-1/-2 have been
shown to facilitate the binding of various sequence-specific transcription factors to their respective DNA binding sites, such as steroid hormone receptors (Boonyaratanakornkit et al., 1998), p53 (Jayaraman et al., 1998), and Oct (Zwilling et al., 1995). In SDS-PAGE, HMG-1 migrates at 29 kDa whereas HMG-2 migrates at 28 kDa. Both HMG-1 and HMG-2 are known to present in the testis and play role in testicular physiology (Bucci et al., 1984). Earlier reports suggest that in testis, the ratio of HMG-2 to HMG-1 increases in parallel with proliferative activity (Seyedin and kistler, 1979). It is also shown that very high levels of HMG-2 in spermatogenesis are associated with non-replicative spermatocytes and spermatids, indicating possible role of HMG-2 in spermatogenesis other than replication (Luke et al., 1984).

Several inflammatory mediators like interleukins (ILs), prostaglandins (PGs), nitric oxide (NO) etc., are produced within the normal testis, where they are believed to be involved in regulating Leydig cell function and spermatogenic development (Je’gou et al., 1995; Hales, 1996).

**Interleukins (ILs)** are a large group of cytokines produced mainly by leukocytes, some are made by polymorphonuclear phagocytes, or by auxiliary cells. They have a variety of functions, but most are involved in directing other immune cells to divide and differentiate. Each IL acts on a specific, limited group of cells that express the correct receptor for that interleukin. There are various interleukins from IL-1 to IL-18. Activated macrophages, endothelial cells, B-cells, and fibroblast cells produce interleukin-1. It induces inflammatory responses, oedema, promotes the production of prostaglandins, IL-2, and the growth of leukocytes. IL-1 also augments corticosteroid release,
induces fever and shivering – useful responses, because elevated body temperature reduces bacterial growth. One of the major paracrine regulatory system in the testis is the interleukin-1 system, which is a family of polypeptides with a wide range of biological activities produced after infection, injury or antigenic challenge (Cavaillon 1996; Dinarello 1991). In testis, IL-1 and IL-6 are known to elicit a broad range of cellular responses, including regulation of germ cell division and differentiation. The autocrine and paracrine mechanisms regulating testicular function in vivo lead to the formation of individuals and perpetuation of the species.

Apart from Leydig cells, the rat testicular interstitial tissue contains a large population of resident macrophages, lymphocytes, and subcapsular mast cells (Hedger, 1997). In some species, including humans, the number of macrophages in particular is quite substantial, representing the second most numerous cell type in the interstitial tissue after the Leydig cells (Miller, 1982; El-Demiry et al., 1987; Pöllänen and Niemi, 1987). Expression of the inducible isoform of nitric oxide synthase (iNOS) is a sensitive and specific marker of macrophage activation (Peng et al., 1998). Recent studies have shown that iNOS is expressed constitutively in the testis in a stage-specific manner in spermatocytes and Sertoli cells, and most prominently, in Leydig cells (O’Bryan et al., 2000a). Nitric oxide (NO), the product of iNOS, is implicated as a crucial regulator in inflammation and influences various signaling pathways (Droge, 2002; Grisham et al., 1999; Davis et al., 2001) and elicits diversified biological effects based on the local concentration. When the cellular concentration of NO is higher than 1 mM, the predominant NO-
mediated effects include DNA deamination, oxidation, or nitration via interaction of NO with either oxygen or superoxide radicals. Thus, the effects of NO are mostly detrimental at high concentrations (Handy et al., 2001).

Prostaglandins (PGs) are potent, evanescent mediators of both inflammation and thrombosis (Fitzgerald, 1992; Dubois et al., 1998). Their formation from arachidonic acid is catalyzed by the enzyme PGG/H synthase, colloquially known as cyclooxygenase (COX) (Smith, 1992). Mammalian cells contain two isoforms of COX (Funk et al., 1991; Smith et al., 2000). They are structurally homologous and have similar kinetic properties, but they are differentially regulated (Jones et al., 1993). COX-1 is expressed in almost all tissues, including platelets, and its PG products are thought to mediate physiological responses, such as vascular homeostasis and gastro-protection, thus plays role in house keeping functions. COX-2, although often undetectable in resting cells, is readily induced as an immediate early gene in response to cytokines, growth factors, phorbol esters, and bacterial LPS. COX-2, however, was shown to be constitutively expressed in the testis (Neeraja et al., 2003).

There is growing evidence that arachidonic acid is oxygenated enzymatically in every cell type and that the oxygenated metabolites regulate a variety of pathological and physiological processes including reproduction (Cooke, 1989). Earlier studies showed the metabolism of arachidonic acid in the testis via cyclooxygenase and lipoxygenase pathways (Grossman et al., 1979; 1986). Evidence has been introduced linking the lipoxygenase products and steroidogenesis in Leydig cells (Dix, 1985), thereby supporting that this
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pathway may be a common event in the hormonal control of steroid synthesis (Mele et al., 1997.). Analysis of the microsomal products on TLC revealed PGF₂α (79.5%) as the main product followed by PGE₂ (20.3%) and PGD₂ (0.17%) whereas analysis of lipoxygenase pathway products revealed the presence of 12-HPETE as the major product both in cytosol and in microsomes. Besides this, 15- and 5-HPETEs were also observed in substantial quantities (Reddy et al., 1992). The metabolites were shown to be the intratesticular factors regulating androgen production (Reddy et al., 1993).

The cyclooxygenated and lipoxygenated metabolites of arachidonic acid (AA) play a diverse modulatory role on testicular steroidogenesis (Romanelli et al., 1995). Mouse spermatozoa can synthesize PGE₂ and 5-HETE in the presence of AA in vitro and that NO is involved in the production of AA metabolites in the male gamete (Herrero et al., 1995).
1.4. Role of reactive oxygen species and oxidative stress in the pathophysiology of testis

Various earlier studies show that there is direct relationship between oxidative stress and male infertility (Aitken, 1994; Sikka et al, 1995; Sharma and Agarwal, 1996). Free radicals have one or more unpaired electrons with unpaired spin in their outer orbital, there by possess increased reactivity with other molecules. In order to overcome this state of unpaired electron, these products participate in hydrogen abstraction, bond scission, radical addition and annihilation reactions. Therefore they oxidize lipids in membranes, aminoacids in proteins and carbohydrates, damage nucleic acids and depolymerize hyaluronic acid. Due to prevalence of oxygen in biological systems oxygen centered radicals called “reactive oxygen species” (ROS) are the most common type found (Table. 1).

<table>
<thead>
<tr>
<th>Table 1. Mediators of Oxidative Stress</th>
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<tr>
<td><strong>Reactive Oxygen Species</strong></td>
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<tr>
<td>Free radicals</td>
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<tr>
<td>Hydroxyl radical (HO·)</td>
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<tr>
<td>Superoxide radical (O₂⁻)</td>
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<tr>
<td>Nonradicals</td>
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<tr>
<td>Hydrogen peroxide (H₂O₂)</td>
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<td>Singlet oxygen (¹O₂)</td>
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<td>Lipid peroxidation products</td>
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<td>Peroxyl radical (ROO·)</td>
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<td>Alkoxy radical (RO·)</td>
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<td>Secondary Products</td>
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<tr>
<td>Malondialdehyde</td>
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<td>4-Hydroxyalkenals</td>
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Thus, the term reactive oxidants or ROS refers to all free radicals or activated oxygen species, which may cause oxidative injury. Though ROS production is essential to normal function or metabolism of most mammalian cells, they are destructive unless tightly controlled (Fig. 7).

![Formation of free radicals](image)

**Fig. 7. Formation of free radicals**

ROS-generating processes were found to be key components in processes such as inflammation, ischemia-reperfusion injury, ageing and carcinogenesis (Halliwell and Gutteritz, 1989; Sies, 1997; Fuchs et al., 1997). At low concentrations, ROS have biopositive effects and act selectively. They are intermediates in the metabolism of prostanoids (Lands, 1985), in the regulation of vasotonus (Ignarro, 1990), in gene regulation, e.g., activation of nuclear factor kappa B (NF-κB) (Schreck et al., 1992), in the regulation of cellular growth, and in the function of intra- as well as intercellular signaling.
and other types of signal transduction (Saran and Bors, 1989; Joseph and Cutler, 1994). Furthermore they are involved in antimicrobial defense and immunological surveillance, i.e., neutrophil oxidative burst and macrophage cytotoxicity (Test and Weiss 1986; Klebanoff, 1992).

An imbalance of oxidants and antioxidants infavor of the former (Sies, 1997), the occurrence of peroxidation products (Spiteller, 1993), and subsequent pathological sequelae (Janssen et al., 1993) is termed oxidative stress. Mammalian cells have developed a battery of defenses to prevent and repair the injuries caused by oxidative stress. The antioxidant defense includes both enzymatic and nonenzymatic ways. The nonenzymatic antioxidant defense includes, water soluble compounds such as ascorbate (mainly extracellularly) and glutathione (intracellularly) (Reed, 1990; ochsendorf, 1998), the lipid soluble, membrane-bound antioxidants tocopherol and ubiquinol/ubiquinone. The enzymatic defense includes superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase and systems that prevent formation or metabolism of prooxidants, such as NADPH ubiquinone reductase and glucose 6-phosphate dehydrogenase (Kehrer and Lund, 1994). Earlier studies have demonstrated clearly that these enzymes are important components of germ cell defensive machinery (Aravinda et al., 1995; Gopalakrishna and Shaha, 1998; Peltola et al., 1991; Bauche et al., 1994; Mruk et al., 2002).

The principle modes of action may be divided into three ways. First, antioxidants may directly scavenge the oxidants produced (prevention). Secondary reactions interfere with processes already initiated (interception).
Examples are interruption of already occurring chain reactions, such as lipid peroxidation by tocopherol. Antioxidants are effective in protecting reproductive tissues below a threshold level of ROS (Ochsendorf et al., 1997). If steady-state free radical concentration exceeds this threshold this will lead to autocatalytic cell injury. The third line of defense, cell renewal, takes place, after ROS produced locally would lead to oxidative injury.

An early and effective mechanism of killing of microbes in the male genital tract is the oxidative burst of polymorphonuclear leukocytes and macrophages and they play major role in male infertility (Roos, 1991; Wolff 1995). Significantly elevated superoxide generation was found in patients with sperm cultures positive for aerobic bacteria (Mazzilli et al., 1994). Leukocytes were identified to be main producers of ROS in semen (Zalata et al., 1998). The nitric oxide and superoxide anions produced by neutrophils react to form highly reactive peroxynitrite ion.

Infections are known to cause induction of reactive oxygen species. *Rickettsia rickettsii* infections led to an intracellular increase of ROS production within 5 hours. The increase of intracellular hydrogen peroxide was demonstrable before rickettsial multiplication and ultrastructural manifestations of cell injury (Hong et al., 1998). The enterocyte damage after *Salmonella typhumurium* infections was accompanied by an increased ROS production (Mehta et al., 1998). The same held true for infections with *Shigella* (Kaur et al 1998), *Helicobacter pylori* (Bagchi et al 1996) and *Entameoba histolytica* (Munoz-Sanchez et al., 1997), and with cyanobacteria toxicity (Ding et al., 1998).
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In different cell types ROS can increase or decrease the proliferation rate, induce apoptosis or necrosis, modulate gene expression and act to stimulate or inhibit several well-characterized cell-signaling components. In the testis developing germ cells, after undergoing complex cellular changes, must migrate progressively from the basal to the adluminal compartment of the seminiferous epithelium where elongated spermatids are eventually released into the tubular lumen at spermiation. This process requires extensive tissue restructuring in the seminiferous epithelium, resulting in the production of reactive oxygen species (ROS) and nitrogen species (RNS) such as superoxide ($O_2^-$), hydroxyl ($OH^-$), peroxyl ($RO_2^-$), hydroperoxyl ($HO_2^-$), nitric oxide ($NO^-$) radicals. If left unchecked, these radicals can induce cell damage and eventually death (necrosis). Germ cells in comparison to somatic cells are more susceptible to oxidative stress due to intimate association of germ cells with free radical generating phagocytic Sertoli cells (Bauche et al., 1994). Germ cell plasma membrane consists of unusually higher concentrations of polyunsaturated fatty acids that are vulnerable to oxidation by free radicals (Beckman and Coniglio, 1979). Docosahexenoic acid found at high concentration in sperm plasma membrane is prone to oxidation (Jones et al., 1979; Storey 1997). Consequent to the increase in these free radicals, increased lipid peroxidation can give rise to lipid hydroperoxides, lipid alkyl and peroxyl radicals and enals, which have various biological implications (Halliwell and Gutteridge., 1989).

The effect of ROS on the mammalian testis has not received much attention and there is paucity of data with regard to the prooxidant effects of
bacterial lipopolysaccharide during infection. This assumes high relevance, since an elevated generation of ROS in testicular compartment can lead to alterations in tissue physiology. Increasing knowledge of the mechanisms whereby ROS and endogenous antioxidant systems influence reproductive processes can assist to optimize the application of exogenous antioxidants to fertility treatment.

1.5. Role of cell death (Apoptosis/Necrosis) in testicular pathophysiology

Cell injury may be reversible (sublethal) or irreversible (lethal). Many causes may result in reversible injury initially. If cell is severly injured, the cell may be unable to recover and cell death follows either by apoptosis or necrosis (Fig. 8).
The mechanisms of cell injury include: a) cell membrane damage by complement-mediated lysis via the membrane attack complex (MAC), bacterial toxins, free radicals; b) Mitochondrial damage leading to inadequate aerobic respiration; c) ribosomal damage leading to altered protein synthesis; d) nuclear damage. Free radicals can injure cells by generating chain reactions, producing further free radicals, which cause cell membrane damage by cross-linking of proteins and by critical alterations of lipids. Failure of ATP synthesis (usually because of hypoxia) can result in the failure of ion transport mechanisms (‘membrane pumps’). Consequently, there is a rise in intracellular calcium and sodium ions and a reduction in intracellular potassium ions. If the endoplasmic reticulum is damaged, sequestered calcium is released resulting in a further increase in intracellular calcium that causes activation of endonucleases that damage DNA. It can also activate proteases and phospholipases causing further damage to cell cytoskeleton and membranes, and thus contribute to necrosis.

Apoptosis known as programmed cell death, occurs in normal tissues as a means of regulating the number of cells in a tissue or organ. It is also seen during embryonic development and various pathological processes. The process of apoptosis is associated with well-defined morphological and biochemical changes, including a reduction in cell volume, blebbing of the cell membrane, chromatin condensation and margination, and formation of apoptotic bodies. Apoptosis is involved in both physiological as well as pathological conditions. Examples of physiological apoptosis include embryogenesis, menstrual cycle (endometrial cell loss), immune cell
development (deletion of T cells that may react with the body’s own tissues) and those of pathological apoptosis include tumours (the balance between apoptosis and cell proliferation is disturbed in neoplasia), atrophy (cell loss in atrophic tissues is by apoptosis), viral illness (during hepatitis individual hepatocytes can be seen in apoptotic forms) and AIDS (loss of lymphocytes is by apoptosis).

Necrosis involves huge inflammation in the tissue. In contrast to apoptosis, necrosis (sometimes called oncosis) is a passive process that does not require energy expenditure by the cell and occurs in response to a wide variety of noxious agents. Necrosis does not occur in a developmental context, usually affects a group of contiguous cells, and is characterized by swelling of the cell and its organelles (as a result of ion pump failure) and results ultimately in membrane rupture. This process involves release of hydrolytic enzymes from damaged lysosomes resulting in digestion and denaturation of cellular proteins and cell lysis.

Germ cell death has long been recognized as a significant feature of mammalian spermatogenesis (Russel et al., 1990). In adult rat testis this loss is incurred mostly during spermatogonial development (75%) and to a lesser extent during maturation divisions of spermatocytes and spermatid development (Huckins, 1978). Studies in humans have demonstrated that both spontaneous (Sinha Hikim et al., 1998) and increased germ cell death in conditions of abnormal spermatogenesis involve apoptosis and implicate a prominent role of programmed germ cell death in male fertility (Dunkel et al., 1997; Lin et al., 1997).
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The members of caspase family play key role in apoptosis (Henkart, 1996). Caspases are synthesized as inactive proenzymes and then activated following cleavage at specific aspartate sites (Alnemri, 1997; Woolveridge et al., 1998). As caspases themselves can cleave a carboxy-terminal to an aspartate residue they have the inherent capacity to activate each other. The Initiator caspases activate other caspases called executioner caspases. The executioner caspases are then involved in the cleavage of a set of proteins, including poly-(ADP) ribose polymerase (PARP), lamin, actin, and gelsolin, and causes morphological changes to the cell and nucleus typical of apoptosis. Two major pathways, intrinsic and extrinsic are involved in the process of caspase activation and apoptosis in mammalian cells (Green, 2000; Hengartner, 2000) (Fig. 9).

![Fig. 9. Apoptotic pathways](image-url)
The intrinsic pathway of apoptosis involves the release of cytochrome c into the cytosol where it binds to apoptotic protease-activating factor-1 (Apaf-1). Once activated (possibly through oligomerization) by cytochrome c (cyt c), Apaf-1 binds to procaspase-9 via the caspase recruitment domain at the amino terminus in the presence of deoxy-ATP, resulting in activation of the initiator caspase-9 and subsequent proteolytic activation of executioner caspases 3, 6, and 7. Members of Bcl-2 family of proteins play a major role in governing this mitochondria-dependent apoptotic pathway, with proteins such as Bax functioning as inducer and proteins such as Bcl-2 as suppressors of cell death (Adams and Cory, 1998). The tumor suppressor protein p53 functions as a transcription factor, thereby up-regulating the transcription of pro-apoptotic genes such as bax, and possibly repressing the transcription of survival genes such as bcl-2 (Miyashita et al., 1994).

The extrinsic pathway of apoptosis involves ligation of the death receptor (such as Fas) to its ligand (FasL). Binding of Fas L to Fas induces trimerization of Fas receptors, which recruit Fas-associated death domain (FADD) through shared death domains. FADD also contains a death effector domain in its N-terminal region. The Fas/FADD complex then binds to the initiator caspase-8 or 10 through interactions between the death effector domain of the FADD. Caspase 8 or 10 then activates the effector or executioner caspases 3 and 7, resulting in cellular disassembly. Cross talk between these pathways occurs at multiple levels. A third subcellular compartment, the endoplasmic reticulum, has also shown to be involved in apoptosis (Nakagawa et al., 2000). Both pathways converge on caspase-3 and
other executioner caspases and nucleases that drive the terminal events of programmed cell death.

Poly (ADP) ribose polymerase (PARP) is a nuclear enzyme responsible for the poly(ADP) ribosylation of chromosomal proteins and nuclear enzymes (Ohashi et al., 1983). The formation of DNA strand breaks during apoptosis is a potent stimulus for PARP activation (Ferro and Olivera, 1982). The induction of PARP may be an attempt by the dying cell to repair DNA damage caused by nuclease activation (Ohashi et al., 1983). PARP is a substrate for the caspases (Lazebnik et al., 1994), in particular for one family member, caspase-3 (Tewari et al., 1995).

However, the mechanisms by which these proapoptotic stimuli activate germ cell death are not well understood during testicular inflammation. Understanding the molecular components of apoptotic program in testicular cells during testicular inflammation is an essential step toward the development of novel therapeutic regimens to control accelerated cell death during abnormal spermatogenesis due to testicular inflammation during localized/gonadal or systemic infections.