

Impaired hypothalamic-pituitary-testicular axis activity, spermatogenesis, and sperm function promote infertility in males with lead poisoning

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Summary

Lead poisoning is a stealthy threat to human physiological systems as chronic exposure can remain asymptomatic for long periods of time before symptoms manifest. We presently review the biophysical mechanisms of lead poisoning that contribute to male infertility. Environmental and occupational exposure of lead may adversely affect the hypothalamic–pituitary–testicular axis, impairing the induction of spermatogenesis. Dysfunction at the reproductive axis, namely testosterone suppression, is most susceptible and irreversible during pubertal development. Lead poisoning also appears to directly impair the process of spermatogenesis itself as well as sperm function. Spermatogenesis issues may manifest as low sperm count and stem from reproductive axis dysfunction or testicular degeneration. Generation of excessive reactive oxygen species due to lead-associated oxidative stress can potentially affect sperm viability, motility, DNA fragmentation, membrane lipid peroxidation, capacitation, hyperactivation, acrosome reaction, and chemotaxis for sperm-oocyte fusion, all of which can contribute to deter fertilization. Reproductive toxicity has been tested through cross-sectional analysis studies in humans as well as *in vivo* and *in vitro* studies in animals.

Keywords: Blood lead level, Hypothalamic–pituitary–testicular axis, Lead poisoning, Male infertility, Premature acrosome reaction, Reactive oxygen species, Semen analysis, Sperm function, Sperm function test, Spermatogenesis

Introduction

Exposure to heavy metals in the workplace is a key global public health issue despite governmental regulations in environmental emissions. In addition to not naturally degrading, heavy metals form complexes inside cells upon entering the body (Tchounwou *et al.*, 2012), adding to their lasting structural, metabolic, and

DNA toxicity. Of the types of heavy metal poisoning, inorganic lead poisoning (i.e., plumbism) is one the oldest known and most ubiquitous hazards. Lead may enter the body through the intestines, skin, or lungs, with the latter being the most prominent route (Gomes *et al.*, 2015). Common environmental sources include paint chips/dust, soil, air, water, food, as well as households (i.e., pottery and ceramics), folk remedies, cosmetics, and drugs of abuse (Kianoush *et al.*, 2015). Examples of occupational sources include automobile factory workers and mechanics, electronics manufacturers and solderers, and construction workers (Kianoush *et al.*, 2015).

As of November 2015, the Centers for Disease Control and Prevention's Adult Blood Lead Epidemiology and Surveillance programme has designated elevated blood lead levels (BLL) at ≥ 5 $\mu\text{g}/\text{dl}$, a threshold that has been lowered through recent years due to newfound health risks associated with even trace

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amounts of lead (CDC, 2016). The US Occupational Safety and Health Administration (OSHA) Lead Standards make it mandatory for workers to refrain from their occupational setting when BLLs are ≥ 50 $\mu\text{g}/\text{dl}$ (construction industry) or ≥ 60 $\mu\text{g}/\text{dl}$ (general industry), and only return when the BLL is < 40 $\mu\text{g}/\text{dl}$ (CDC, 2016). Greater BLL threatens the body with immune, neuropsychological, cardiovascular, bone, liver, gastrointestinal, or renal damage (Lopes *et al.*, 2016). Although some damage from acute exposure is reversible, long-term exposure can lead to permanent damage in various physiological systems.

Global male fertility rates have declined in recent years with environmental pollution and occupational exposure to lead cited as a potential contributor. By measuring birth rates, a retrospective cohort study demonstrated the decreased fecundity of lead-exposed workers compared with non-lead-exposed workers. The effect was also amplified with longer duration of lead exposure (Lin *et al.*, 1996). Lead toxicants may cause issues with fertility by infiltrating the male reproductive system (Benoff *et al.*, 2000). Lead may also affect hormonal regulation by disrupting the hypothalamic–pituitary–testicular axis or directly affect the testes and sperm at multiple levels. We review the means through which male fertility is interrupted by lead toxicity.

Lead toxicity and the hypothalamic-pituitary-testicular axis

Moderate exposure to lead has been tied to dose-dependent changes in male endocrine functions, as indicated by the effects of lead on the hypothalamic-pituitary-testicular (HPT) axis (Ng *et al.*, 1991). Studies suggest that the hypothalamus is the primary site of the neurotoxic action of lead (Sokol, 1987). A clinical study on boys undergoing sexual development showed that marginally increased BLL may delay puberty and ultimately limit final testicular volume (Staessen *et al.*, 2001). The effect is potentially due to impaired gonadotropin-releasing hormone (GnRH) mRNA expression at low levels of long-term lead exposure (Sokol *et al.*, 2002). Lead may also disturb hypothalamic GnRH release by arresting prostaglandin E_2 synthesis or norepinephrine-stimulated release of prostaglandin E_2 (Sokol *et al.*, 2002). A study looking at moderate occupational exposure to lead found that serum LH decreased with increasing duration of lead exposure (Ng *et al.*, 1991), which would ultimately impact testosterone production (Alexander *et al.*, 1998) in Leydig cells. Lead may act at the hypothalamic–pituitary unit by interfering with the calcium-dependent secondary messenger systems that

regulate LH release from secretory granule storage (Ronis *et al.*, 1996); this disrupts the hormonal feedback loop and serve as an explanation for an increase in stored β -LH (causing vacuolization of gonadotropic cells). Increased LH mRNA and stored LH may also be caused by the interaction between lead and the metal-dependent testosterone receptor at the pituitary unit. At the testicular level, lead impairs testicular LH receptor concentrations and steroidogenesis at the pubertal stage (Ronis *et al.*, 1996). Another animal study with lead exposure during sexual development showed degenerative changes in gonadotropic cells of the pituitary gland, thus accounting for suppressed plasma LH and testosterone levels into adulthood (Ait Hamadouche *et al.*, 2013).

Decreased follicle-stimulating hormone (FSH) levels have also been observed in moderately exposed lead workers (Gustafson *et al.*, 1989), potentially affecting spermatogenesis. In contrast, increased (Ng *et al.*, 1991) or unchanged (Assennato *et al.*, 1987) FSH levels have also been reported. This disparity in reporting could be attributed to differing concentrations of lead, differing duration of exposure, and the physiological state of the reproductive axis and the testes. Metabolic function of Sertoli cells may also be impacted by lead exposure during pubertal development (Nathan *et al.*, 1992), thus impairing spermatogenesis. Sertoli cell dysfunction from high lead exposure have been associated with inhibin B overproduction (Mahmoud *et al.*, 2005), which may account for the decreased FSH levels previously discussed. In contrast, a lead exposure study in the primate model also reported a decreased inhibin/FSH ratio as a cause of Sertoli cell dysfunction. However most evidence, such as the lack of change in Sertoli cell morphology upon lead exposure (Nathan *et al.*, 1992), points to reproductive axis dysfunction rather than Sertoli cell dysfunction in adult cases of lead poisoning.

Along with testosterone suppression, rat studies have shown increased epididymal androgen binding protein levels (Nathan *et al.*, 1992). *In vivo* lead exposure studies on prepubertal rats have depicted decreased activity of 3β -hydroxysteroid dehydrogenase, an enzyme necessary for testosterone synthesis, as well as reduced gonadotropin-receptor binding and associated cyclic AMP production (Wiebe *et al.*, 1983). Impaired feedback response to plasma testosterone (Vigeh *et al.*, 2011), pituitary LH dysregulation (Sokol *et al.*, 1985), and Leydig cell degeneration (Saxena *et al.*, 1986) may also contribute to testosterone imbalance.

Many of the toxic effects we have presently discussed may be ascribed to the lead-induced generation of reactive oxygen species (ROS) (e.g. hydrogen peroxides and superoxides), which causes oxidative stress. ROS halts synthesis of sulfhydryl antioxidants and prevents enzymatic reactions (Vigeh *et al.*, 2011). In

fact, lead itself has high affinity to sulfhydryl residues in proteins, thus acting as a nonspecific enzyme inhibitor and arresting their antioxidant activity (Ait Hamadouche *et al.*, 2013). As oxidative stress disturbs chromatin condensation, it has been suggested that the under-protamination of sperm DNA could automatically lead to DNA fragmentation (Henkel *et al.*, 2010). A study performed on Swiss mice found that lead-induced ROS was associated with a significantly reduced sperm count and increased sperm abnormalities. Intraperitoneal injections of lead acetate, used to induce lead poisoning in the mice, led to an increase in lipid peroxidation potential, an indicator of oxidative stress, in the testes of the study mice as compared with the control group. According to the study, the significant decrease in sperm count may be attributed to lead-induced ROS damage on the polyunsaturated fatty acids of cell membranes of germ cells, and subsequently the spermatozoa and mature sperms (Mishra & Acharya, 2004). A computer-assisted semen analysis demonstrated that excessive ROS in semen damaged sperm concentration, motility, and other sperm motion parameters (Takeshima *et al.*, 2016).

Brain catalase activity was found to be significantly decreased in a rat study looking at lead-induced HPT dysfunction; this will ultimately permit oxidative damage in cells (Ait Hamadouche *et al.*, 2013). Increased IL-7, IL-10, IL-12, and TNF- α cytokine levels have been found to accompany lead-related oxidative stress, particularly in semen (Kasperczyk *et al.*, 2015). ROS may account for decreased serum testosterone through several means.

Lead toxicity and spermatogenesis

At various BLLs, men occupationally exposed to lead have exhibited decreased sperm concentration density per unit of seminal fluid, oligospermia, hypospermia, as well as teratozoospermia (e.g. widened, rounded, or shortened) and immature sperm (Vigeh *et al.*, 2011). To circumvent the ethical dilemma of testing in humans, animal experimentation has demonstrated lead toxicity may account for decreased weight of testes, seminal vesicles, epididymis, and ventral prostate (Ronis *et al.*, 1996); this may be paralleled with chronic lead exposure affecting accessory gland development beginning in childhood. Animal studies have further established the effect of lead on aberrant testicular tissue morphology (Ait Hamadouche *et al.*, 2013; Saxena *et al.*, 1984), testicular germ cell death (Adhikari *et al.*, 2001), interstitial edema (Ait Hamadouche *et al.*, 2013), seminiferous tubule degeneration (Ait Hamadouche *et al.*, 2013; Graca, Ramalho-Santos & de Lourdes Pereira, 2004), and abnormal seminal

cytology (Cullen, Singh, Dykeman, Rice & Foster, 1993). Developmental exposure has also revealed limitations in the number, size, shape of seminiferous tubules and spermatogonia (Garu *et al.*, 2011).

In rats, dose-dependent reductions in the testicular and epididymal enzymatic activity of alkaline phosphatase and sodium-potassium ATPase were found with increased lead dosing (Batra *et al.*, 2001). The same study showed disrupted spermatogenesis with immature sperm deposited in the epididymal tubules, with complete arrest of spermatogenesis (i.e., empty epididymal tubules) at higher levels of lead (Batra *et al.*, 2001). The increased concentration of lead was found to not only vacuolize the cells, but also damage the basement membrane and epithelium of the caput and corpus epididymis (Batra *et al.*, 2001). Increased lipid peroxidation, another consequence of ROS that harms cell function, has been found in the testes and epididymis in rats chronically exposed to lead (Marchlewicz *et al.*, 2007). A microanalysis study illustrated that though the blood–testes barrier generally protects the seminiferous epithelium from lead toxicity, the blood–epididymis barrier does not (Marchlewicz, 1994). This study also identified lead accumulation in smooth myocytes, epithelial cells and in the lumen of epididymal duct, as indicated by diminished epididymal spermatozoa (Marchlewicz, 1994).

Lead toxicity and sperm function

It is generally affirmed that a BLL of ≥ 40 $\mu\text{g}/\text{dl}$ is associated with declined sperm quality and fecundity (Oliveira *et al.*, 2009). As mentioned before, the lack of sperm maturity may counteract sperm function. Asthenozoospermia and delayed semen liquefaction time have been found to compromise sperm quality in men with occupational lead exposure (Naha *et al.*, 2005). Lead toxicity has also been associated with delayed semen melting, further negatively impacting quality (Xuezhi *et al.*, 1992).

In addition to impaired sperm count and morphology, decreased sperm function is another potential consequence of lead toxicity (Hsu *et al.*, 1997; Taha *et al.*, 2013). Generation of ROS may increase lipid peroxidation in sperm plasma membranes, causing declined fluidity essential for motility, integrity, and viability (Kasperczyk *et al.*, 2008; Oliveira *et al.*, 2009). Lipid peroxides are capable of inducing DNA damage and reducing fertility during semen storage. The sperm plasma membranes are rich in polyunsaturated fatty acids containing double bonds vulnerable to free radical attack and initiation of the lipid peroxidation cascade, which is characterized by an autocatalytic, self-propagating reaction, giving

rise to cell dysfunction accompanied by the loss of membrane functions and integrity. Decreased membrane functionality hinders binding with homologous and heterologous zona pellucida (i.e. sperm–oocyte fusion) and the ability to undergo acrosomal exocytose (Bansal & Bilaspuri, 2010). ROS may also reduce phosphorylation of sperm axonemes to further weaken motility (de Lamirande & Gagnon, 1992). ROS impact on motility may also be ascribed to ATP deficiency from the lack of axoneme protein phosphorylation, as glyceraldehyde-3-phosphate dehydrogenase in the fibrous sheath become limited to produce sufficient energy for motility through the glycolytic pathway (Armstrong *et al.*, 1999; Gomes *et al.*, 2015). Even insignificant oxidative stress for broad periods could compromise the condensation state of sperm DNA, an effect that would not be observable before fertilization (de Lamirande *et al.*, 1997; Gavrioliouk & Aitken, 2015). In terms of direct effects on motility, studies show that lead impairs axonemal microtubule sliding and displaces the role of calcium in binding to calmodulin for tail protein tyrosine phosphorylation (Oliveira *et al.*, 2009). An *in vitro* study found an increase in flagellum abnormalities, with an increased presence of coiled tails (Gomes *et al.*, 2015). Lead may interact with the sulfhydryl groups on the proteins of the outer dense fibres and fibrous sheath, which are cytoskeletal components of the flagellum, causing its detachment from the plasma membrane (Gomes *et al.*, 2015). Finally, while still poorly understood, sperm apoptosis is the most fatal consequence of oxidative stress (Aitken *et al.*, 2015).

Previous studies indicate Cys₂His₂ zinc finger protein transcription factors as targets in lead-poisoning disease, in which low concentrations of lead are shown to replace zinc in the binding to closely spaced thiol groups within the zinc finger domains of proteins. This in turn irreversibly inhibits the binding of zinc to the zinc finger domain, causing a conformational change of the domain and a loss of specific DNA binding. Accumulation of lead ions has been shown to occur within cellular nuclei. Given the prevalence of Cys₂His₂ zinc finger factors near the amino terminus of proteins in eukaryotes, and their nucleic acid binding potential, it is suggested zinc finger protein transcription factors play a role in gene expression and regulation, in signal transduction, cell growth and differentiation, and chromosome structure (Hanas *et al.*, 1999).

Inhibition of such regulatory proteins could be a potential cause for the disorders observed in spermatogenesis, under lead-poisoning conditions. A separate study exploring the role of Cys₂His₂ zinc finger protein transcription factors in spermatogenesis suggests they play an essential role during the meiosis prophase of the germ cells, possibly acting

as regulatory factors during progression of meiosis in spermatogenesis (Ishizuka *et al.*, 2016). When testing the effects of varied lead doses on rat testis, it was found that with increasing dose, there was an accumulation of lead in the testis and epididymis, along with a respective decrease in the activities of enzymes alkaline phosphate and Na⁺/K⁺-ATPase, both known to play a role in spermiogenesis and spermatogenesis. At higher doses of lead, histological changes such as accumulation of immature cells in the lumen of seminiferous tubules, complete arrest of spermatogenesis, and damage to basement membrane of epididymis were observed (Batra *et al.*, 2001; Rafique *et al.*, 2009). However, with a concomitant administration of zinc, lower levels of lead accumulation occurred, accompanied with a significant improvement of enzyme activities, and prevention of histological damage to testis and epididymis. Therefore, it is thought that the significant competition of zinc and lead can help zinc serve a protective role during the event of lead poisoning (Batra *et al.*, 2001; Rafique *et al.*, 2009). Studies looking at oxidative stimulation and sperm injury in assisted reproductive technology have shown that zinc could inhibit hydrogen peroxide-induced impairment of sperm motility, vitality, membrane integrity, and DNA damage (Wu *et al.*, 2015). Zinc and lead measurements in the semen and serum of human males suffering from infertility for almost a year, suggest an optimal level of zinc serum (80 to <90 µg/dl) for best sperm parameters and which lowest values of lead in semen (Fatima *et al.*, 2015).

Lead appears to also play a role in displacing zinc from its binding sites on zinc-binding proteins and zinc-dependent proteins as indicated by an increase in free seminal zinc. As lead binds tightly to zinc outside of the sperm cells, zinc is prevented from entering the cell and playing its role in the sperm chromatin condensation–decondensation process (Hernandez-Ochoa *et al.*, 2005). Another supporting hypothesis for unstable sperm chromatin structure is that lead binds to cysteine residues of protamines, thus preventing protamine–DNA binding complex (Hernandez-Ochoa *et al.*, 2005). During the maturation stage at the epididymis, lead appears to increase chromatin condensation by interacting with adjacent sulfhydryl moieties during the formation of disulphide bonds (Johansson & Pellicciari, 1988). Sperm DNA fragmentation, reportedly occurring at BLL of ≥45 µg/dl (Bonde *et al.*, 2002), as well as elevated seminal ROS (Taha *et al.*, 2013) attest to the reduced viability of sperm.

Aside from sperm motility, which is critical for sperm to manoeuvre both male and female reproductive tracts pre- and post-ejaculation, increased seminal plasma lead has been shown to also impair the fertility potential of sperm in *in vitro* fertilization (Benoff *et al.*,

2003; He *et al.*, 2016). Lead causes premature acrosome breakdown of sperm. The pathogenesis of acrosome breakdown is attributed to lead infiltrating potassium channels of mature spermatozoa; susceptibility to potassium channel poisoning depends on ion channel polymorphisms which confer specific sensitivities to lead (Benoff *et al.*, 2000). *In vitro* studies have shown that hydrogen peroxide, a ROS present from lead toxicity, may also initiate premature acrosome reaction (Hsu *et al.*, 1999). It should be noted that while excessive ROS is detrimental, normophysiological ROS levels are important in the full maturation of spermatozoa, *e.g.*, during capacitation, continuous production of ROS during sperm maturation is vital for the intracellular pathways of phosphorylation to remain functional (de Lamirande *et al.*, 1997; Du Plessis *et al.*, 2015). Another *in vitro* study found that lead dose-dependently decreased the intracellular cAMP levels (explicated by potential lead/ROS-mediated breakdown of adenylate cyclase, which synthesizes cAMP (He *et al.*, 2016), calcium levels, and progesterone-induced intracellular calcium increases, leading to decreased sperm protein tyrosine phosphorylation. This result explains reduced sperm motility, inhibited capacitation, and the inhibited progesterone-induced acrosome reaction (He *et al.*, 2016). The latter appears to occur due to lead inhibition of CatSper channels (He *et al.*, 2016). Premature acrosome breakdown reduces the capability of sperm to express sufficient mannose-binding lectins. This action impairs the mannose-stimulated acrosome reaction necessary for sperm penetration into the zona pellucida and corona radiata of the ovum (Benoff *et al.*, 2003). Lead-induced ROS generation leading to impaired capacitation of sperm may also compromise penetrative ability (Hsu *et al.*, 1998). Subsequent capacitation, spermatozoa utilize chemotaxis to migrate through the lower female reproductive tract against a concentration gradient of progesterone, a sperm chemoattractant, secreted by oocytic cumulus cells. High levels of ROS produce a state of oxidative stress leading to a lower percentage of chemotactic spermatozoa (Sanchez *et al.*, 2010; Du Plessis *et al.*, 2015). The privation of ROS scavenging may also initiate spontaneous sperm hyperactivation, impairing sperm transport in the lower female reproductive tract or leading to premature capacitation (de Lamirande *et al.*, 1997; Ichikawa, Oeda *et al.*, 1999). Furthermore, lead exposure studies in animals have shown a dose-dependent decrease of sperm adherence to the ova along with compromised DNA, RNA, and protein synthesis under the sperm–zona pellucida binding conditions (Chowdhuri *et al.*, 2001). Damaged nucleic acids and inhibited DNA repair has been shown to be caused by increased ROS in spermatozoa (Vigeh *et al.*, 2011), which has been correlated with high lead levels in seminal plasma (Kiziler *et al.*, 2007).

A study with male mice eating lead-exposed chow was associated with an increase in post-implantation embryo losses *in utero*, leading to below-average litter sizes (al-Hakkak *et al.*, 1988). Similarly in humans, paternal lead exposure has been linked to increased risk of miscarriages in the men's wives (Lindbohm *et al.*, 1991). Lead has also been shown to affect male fecundity, quantitatively estimated by the amount of time to pregnancy, at higher exposure levels of ≥ 40 $\mu\text{g}/\text{dl}$ (Apostoli *et al.*, 2000). Despite the presence of epidemiologic studies, the etiologies for miscarriages, embryonic developmental complications, and similar scenarios have not been well studied (Gomes *et al.*, 2015).

Discussion

Although exact pathogenic mechanisms have yet to be fully mapped out, it is clear that lead toxicity has a pleiotropic, dose-related suppression effect at the HPT axis, spermatogenesis, and sperm function, with dysfunction at these three levels ultimately contributing to infertility. By being the overall limiter of spermatogenesis, the HPT axis is the precedent site of impairment and thus it is likely to be the most imposing of the three levels. The axis is more prone to exposure from lower BLL, while the blood–testis and blood–epididymis barriers serve to protect the gonads. Several studies have demonstrated that low-dose chronic exposure may be more harmful and irreversible than high-dose acute exposure; this situation is a particularly a concern for workers facing occupational lead exposure. Although minimizing exposure or chelating agent treatment have successfully restored or improved fertility, long-term damage may be more challenging to correct, as all three levels have potentially been compromised. Because polymorphisms, sexual developmental stage, other physiological issues, and environmental factors should all be accounted for when determining individual susceptibility to lead reprotoxicity, isolating the cause(s) and mechanism(s) for infertility are very unpredictable.

Clinicians should run sperm function tests and consider lead measurement tests when dealing with couples with unexplained infertility. Although semen analysis and sperm function tests reveal the pathophysiological implications of lead toxicity, the mechanisms are mostly theorized and not fully understood. Aside from molecular and cellular biology studies, more cross-sectional studies should be piloted to observe the collective effects of dosage level and duration of exposure not only on overall fertility rates but on the viability of embryo implantation.

Semen parameters such as fructose level, pH, and MOT, which have generally not been checked in previous studies, may provide additional evidence for low-level lead toxicity. Sperm function tests can better evaluate the genetic integrity of sperm in order to determine association with lead exposure and fitness for fertilization. As lead-related infertility is multifactorial, pituitary function, testosterone, and sperm function tests should be consolidated to provide a clearer diagnosis. Reproductive lead accumulation should also be explored; glutathione and ATP-dependent efflux pump-multidrug resistance protein 1 have been found to mediate lead excretion in mouse Sertoli cells (Huang *et al.*, 2014).

Conflict of interest

The authors have no conflicts of interest to declare.

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