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Recent Advances in Heavy Metals Induced Effect on Male Reproductive Function—A Retrospective

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Abstract: Reproductive hazards from metal exposure in males are one of the fastest growing areas of concern in toxicology today. Exposure to different heavy metals causes irreversible toxic insult to male reproductive system. Heavy metals produce cellular impairments at structural and functional level in male reproductive system. The effect of heavy metals, such as lead, mercury, cadmium, chromium and arsenic on male reproduction has been studied in details in various experimental species. But data on humans are steadily building up. Metals could interfere with the gametogenic cells or Leydig cell or spermatozoa directly in semen. These effects may results in reduced fertility or associated with pregnancy wastage, congenital malformation associated with genetic diseases. Moreover, the features of heat stress protein (hsp), Androgen-Binding Protein (ABP), Cadherin and many other stressor protein along with reactive oxygen species (ROS) and neuro-endocrine mechanism are highly affected by these heavy metals exposure. Still the data are inadequate and need confirmation.

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[1-2]. The evidence of the past twenty years have shown disturbing trend in male reproductive health hazards due to careless use of these chemicals which caused detrimental effects on different organs. Therefore, broad-spectrum irreversible toxic actions at cellular and molecular level were observed mainly on reproductive system of human and experimental animals [3-4].

Adverse effect of heavy metals on male reproduction: 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.

Lead: Lead is widely used in acid battery plant refinery, smelter, fuel combustion industry, printing press and automobile exhaust where tetraethyl lead acts as anti-knocking agent. Toxicity is manifested in male reproductive system by deposition of lead in testes, epididymis, vas deferens, seminalvesicle and seminal ejaculate. Lead has an adverse effect on sperm count and retarded the activity of alive sperm. Moreover, motility as well as prolonged latency of sperm melting both in exposed person and experimental animals were observed after lead exposure [5-6].Study with male CF-1 mice indicated the significant decrease in epididymal sperm count at low dose of lead exposure (0.25% via drinking water). Moreover the decreased motility and increased incidence of teratospermia at higher dose of lead exposure (0.50%) along with inhibition of post-meiotic cells mainly pachytene spermatocyte were noted. In the same experiment the detachment of gemminal cell layer from basal membrane, atrophy of Leydig cells plus interstitial edema and low density of seminal plasma were also observed. Experimental studies were conducted to note the potential comparative effects of lead acetate considering different routes of

administration, dosages and durations. The doses of 1 mg/kg, 2 mg/kg, 4 mg/kg and 6 mg/kg lead acetate were administered intraperitonially to rats over a period of 30 days. On the other hand, 0.25, 0.50 and 1.0 g/lit lead acetate were fed to rats over a period of 60 days. Highest dose (1.0 g/lit Pb-A) showed the testicular degeneration along with high deposition of lead in the testicular tissues. Relative toxic manifestation in animal system was confirmed by estimating delta-aminolevulinic acid in urine (ALA-U), which was 1.40 mg/100 ml in comparison to control (0.30 mg/100 ml). Higher dosages of lead through i.p. route (4 mg/kg and 6 mg/kg) indicated similar finding as above. In human study, the semen lead was correspondingly increased with blood lead in the subjects those who were exposed to lead over a period of six years (average) and six hours daily. The sperm analysis from those subjects revealed the morphological abnormalities of sperms (mainly, the tail abnormality) and significant decreased in certain key seminal constituents like, fructose and succinic dehydrogenase which indicated the male reproductive functional impairment in system exposed to lead [7-8].The distribution pattern of lead in the whole male reproductive tract of rats was observed after treatment with lead acetate. Lead may form strong bondage with structural proteins. Morphological impairment along with the inhibition of certain membrane bound enzymes, like, 5′-nucleotidase, ATPase and alkaline phosphatase were also observed in the testicular tissues with high dosages of lead treated rats. Significant lowering of reaction intensity in the localization of testicular Δ^5-3β-hydroxysteroid dehydrogenase along with decrease in testicular ascorbic acid and increase in cholesterol suggested the inhibition of steroidogenesis. Low activity of ATPase and AMPase at the basement membrane of seminiferous tubules was observed in rats exposed to lead at dose of 6 mg/kg i.p over a period of 90 days[6]. The exact reason behind the event is not fully known, but very recently scientists suspected that lead interfered with the enzymes which contain (–SH) group or with the intervening redox systems and tissue respiration. Low fructose content decreased the activity of succinic dehydrogenase and alkaline phosphatase in seminal plasma were observed among the workers of printing press exposed to lead more than 8h/day over a period of 10 years[8]. Moreover, workers occupationally exposed to lead exhibited moderately high blood lead levels associated with sexual disorders like decreased libido that was followed by an increased frequency of atheno, hypo- and teratospermia. Lead adversely affects the testicular tissues and steroidogenic processes either directly or through endocrinological system; but the observations were not very conclusive. Several recommendations could possibly be made out of these observations (i) to note the structural and functional status of pituitary along with testosterone, FSH and LH levels in blood; (ii) to note the detoxifying enzymes status in the testicular tissues after lead treatment in the experimental animals.

Cadmium: Cadmium released from tannery, smelter, battery crushing unit. The action of cadmium is spermatogenic stage specific. High dose of cadmium chloride exposure caused rapid testicular edema, haemorrhage and necrosis. Cadmium exerted deleterious effect on the vascular structure of testis that may be the result of varying degrees of cadmium induced ischemia. Degeneration of testicular tissue after

different doses of cadmium exposure caused rupture of blood vessels [9]. Electron microscopic observation revealed that DNA fragmentation in mouse testicular tissue after cadmium exposure showed a positive effect. Zinc plays an important role in maintenance of structure of superoxide dismutase (SOD), which scavenges the free radicals and maintains appropriate spermatozoal milieu[10-11]. Cadmium replaces Zn^{2+} leading to enzyme structural distortion, which was manifested by reduced activity of SOD. Viability of spermatozoa was also reduced in cadmium exposed groups. Moreover cadmium directly or indirectly targets GSHB-Px which catalysed the destruction of H_2O_2 and lipid hydroperoxides by reduced glutathione (GSH) and protecting the membrane lipids from peroxidative damage in a highly oxidative stress condition. The ultimate result is membrane degeneration of spermatozoa leading to abnormal and dead sperm in semen. Selenium (Se) and vitamin E exhibited protective role against LPX because Se maintained GSH-Px activity in spermatozoa and seminal plasma. High vitamin E concentration in spermatozoa was associated with reduction in its susceptibility to LPX. Metallothione – a low molecular weight metal binding protein, plays an important role in cadmium detoxification. Cadmium showing high bonding with metalothionin so that the metal may no longer be available to produce toxicity.

Chromium : The nephro- and dermatotoxic heavy metal, chromium are widely used in refractory, pigment and stainless steel factory, tannery, welding, engraving and photo processing unit. It caused severe reproductive injury among the exposed persons. Industrial workers exposed to chromium over 25 months for 6h daily showed high metal level in blood and semen. The welding fumes contain high percentage of chromium and so the welders exposed to smoke generated by welding, suffered from an increased risk of reduced semen quality leading to infertility[12]. Experimental observations indicated that different doses of chromium, i.e., 20, 40, 60 mg/kg sodium chromate in rats caused diminution of tubular diameter, nuclear size of testicular cells and reduction in cell population of spermatogenic cells. The degree of damage is directly proportional to the dose applied[13].

Selenium : Selenium has long been known to have a damaging effect on different tissues of several species of animals. On the other hand, selenium is considered to be one of the most efficient protective agent against cadmium induced injury. Initial report from our laboratory showed that selenium dioxide (SeO_2) has got an adverse toxic effect on testis. Among two dosages of SeO_2 (2, 6 and 10 µg/rat daily for 90 days), 10 µg SeO_2/rat (i.p) caused significant testicular degeneration along with Leydig cell atrophy[14].

Mercury : Mercury is widely used in refinery, plastic and paints, antiseptic, scientific instruments, photography, fuel combustion and agriculture field. This metal is spermato, steroido- and fetotoxic agent. Mercury chloride exhibited structural alteration of testicular tissue along with biochemical change. The control testis of albino rat of CF strain showed sharp localization of ACPase, ATPpase, ALKPase in PTM, spermatogeic cell and Leydig cell membrane[15]. Mercury and its compound, methyl mercury chloride, affected these membrane bound hydrolytic enzymes in rats resulted in sharp decrease of these enzymes, co-related with progressive degeneration

of peritubular membrane. Mercury also caused the structural and functional disintegration of these enzymes due to its high affinity towards the enzyme's (SH) group[16]. The prominent feature of Mercury induced toxicity are : (1) depletion and clogging of different spermatogenic cells, (2) presence of pyknotic or karyotectic pachytene nuclei, (3) absence of nuclear chromatin at stage XII in dividing ells, (4) absence of noticeable lumen and (5) presence of vacuolated early elongated spermatid along with dispositioning of acrosome. The intensity of damage is directly proportional to the duration of exposure [17].

Arsenic : Arsenicals are widespread in the environment as a result of natural and anthropogenic occurrence[18]. Male reproductive effect of arsenic was first studied in mice, then in fishes [6]. Arsenic exposure in experimental rats has shown to produce steroidogenic dysfunction leading to impairment of spermatogenesis[19]. Recent publication by Sarkar et al. revealed that arsenic effects mainly the processes of meiosis and post-meiotic stages of spermatogenesis and acute exposure to arsenic causes rapid and extensive disruption of spermatogenesis in mice [20]. Still, the male reproductive toxicity study in relation to arsenic exposure is spare. Therefore, without more experimental investigation, it is difficult to prove the specific mechanism of action of arsenic which affect the male reproductive function.

Molecular basis of metal induced male germinal toxicity: Recent studies on the molecular basis of metal toxicity demonstrated the function of different types of stressor protein after chronic exposure of metals and their effect on sperm DNA, core protein Histone and other proteins that are secreted from sertoli cells and passed through lymphatic channels of the Sertoli cells but the exact reason behind the effects is yet to be known. Damage of Sperm protein :Androgen binding protein (ABP) secreted from sertoli cells pass though the sertoli cells-lymphatic channels, bind with androgens and maintain their activity. Studies revealed that in exposed condition one of the components ABP-heat shock protein 27 (hsp 27) plays an important role during spermatogenesis [21]. This stress protein co-localized with microfilaments in Sertoli cells. N-cadherin and desmoglein are adhesive type of proteins found in Sertoli cells which are retained in seminiferous tubules. They are essential for maintenance of cellular adhesion of seminiferous tubules and exerted their function as signal receiver. The nature of the signal is unknown. A group of scientists of Kent State University, Ohio, USA, detected a kinase type anchoring protein—AKAP 110 in human spermatozoa and round seminiferous tubule were present. This protein binds with regulatory subunit of protein kinase APKA protein in acrosomal region of sperm head which inactivate and proteins alter the structional and functional activity of protein nature after exposure of different types of metal exposure and leads to infertility due to inactivation of sperm.

Conclusion

The above findings from different scientific studies also indicated that the degree of toxic manifestation of different metals depends on dose, duration, route of administration and other physiological factors specially nutrition. Toxic manifestation by different metals varies from species to species (Table 1). The signs and symptoms of metal toxicity depend on the duration of exposure, type of metal, condition of workplace, socio-economic status and history of disease. But extensive

literature study explored that there is a gap of knowledge in the proper toxicity survey. Thus, further efforts should be made to widen our knowledge in this unmapped area of research.

Table 1
Effect of other metal on male reproduction

Metal	Species	Sex	Route of Administration	Duration	Observation
Aluminium	Rat	Male	Subcutaneous (dosage not reported)	30 days	Spermatogenic arrest without Leydig cell damage
Boron	Human	Male	Feeding and inhalation	-	Oligospermia and decreased libido among men working in Boric acid manufacturing factories
Nickel	Rat	Male	Subcutaneous (dosage not reported)	-	Arrest spermatogenesis with testicular damage
	Rat	Male	Fooding Nickel sulphate 25 mg/kg	120 days	Completely infertile
	Human	Male	No report available	-	-
Cadmium	Rat	Male	9 mg/kg (i.p)	6-14 days	Testicular atrophy and cellular degenerations

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