Review Article

Oxidative Stress on Male Reproductive Toxicity

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ABSTRACT

Globally Harmonized System defines *Reproductive Toxicity* as "an adverse effects (of chemicals) on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring." Several toxicity assessment studies have been performed taking into account the action of toxicants on reproductive activity. Testis is the main organ of male reproductive system and spermatogenesis by itself is a highly complex differentiation process in which degeneration of spermatogenesis is an integral part of normal sperm synthesis. However, spermatogonial degeneration can also occur on exposure to toxic chemicals. This article highlights the role of environment toxicants in male infertility.

Keywords: Infertility, toxicants, antioxidants, spermatogenesis.

INTRODUCTION

The environmental toxicants and even clinically useful drugs are found to cause severe cellular damage in different organs of the body by metabolically activating the production of highly reactive substances such as free radicals.[1] Many research works have been performed to access the role of toxicants on reproductive activity. Testis is the main organ of male reproductive system where normal sperm synthesis occurs.[2] Normal sperm synthesis can be hindered either by direct interaction of toxicants on the cells within the testis or indirectly by acting on hormones involved in sperm synthesis. In view of the fact that Spermatozoa has got high amount of polyunsaturated fatty acid in its membrane, it is greatly susceptible to oxidative stress which also happens due to the intracellular deficiency of antioxidant enzymes.[3] Normally spermatozoa are equipped with antioxidant defense mechanism so as to quench ROS, thereby protecting gonadal cells and mature sperm from oxidative damage. When reactive oxygen species (ROS) level exceeds, it attacks polyunsaturated fatty acids in sperm plasma membrane leading to lipid peroxidation and induce oxidative stress. Low ROS levels are required so as to regulate the principal functions of sperm such as capacitation, acrosome reaction and fertilizing ability.[4]

Spermatogenesis and Hormonal Regulations

Testes are the main organ in male reproductive system involved in carrying out the function of spermatogenesis and androgen production, primarily testosterone. It happens in two compartments within the testis; seminiferous tubules and interstitium respectively. Spermatogenesis includes all the processes involved in production of spermatozoa from male primordial germ cells by means of mitosis and meiosis. Steroidogenesis refers to production of male steroid hormones. Seminiferous tubules and interstitium is anatomically divided but are functionally connected to each other which is necessary for normal germ cell production. The functions of testes and its compartments are governed by hypothalamus and pituitary gland by means of endocrine hormones. Seminiferous tubule contains sertoli cells and germ cells at different stages of development indicating the respective mitotic or meiotic process and the spermatid development. Sertoli cells support growing germ cells by providing nutrients. Interstitium is populated with androgen producing Leydig cells, in addition to vascular smooth muscle cells, macrophages and endothelial cells.[5]

Spermatogenic cycle starts with division of stem cells and ends with formation of mature sperm. The germ cells formed are found at different levels from base of the tubule to the lumen. Spermatogenic cell types formed in successive stages of cycle includes spermatogonia, primary spermatocyte, secondary spermatocyte, spermatids and spermatozoa.[6] Figure 1 shows different stages of spermatogenesis. Spermatogonium divides mitotically and matures into primary spermatocyte. Meiosis I produces two haploid secondary spermatocyte. Meiosis II produces four haploid germ cells, round spermatids. Round spermatids undergo differentiation to form elongated spermatids which is released into the lumen. The entire spermatogenic process can be divided into four different phases. The first phase is spermatogoniogenesis – Mitotic proliferation and differentiation of diploid germ cells. In second phase meiotic division of tetraploid germ cells into haploid germ cells (spermatids) occurs, followed by Spermiogenesis - Transformation of spermatid into elongated spermatozoa and Spermiation – Release of spermatozoa from germinal epithelium into tubular lumen. Meiotic process is a critical event in gametogenesis during which recombination of genetic material, reduction of chromosome number and

development of spermatids takes place. The number of stages of spermatogenesis depends on morphological criteria. For rats it is 14 stages and for humans it is in 12 steps. The duration of one spermatogenic cycle lasts within 8- 17 days. For the development of a mature sperm, atleast four cycles are necessary. Overall duration is calculated as 50 days for rats and 64 for humans.[7]

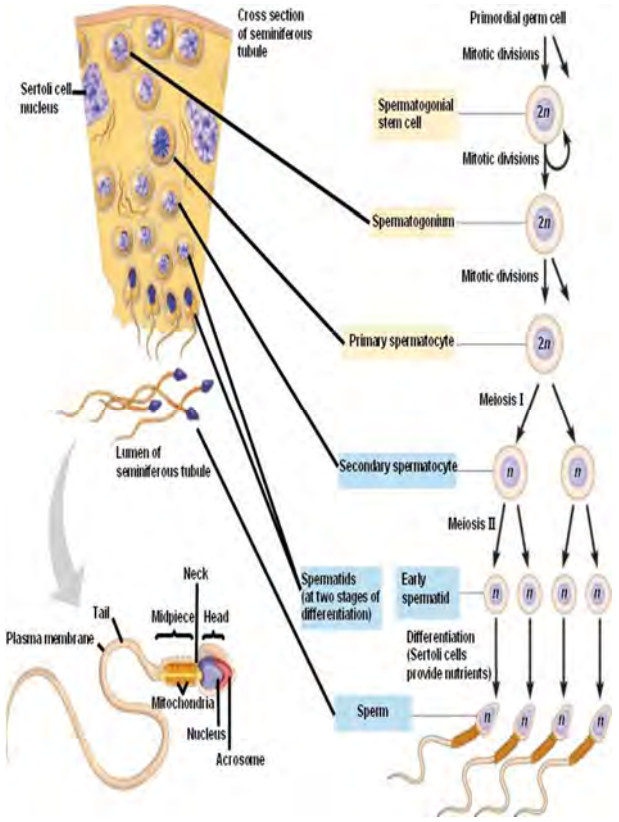

Figure 1: Process of spermatogenesis (Weinbauer)

Leutinizing hormone (LH), Testosterone and Follicle stimulating hormone (FSH) are the prime endocrine regulators of spermatogenesis. LH binds to receptor on leydig cells and stimulates testosterone secretion and synthesis. FSH supports sertoli cell functioning and thereby governs spermatogenesis at different stages of maturation. Reduction in availability of these hormones or testosterone results in stage specific degeneration of germ cells present in testis accompanied by significant rise in apoptosis.[5]

LH and FSH secretion and in turn testosterone production is controlled by hypothalamus and pituitary and a feedback system that exists between these hypothalamic pituitary gonadal axes. Figure 2 shows hypothalamic pituitary gonadal axes and the negative feedback loop present. It depicts gonadal stimulation for LH secretion which in turn acts on leydig cells resulting in stimulating the synthesis and secretion of testosterone. FSH released from anterior pituitary acts on sertoli cells which helps in supporting different stages of cycle. Inhibin functions to suppress FSH secretion. Testosterone sends negative signal to hypothalamus and anterior pituitary and controls hormone levels in normal limits. Therefore impaired hormone levels can lead to spermatogenesis inhibition and can effect tubular action which in turn can result in low number of functional sperms and reduced fertility.[8]

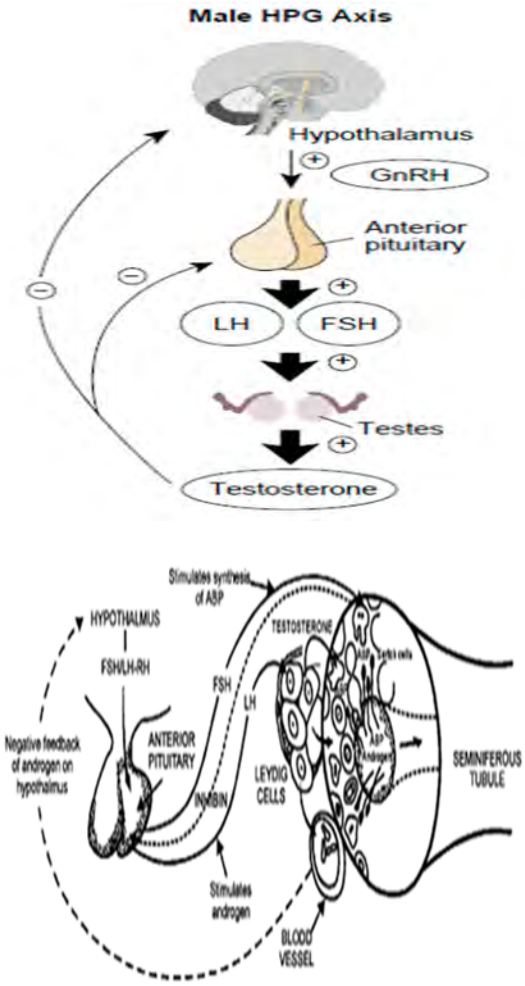

Figure 2: Feedback system between Hypothalamic pituitary gonadal axes

Oxidative Stress and Male Fertility

Oxidative stress is a condition due to imbalance between reactive oxygen species (ROS) and biological system's ability (antioxidant defense system) to readily detoxify the reactive intermediates formed or to repair the damage caused by toxicant.[9,10] ROS or free radicals are oxidizing agents containing one or more unpaired electrons which are produced as by products during normal enzymatic reactions of inter or intracellular signaling. Cellular damages are induced following transfer of these unpaired electrons on to nearby cell structures. For a healthy subject pro oxidants and antioxidants remain in balance. Oxidative stress can result in damage of intracellular macromolecules like glutathione, DNA, RNA, protein, lipid and ATP. Marked changes in the levels of these substance is indicative of extensive cell damage and death.[11,12] Oxidative stress is of great concern because it has resulted in poor fertilization, poor embryonic development, pregnancy loss, and birth defects like autism and childhood cancer.[13,14]

Spermatozoa contain antioxidant defense mechanisms in it thereby protecting gonadal cells and mature spermatozoa from oxidative damage by means of overcoming ROS action. ROS in one way plays a major role in sperm physiology which includes processes like sperm maturation and capacitation whereas on the other hand excessive ROS production alters normal sperm functioning. Therefore proper balance between ROS production and its removal is essential for spermatogenesis. Excess seminal ROS causes male infertility.[15] Spermatozoal membranes are rich in polyunsaturated fatty acids and are sensitive to oxygen induced damaged mediated by lipid peroxidation, making it highly susceptible to ROS attack bringing about deleterious effects on sperm physiology and functioning.[16] In some conditions, oxidative stress induced damage can be repaired. In the case of spermatozoa, damage caused cannot be mend as they lack necessary cytoplasmic-enzyme repair system making it potentially susceptible to oxidative damage.[17] The extend of oxidative damage can be detected quantitatively by measuring Malondialdehyde (MDA) levels, one of the final product from lipid peroxidation. Increased MDA levels are seen associated with decreased sperm motility.[18] Excess free radical generation results in defective spermiogenesis leading to the release of spermatozoa exhibiting abnormally high levels of cytoplasmic retention.[3] ROS generation can happen through two ways: 1) Nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) system at the level of sperm plasma membrane or 2) Nicotinamide adenine dinucleotide dependent (NADP) oxido reductase reaction at mitochondrial level. The latter mechanism seems to be the main source for ROS since spermatozoa is rich in mitochondria and it requires constant energy source for its movement.[19] Therefore the presence of dysfunctional spermatozoa can be an indicator for production of ROS. Figure 3 summarizes the relationship between oxidative stress inducing agents and its impact on male fertility.

Seminal plasma and sperm is provided with an array of antioxidant enzymes acting as free radical scavengers which includes glutathione peroxidase, glutathione reductase, glutathione S transferase, superoxide dismutase, Catalase, low molecular weight antioxidants, vitamin E and vitamin C etc.[20] Antioxidant defense mechanism is of great importance because peroxidative damage caused by free radicals is considered to be the most important cause for impaired testicular function.

Antioxidants in general are compounds that dispose, suppress or scavenge ROS formed. Superoxide dismutase is considered to be the first line of defense against free radical action. It spontaneously catalyses dismutation of superoxide anions (O^{-}_{2}·) to O_2 and H_2O_2 which is further acted upon by catalase to form O_2 and H_2O. Catalase hence has a potential role in ageing process and controlling oxidative stress that results from H_2O_2.[16] Glutathione peroxidase is non-specific for H_2O_2 and catalyses metabolism of substrates varying from H_2O_2 to organic hydroperoxides. It has got glutathione as electron donor which helps in removing peroxyl (ROO) radicals from its native form. Glutathione reductase, member of pyridine nucleotide disulfide oxidoreductase family of flavor enzymes can directly act as antioxidant enzyme to inhibit sperm lipid peroxidation. It helps in catalyzing the conversion of glutathione disulphide to glutathione in the presence of NADPH. Glutathione S transferase helps in conjugating glutathione to electrophiles thereby protecting cellular constituents from oxidative damage.[21]

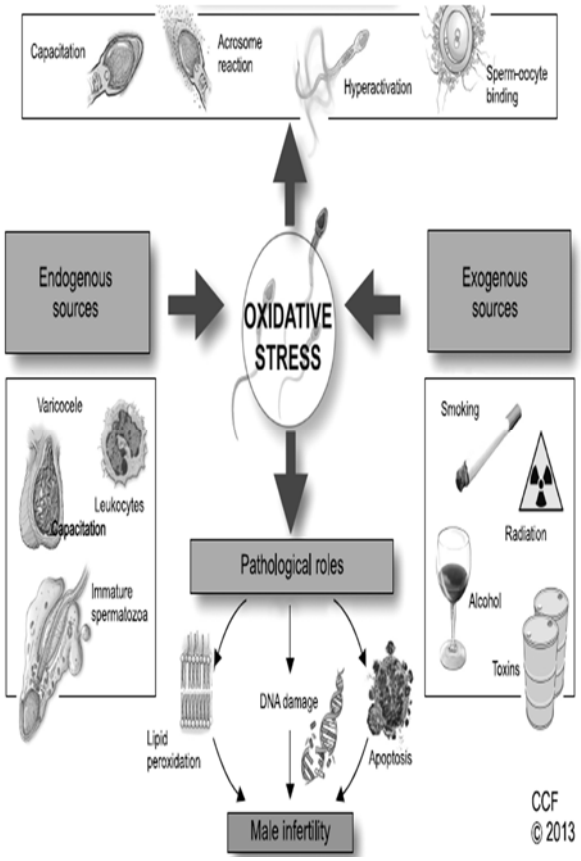

Figure 3: Source of oxidative stress and its impact on male fertility

Glutathione is the most abundant non protein thiol found in mammalian cells. It plays a major role in biological processes like protein and DNA synthesis, amino acid transport. However, it's most important role is supposed to be protecting cells from oxidation and controls male fertility.[22] It participates directly or in conjugation with GPx in neutralizing free radicals formed. It also helps in maintaining Vit C and E in their reduced (active) form. Vitamin E is a chain breaking antioxidant that is found in sperm cell membrane. It neutralizes H_2O_2 and reduces free radicals thereby breaking the chain of peroxide formation and protecting the membrane from oxidative damage.[23] It also improves the activity of other scavenging oxidants and helps in preserving sperm motility and morphology.[24,25] Vitamin C is another chain breaking antioxidant that reacts with OH-, O_2^{-}, and H_2O_2 in the extracellular fluid thereby protecting sperm motility and viability.[23] Henceforth these antioxidant mechanisms are inevitable so as to maintain sperm

motility, rate of hyper activation and ability for acrosome reaction.[26,27]

However an increased ROS activity impairs the sperm functioning resulting in infertility through mechanisms involving induction of peroxidative damage to sperm membrane, DNA damage and apoptosis.

Several studies have been reported depicting the reproductive toxicity effect of some compounds which include Fytolan induced toxicity,[28] Mercuric chloride induced toxicity,[29] Chlorpyrifos induced toxicity, Cypermethrin toxicity,[30] Sodium meta bisulphate toxicity[31] and goes on.

Most of these studies cover the effect of compound on testicular antioxidant activity which is supported by additional evidences.

Hence it is apparent that toxicant action is greatly evident from the antioxidant status which can be used as a confirmative option for free radical induced reproductive toxicity.

CONCLUSION

This brief overview of mechanisms indicates that disturbance in antioxidant enzymes creates adverse effects in the reproductive system. Metals and metal compounds distribution in the environment raises increasing concern for ecotoxicological effects. The major mechanism behind metal toxicity is related to oxidative stress. This stress may potentially affect spermatogenesis, either by affecting genetic integrity or hormone production. Exposure to metals has been long associated with low sperm motility and density, increased morphological anomalies and male infertility.

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