Universal Journal of Environmental Research and Technology
 eISSN 2249 0256
Available Online at: www.environmentaljournal.org
Volume 1, Issue 4: 390-398

Open Access **Review Article**

Effects of Lead Toxicity on Developing Testes in Swiss Mice

Ragini Sharma and Umesh Garu

Environmental and Developmental Toxicology Research Laboratory, Department of Zoology, University College of Science, Mohanlal Sukhadia University, Udaipur

Corresponding author: taurasragini@yahoo.com, umesh_garu@yahoo.com

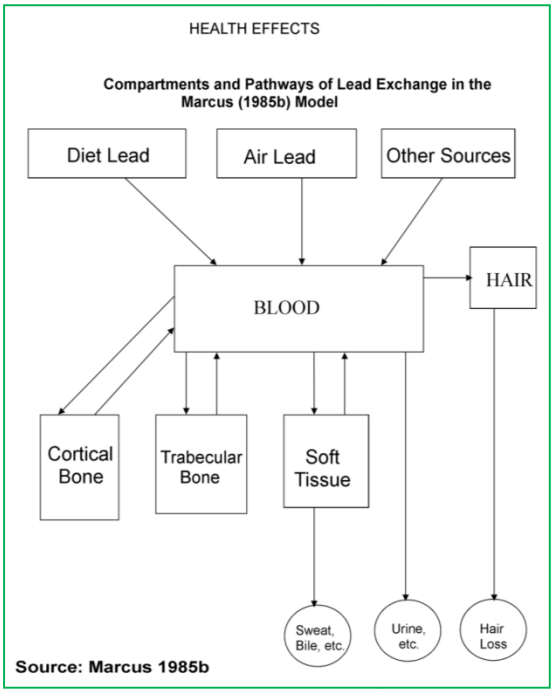
Abstract:

Environmental toxicology studies the effect of environmental toxicants on the health of all organisms and on the different compartments of the environment. Despite the scientific studies carried out over the years, on the toxic effects of lead on development of organism, still there are uncertainties over the reproductive effects of different levels of lead exposure. The reproductive effects of lead are complex and appear to involve multiple pathways, not all of which are fully understood. Reproductive dysfunctions by lead have distinct morphological changes, decrease sperm quality and alter sperm morphology. The effect of different toxicants is registered at different levels of development of the male reproductive tract, thus rendering the analysis of reproductive damage much more complicated. In the animal model, lead has a primary effect on the testes, and acts at all levels of the developing reproductive axis. The present review is undertaken to investigate the chronic effects of lead acetate on development of reproductive system in Swiss albino mice. Previous reported studies shown that lead exposure suppresses the hypothalamic-pituitary-testicular axis, thus alters the histology of testis, the morphology of the spermatozoa and the relationship of cell types in the testes. Mating of lead-treated males with non-treated females confirmed the reduction of fertility in the exposed males.

Keywords: Lead toxicity, Testis, Development, Spermatogenesis, Swiss mice.

1.0 Introduction:

The humans are exposed to various types of environmental contaminants at different stages of their life span, majority of them are harmful. In recent years, there has been growing concern about the deleterious effects of chemical on developing male reproductive system. Exposure of heavy metals during pregnancy has been associated with adverse effects on development of gonads. These substances may act as testicular toxicants and correspond to different compounds, which are related to social habits, life conditions, working hazards or use of drugs and medicines (Bustos-Obregón, 2001; Johnson, *et al.*, 1970; Pomerol & Arrondo, 1994). Although, many studies have reported the toxic and carcinogenic effects of metals in human and animals, it is also well known that these metals form a crucial part in normal biological functioning of cells. Many heavy metals are classical testicular toxicants, though the mechanism of their action may differ. Lead toxicity is known to humanity since ancient times and mentioned in documents left by the Greeks, Romans and Arabs, and even the Egyptians reported by Ahmad, *et al.,* (2003). One of the oldest harmful agents known to mankind is lead. Lead is of public health concern due to their toxic effects on various developing organs, persistence in pregnant and breast feeding mothers. Lead became popular because of its dense, ductile, malleable and corrosion resistant properties. (Florea, *et al.,* 2006). Lead is a ubiquitous environmental and industrial pollutant that has been detected in every facet of environmental and biological systems. Lead is a heavy soft metal, occurs in nature as an oxide or salts. Lead can be found in water pipes, insecticides, lining of equipment where corrosion resistance and pliability are required, in petroleum refining, in construction, bullets of gun, x-ray and atomic radiation protection and is a major industrial by product. Lead appears in homes in many forms as lead piping, lead-containing solders, paints, ceramic glazes, base metal utensils and fixtures. Also, cream powder, lipstick and hair colour have lead. Agricultural soil contamination may be responsible for lead found in many herbal medicines and cigarettes. On the contrary, their detrimental effects on physiological, biochemical, and behavioural dysfunctions have been documented in animals and humans by several investigators (Goyer, *et al.,* 1979; Ruff, *et al.,* 1996).



Marcus model is a classical multicompartmental model of lead uptake and disposition in children and adults. Model shows representation of the model including the movement of lead from exposure media (i. e., intake via inhalation or ingestation) to the lungs and gastrointestinal tract, followed by subsequent exchanges between blood plasma, soft tissue and bone compartments and excretion from liver, kidney and sweat and reproductive tracts.

Experimental animal studies, mainly in rats, have also reported that lead is an active element responsible for male reproductive parameter imbalances (McGivern, *et al.,* 1991; Nathan, *et al.,* 1992). Recent research examining the etiology of lead toxicity-induced hypertension reveals that the free radical production and lowering of inherent antioxidant reserves resulting from lead toxicity. Significant quantities of lead transfers to the developing fetus, however the placenta appeared to greatly limit the passage of lead. These factors range from indirect effects of lead on maternal nutritional or hormonal status before and during pregnancy that could affect parental fertility in both sexes (ATSDR pg no.93). An ongoing prospective study of the effects on child development following prenatal and postnatal lead exposure in the lead smelter town of Port Pirie, South Australia, and its surrounding areas, has provided information on congenital anomalies, length of gestation, birth weight, and stillbirth or miscarriage (McMichael, *et al.,* 1986), and on neurobehavioral development (Baghurst, *et al.,* 1987; Vimpani, *et al.,* 1985). Higher lead levels in milk and blood of infants is due to the transfer of lead via placenta and human milk. A great number of studies demonstrate that gestational and lactational exposure represents a substantial aspect of lead transmission to neonates.

In humans, there is increasing evidence that the birth sex ratio is altered in areas close to industry and exposed to environmental and industrial chemicals. It is well known that Pb influence biological enzyme system and it can be assumed that multiple mechanism of interaction is yet to be elucidated. The regulatory mechanisms of development of male reproductive system are very complex and not fully understood. Sperm counts may have decreased for multiple reasons as the regulation of spermatogenesis remains poorly understood, but is known to involve complex endocrine, intratesticular, and intracellular regulation processes. The stock of gonocytes is determined during fetal development and takes part in determining the number of germ stem cells present in adulthood, since experimentally induced decreases in the number of gonocytes during fetal development lead to decreases in sperm count in adulthood (Moreno, *et al*., 2001).

In rodents testicular necrosis and atrophy demonstrates the effects of lead on adult rat testis. Sokol and Berman (1991) indicated that prepubertal rats may be less sensitive to the toxic effects of lead than rats whose exposure begins after puberty has been initiated. Mehran Dorostghoal, et al., (2010) reported that degrees of reductions in testis volume, seminiferous tubules diameter and germinal epithelium height increase from early weeks to 60 days of age, nearly by the onset of puberty, but decrease afterward, so it seems that lead has transient effects and testicular parameters become better gradually until 120 days of age. One of the mechanisms involved in lead toxicity is the loss of tissue homeostasis by an imbalance between pro and antioxidative factors, which elicits oxidative damage of proteins, lipids and DNA reported by EL-Missiry (2000). Depending on the dose, Pb can enter the tight junctions that form the inter-Sertoli barrier, damaging the epithelium, with a decrease in its height due to germ cell loss, thus increasing the tubular lumen. All these events are caused by an excess of ROS, elicited by Pb toxicity.

In the present article, we have addressed the lead acetate toxicity on the developing male reproductive system. The article is divided into three major parts; 1) Testis, 2) Sperm function, 3) Spermatogenesis. Our review also elucidates the

most likely responsible mechanism of lead on the male reproductive system, as this is still not clearly understood.

2.0 Testis:

Testis is the major organ for male sexual development and fertility (Brennan and Capel, 2004; Wilhelm, et al., 2007). It secretes hormones to promote male-specific traits and produces sperm for reproduction. The key structural components in testes are seminiferous tubules, which provide physical barriers and nutrient supplies for the survival and maturation of sperm. Seminiferous tubules are formed from epithelial-like Sertoli cells, along with associated myofibroblast-like peritubular myoid (PM) cells at the periphery. During testis development, Sertoli cells and PM cells secrete specific extracellular matrix proteins and assemble a layer of basement membrane (BM) (Tung, et al., 1984a), to separate the seminiferous tubules from the interstitial space, where Leydig cells, endothelial cells and other unidentified mesenchymal cells reside.

The major gonads, testis or testicles, begin their development high in the abdominal cavity, near the kidney. During development testis got short after birth, they descend through into the scrotum, a pouch that extends below the abdomen, posterior to the penis. Each testis is an oval structure about 5cm long and 3cm in diameter which outer surrounds by tunica albuginea. There are about 250 lobules in each testis. Each lobule contains 1 to 4 highly coiled seminiferous tubules. Toxicity is manifested in male reproductive system by deposition of lead in testes, epididymis, vas deferens and seminal vesicle. Lead has an adverse effect on sperm count and retarded the activity of live sperm reported by Chowdhury (2009). The potential toxicity of Metals, i.e., lead, cadmium, chromium, selenium and arsenic, caused alteration in sperm morphology, count, motility as well as biochemical disruptions of enzymes and hormones. The rapid industrialization and overgrowing urbanization, the toxic effects of heavy metals on male reproduction system have become a major health concern in the globe (Waldron and Ediing, 1997). Allouche, *et al.,* (2009) found that there were no changes in body weight gain and in absolute or relative weight of testes, epididymis and seminal vesicles in adult albino Wistar male rats that were given 0.0%, 0.025%, 0.05%, 0.1% and 0.3% lead acetate in distilled drinking water for 24 weeks. Results indicate that the statistically significant reductions in testis volume, seminiferous tubule diameter and germinal epithelium height observed in lead treated groups are dose-related and highest at 60 days of age. It has been shown that lead acetate intoxication during spermatogenesis can delay spermiation as well as release of immature spermatogenic cells in the tubules of testes (Corpas, *et al.,* 2002).

(a) Seminiferous Tubules:

The bulk of each testis consists of seminiferous tubules embedded in relatively sparse interstitial tissue. Sperm cells are produced by the tubules. Unlike the tubules in a typical exocrine gland, each seminiferous tubule forms a tightly coiled loop, nearly a meter in length, which opens at both ends into the rete testis. Al-Omar, *et al.,* (2000) reported that lead causes decrease in seminiferous tubules diameter in adult rats. Corpas, *et al.,* (2002) showed that lead acetate causes decrease in the diameter and epithelial thickness of rat seminiferous tubules. Low activity of ATPase and AMPase at the basement membrane of seminiferous tubules was observed in rats exposed to lead at dose of 6mg/kg i.p over a period of 90 days reported by Chowdhury, *et al.,* (1986). Al-Omar, *et al.,* (2000) reported that lead causes decrease in seminiferous tubules diameter in adult rats. Corpas, *et al.,* (2002) showed that lead acetate causes decrease in the diameter and epithelial thickness of rat seminiferous tubules.

(b) Leydig cells:

Leydig cells of the testis are responsible for the biosynthesis and secretion of androgens, critical for developmental and reproductive function in the male. Between the seminal canals lie Leydig's interstitial cells. These are endocrine cells that mainly produce testosterone, the male sexual hormone, and release it into the blood and into the neighbouring tissues. An initial active stage of these cells occurs during the embryonic development of the testis. Later in juvenile life, due to the influence of the LH (luteinizing hormone), Leydig's interstitial cells enter a second, long lasting stage of activity. Testosterone production is directed by LH. The second hormone FSH (follicle-stimulating hormone) affects Sertoli's cells, in that it triggers the formation of a testosterone binding protein.

(c) Sertoli cells:

Testosterone is decisive for spermatogenesis. Any of the elongated cells in the tubules of the testis to which the spermatids become attached; they provide support, protection and, apparently nutrition until the spermatids are transformed into

mature spermatozoa during spermatogenesis, that is Sertoli cells. Sertoli cells control the entry and exit of nutrients, hormones. During the maturation phase of spermiogenesis, the sertoli cells consume the unneeded portions of the spermatozoa. Prospermatogonia of the neonate testis, identified by their morphology and position in the centre of the seminiferous tubule, demonstrated an even lower ability than adult stem cells to colonize recipient testes. The neonatal mouse testis contains '20,000 prospermatogonia (Vergouwen, *et al.,* 1993).The effects of lead on adult rat testis have been widely studied and observations demonstrate that Pb in particular alters those organs, as evidenced by testicular necrosis and atrophy in rodents (McGivern, *et al.,* 1991). Corpas, *et al.,* (2002) reported that lead ingested by experimental pups, caused decrease in mean testis weight on postnatal days 21. Mehran Dorostghoal (2011) study was designed to determine short- and long-term developmental effects of maternal exposure to different doses of lead acetate during lactation on testicular structure in offspring Wistar rats. They have found that testicular parameters decrease in offspring Wistar rats following maternal exposures of lead acetate at doses above 100 mg/kg/day. These dose-related changes suggest that the effects of maternal lead exposure are expressed in offspring. The results revealed that weight of testis in 100 and 300 mg/kg/day doses groups increased significantly during early postnatal development from 14 to 28 days of age. McGivern, *et al.,* (1991) observed a decrease in the intra testicular sperm count in rats prenatally exposed to 0.1% lead acetate supplemented to drinking water and attributed that to the dysfunction of the Sertoli cells as these cells are responsible for the environment of germ cell proliferation and maturation. Corpas, *et al.,* (1995) was found that lead could disturb mitosis of spermatogenic cells and cause alterations in the proliferation of Sertoli cells, therefore an important decrease in the testicular sperm count within testes of adult offspring, and subsequently reduction of epididymal sperm count.

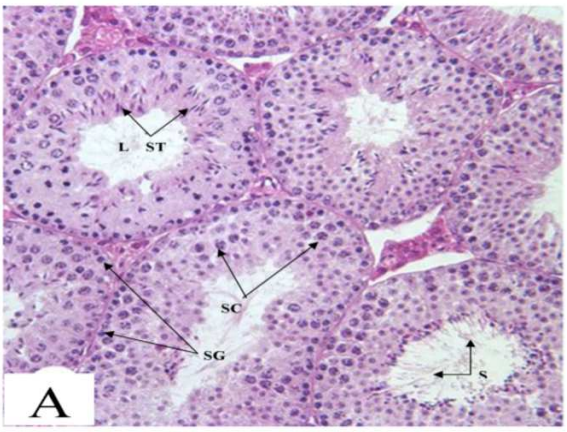


Fig. 2 (A). This slide showing normal developmental stages of spermatogenesis. Magnification X 10. L= Lumen, ST= Spermatid, SC= Spermatocytes, SG= Spermatogonia, S= Sperm.

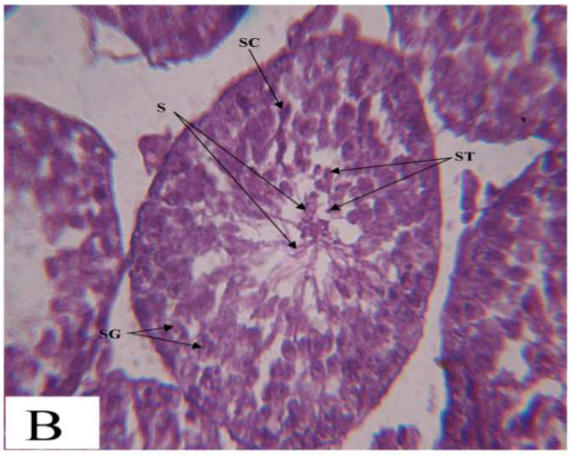


Fig. 2 (B). This slide showing normal developmental stages of spermatogenesis. Magnification X 45. ST= Spermatid, SC= Spermatocytes, SG= Spermatogonia, S= Sperm.

(d) Rete testis:

All of the seminiferous tubules converge onto a network of interconnecting channels, the rete testis, which are lined by a variable cuboidal epithelium. The rete testis in turn leads through numerous small efferent ductules from the mediastinum into the epididymis. It is possible that Pb flows in a retrograde manner via the tubular lumen, after entering the rete testis.

3.0 Sperm Function:

Pinon-Lataillade, *et al.,* (1995) showed that exposure to 0.5% of lead acetate in drinking water from day 1 of intrauterine life until day 60 after birth did not exert significant changes in mice testicular histology or sperm parameters. Acharya and co-workers (2003) described an increase in the number of sperm with abnormal morphology and a decrease in sperm counts in mice after a single intraperitoneal injection of 100 mg lead acetate/kg of body weight (BW). They reported that significant decline in sperm count is due to the genotoxic activity of lead. The lead induced ROS have involved in damaging in the germ cells and involved in gene alteration in germ cells that lead to varieties of abnormal sperm.

In another study, where 10 mg lead acetate/kg bw was administered to rats once a week, for 6 and 9 weeks, a decrease in sperm counts and absolute concentration of motile sperm was described in the latter period (Hsu, *et al.,* 1997). During the later stages of spermatogenesis in mammals, chromatin structure is reorganised and nucleosomal histones are replaced by protamines. An adequate chromatin structure is essential for fertilizing capacity of the sperm cell, and abnormalities in sperm chromatin structure may affect its fertilizing ability (Fuentes-Mascorro, *et al.,* 2000). Hernandez-Ochoa and co-workers (2006) reported that 72% of lead incorporation into the sperm nucleus took place during its development in the testis, and the remaining 28% during its maturation in the epididymis. The decrease in sperm motility can be due to indirect effects of lead, such as increase of ROS generation in sperm cells. The effects of ROS may involve lipid oxidation, in particular, membrane lipids that are required to give the plasma membrane fluidity, which is essential for sperm motility, and structural integrity, and ultimately, for sperm viability (Aitken, *et al*., 1989; Baumber, *et al.,* 2000; Wathes, *et al.,* 2007).

4.0 Spermatogenesis:

An important part of male infertility of unknown etiology may be attributed to various environmental and occupational exposures to toxic substances, such as lead. Spermatogenesis is a complex differentiation process of male germ cells that originates from spermatogonial stem cells leading to the production of a vast number of spermatozoa. Spermatogenesis is initiated in the male testis with the beginning of puberty. This comprises the entire development of the spermatogonia (former primordial germ cells) up to sperm cells. Spermatogonia are the stem cells of the germ cell population. It divided mitotically to produce primary spermatocytes as well as more spermatogonia. These cells are in the first meiosis, during which DNA replicates twice and produce secondary spermatocytes. Secondary spermatocytes divide one more time, without further DNA replication, to produce spermatids. Spermatids relatively large round nuclei and are found at mid-levels within the tubular epithelium. Spermatids are the final product of meiotic division. They are found near the lumen of the tubule and have small round nuclei. Now it does undergo an elaborate process of maturation called Spermiogenesis to become spermatozoa. Spermatozoa are highly specialized, motile cells, each with a single large flagellum and found near the lumen.

Examination of experimental data from both epidemiological and animal research suggests that lead in different concentrations has a wide spectrum of toxicity on the male reproductive system, including spermatogenesis, sperm functional parameters and reproductive hormones. Although unfavourable reproductive effects usually occur at relatively high levels of lead exposure (Teijon, *et al.,* 2006), lower doses for longer time periods may also alter the male reproductive system in a manner similar to that previously reported at higher doses for shorter periods (Sokol, *et al.,* 2002). In our own studies (Garu et al., 2011) we observed that different doses of lead impaired the development of various components of testis. The gestational exposure may lead to significant histopathological and functional disturbances in adult. Various studies suggest an interaction of heavy metal lead with hypothalamic-hypophysis axis that is responsible for controlling the development of testis and spermatogenesis. Lead may also interact directly with sertoli cells and disturbs the production of testosterone.

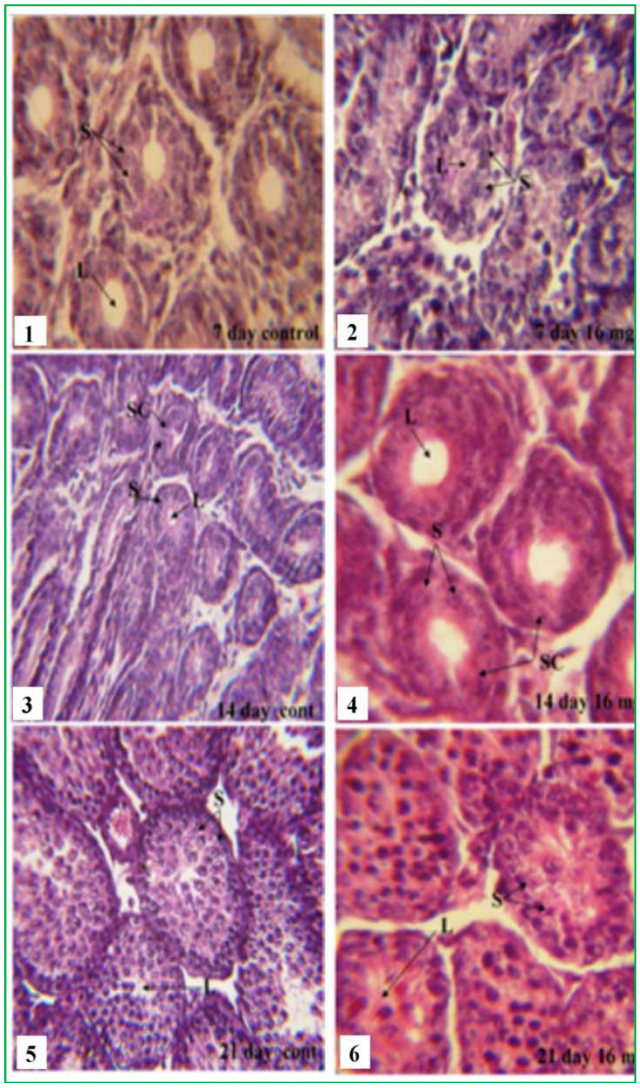


Fig.1. Photomicrograph of control mice testis showing normal development and distribution of seminiferous tubules at PND 7. Eosin and Haematoxylin stain. X10. (S= Spermatogonia, L= Lumen)

Fig.2. Photomicrograph of Lead treated (16mg) mice testis showing altered distribution and development of seminiferous tubules at PND 7. Eosin and Haematoxylin stain. X10. (S= Spermatogonia, L= Lumen)

Fig.3. Photomicrograph of control mice testis showing normal development of spermatocytes and development of seminiferous tubules at PND 14. Eosin and Haematoxylin stain. X10. (S= Spermatogonia, L= Lumen, SC= Spermatocytes)

Fig.4. Photomicrograph of Lead treated (16mg) mice testis showing altered development of spermatocytes and distribution of seminiferous tubules at PND 14. Eosin and Haematoxylin stain. X10. (S= Spermatogonia, L= Lumen, SC= Spermatocytes)

Fig.5. Photomicrograph of transverse section of 21day control mice testis showing normal structure and round shape of seminiferous tubules at PND 21. Spermatogonial cells are well define and small interstitial space in between seminiferous tubules. Eosin and Haematoxylin stain. X10. (S= Spermatogonia, L= Lumen)

Fig.6. Photomicrograph of transverse section of 21day Lead treated (16mg) mice testis showing reduction number in the spermatogonial cells and regular arrangement is disturb in seminiferous tubules. Eosin and Haematoxylin stain. X10. (S= Spermatogonia, L= Lumen)

Histological observation of testicular sections of Pb treated mice reveals germ cell disorganization, epithelial vacuolization and cell loss as described by other authors (Batra, *et al.,* 1998). According to Adhikari, *et al.,* (2001), high doses of Pb elicit apoptosis of germ cells which is a common mechanism of action of many toxicants, including lead and phosphorated pesticides. Garu, *et al.,* (2011) also monitored the developmental effects on testes of male offspring of lead exposed swiss mice during gestation and lactation. The results revealed that lead induced apparent damage and reduction in the number, changes in shape and size of developing seminiferous tubules. Oral exposure of lead acetate changed the arrangement and shape of spermatogonial cells and reduced the number of sertoli cells. It also diminished the development of Leydig cells.

Furthermore, higher percentages of immature and abnormal spermatozoa such as wide, round, and short sperm in lead exposed workers have been reported at both high (40µg/dl) and low (<15µg/dl) blood lead levels (Telisman, *et al.,* 2007). An epidemiological study of the male reproductive system has demonstrated positive correlations between seminal plasma lead and spermatozoa ROS levels (Kiziler, *et al.,* 2007). However, from low to high doses, there are known to be different responses of lead-induced oxidative stress in various target sites, including sperm (Hsu and Guo, 2002). In our recent investigation we have studied the weekly changes in developing testes of lead exposed pups during gestation and lactation. The pregnant mothers were exposed (16mg/animal) from 10^{th} day of gestation to the end of lactation. The pups were selected from each group and sacrificed on PND 1,7,14 and 21. Histopathological changes in lead exposed developing testes were quite noticeable and there was apparent damage in all the basic precursor of the testes in pups. One feature that was common at all stages of developing testes was reduction in thickness of

epithelium and seminiferous tubules diameter. This may be due to the effects of lead on developing spermatogonia and spermatocytes. Many spermatogonial cells disarranged and sertoli cells became shirked and the lumen of tubules obliterated by cell debris. (Fig.1-6)

Additionally, the effect of environmental lead on the male reproductive system has been a major area of concern for several years by which the testicular spermatogenesis and spermatozoa within the epididymis are the major targets for lead action to produce toxicity on reproduction (Wadi and Ahmad, 1999). They reported that the major function of testes is spermatogenesis and hormone synthesis to produce spermatozoa. So when the testicular tissue is damaged by the toxic effects of lead, the process of spermatogenesis would be impaired and sperm production rate will also reduced.

5.0 Conclusions:

The present review demonstrates that when the lead exposed during gestation and lactation, it passes through the placenta of pregnant mice. As the developmental stages are more sensitive to toxins, they create several damages to developing male gonads through different path ways. The pre and postnatal exposure of lead not only disturb the histology of gonads in neonates but also create major alteration in morphology, histology and physiology of gonadal axis in adult male. Lead is capable of reaching embryonic tissues at different period of gestation and disturbs the normal development of testis, which leads to the harmful effects on the process of spermatogenesis at the time of puberty. When embryonic testicular tissues are damaged by lead exposed mothers, the maturation of testis, process of spermatogenesis and reproductive performance would be impaired in adults.

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