Chapter

Effect of Heavy Metals on Tyrosine Kinases Signaling during Sperm Capacitation

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Abstract

Sperm capacitation is the key event prior to fertilization. Success rate of currently used assisted reproductive technology like *in-vitro* fertilization is 50% dependent on sperm maturation or capacitation. *In-vivo* capacitation occur almost in female reproductive tract in response to various signaling or enzymatic molecules. Interestingly, both early and late events of capacitation are centrally regulated by protein kinase A (PKA). Influx of Ca^{2+} and HCO3-transmembrane drive leads to change in pH and intracellular cAMP which ultimately activate PKA regulated capacitation. PKA phosphorylates several target proteins that are presumed to initiate different signaling pathways. Some divalent heavy metals like lead, mercury, arsenic and cadmium mimic Ca^{++} entry and its functions and ultimately affect capacitation by inhibiting or inducing tyrosine phosphorylation. In this chapter we review the mechanism of heavy metals by which they affect the tyrosine phosphorylation during sperm capacitation.

Keywords: Tyrosine Phosphorylation, Spermatozoa, Capacitation, Heavy Metals

1. Introduction

Heavy metals are known to be harmful to humans, animals as well as plants in large amounts. Heavy metals are distributed throughout the environment from both natural sources (inorganic form) and human activities (organic form) and thus accumulating in biosphere including humans and animals' body [1, 2]. Most of these non-degradable toxic elements, such as Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Mercury (Hg), Nickel (Ni), Lead (Pb), and Zinc (Zn), are listed as hazardous contaminants by the EPA [3, 4]. Potential health hazards as toxic manifestations and subtle effects of heavy metals are matter of concern because of daily and wide-spread exposure of humans and animals' consequent to their daily life. The molecular mechanisms for metal carcinogens are still poorly understood. Mercury containing compounds have been used for thousands of years in preservation of various vaccines, treatment of syphilis, skin creams, dental amalgams, and extraction of gold [5]. Direct application of cadmium, lead and arsenic in soil fertilizers and fungicides, leather tanning, waste-water treatment facilities, paper mills and disposal of solid wastes as well as batteries and thermometers in landfills

are the chief sources within the environment which may influence animal and human health [6]. The cause of male infertility in 50% cases is still not clear; thus, it is very important to flash a light on role of heavy metals in infertility [7]. Some malformations of male reproductive system, such as cryptorchidism, hypospadias, and prostate and testicular cancers may originate from exposure to endocrine disruptors [8, 9]. In addition, metals can cause hormonal imbalance by affecting the neuroendocrine system, disrupting the secretion of androgens from Leydig cells or inhibin-B from Sertoli cells [10]. Evidence also exists linking mercury with erectile dysfunction [11, 12]. Loss of libido have been reported in men acutely exposed to metallic mercury vapor [13]. Choy et al. [14] did a study in Hong Kong on 150 infertile couples undergoing *In-vitro* fertilization versus 20 fertile couples. The infertile couples had significantly higher blood mercury levels than the fertile group. About 1/3 of the infertile men and 1/4 of the infertile females had high mercury levels and they attributed it to seafood consumption. Considering the fact that they looked only at blood, fish may have been the culprit. However, fish is not usually a major direct source of exposure. Nevertheless, this study reinforces the fact that mercury levels need to be investigated when dealing with infertility, both in males and females. Evidently, metal dependent and/or species-dependent differences in signaling mechanisms seem to exist in mediating toxic effects of metals; however, further studies on these aspects are required.

Arsenic is reported in human tissues ranging 100–6000 ppb [15]. Arsenic toxicity has been reported in case of respiratory, dermatological, cardiovascular disorders including diabetes and obesity [16–19]. Cd is also reported to have toxic effects including endocrine nephrotoxicity, carcinogenity, and neurotoxicity [20–22]. These heavy metals affect the these heavy metals effect the molecular mechanism of tyrosine kinase that plays a central role in the response of cells to various kinds of stresses or growth factors and acts as switch in many cellular functions. For example, in regulation of cell proliferation regulation of cell proliferation, differentiation, cell-cycle regulation, and cell signal transduction [23] specifically in cAMP-dependent pathway, which is a hallmark event of capacitation, that leads to sperm hyperactivation which is necessary for fertilization [24]. Dysfunctional tyrosine phosphorylation mechanisms linked to abnormal cell signaling, frenzied cell growth leading to development of leukemia, lymphoma, multiple endocrine neoplasia type 2, small lung cancer, breast cancer, and colon cancer [25, 26]. Proteins are building blocks of the living systems and alterations in protein function indicate the response to abnormal or stress condition [27].

Tyrosine kinase-dependent pathways are mediated by the activities of receptor (RTKs) and non-receptor tyrosine kinases (NTKs) [28, 29]. The RTK are transmembrane-spanning receptor and an intrinsic protein and further classified as EGF receptor (EGFR), PDGF receptor (PDGFR), FGFR, VEGF receptor (VEGFR), while NRTKs act as substrates of RTKs, include Src family members [30] and, are classified as SRC, ABL, FAK and Janus kinase [31]. Upon stimulation, RTKs undergo autophosphorylation on the tyrosine residues located in their own carboxy terminus and induce conformational changes. This enhances kinase activities and creates binding sites for cellular substrates through SH2 domain interactions [30]. Some proteins which get phosphorylated at tyrosine residue during capacitation are A Kinase Anchoring Protein-4, dihydrolipoamide dehydrogenase, pyruvate dehydrogenase-A2, glycerol-3-phosphate dehydrogenase-2, pyruvate dehydrogenase, and phospholipid hydroperoxide glutathione peroxidase [32–38]. The molecular events of the acrosome reaction overlap substantially with those of capacitation, including phosphorylation of similar tyrosine proteins, influx of Ca^{2+}, and increased cAMP and PKA levels. The role of ROS in the in-vivo acrosome reaction involves the spermatozoa's actions on ZP via phosphorylation of plasma membrane

proteins. *In-vitro* activation of the acrosome reactions (AR) is also reported against stressors like heavy metals, O^{2-}, H_2O_2, and NO. Cyclic-AMP regulation and Ca^{2+} influx are the key events of capacitation. In-vitro exposure of goat's spermatozoa to mercuric chloride is reported to increase the intracellular Ca^{2+} release and alter the cAMP levels that leads to spontaneous acrosome reaction and inhibition of tyrosine phosphorylation [39, 40]. The primary downstream target of cAMP is protein kinase-A (PKA), whose activity increases during sperm capacitation [41]. Sperm motility stimulant, pentoxifylline (PF) significantly increased sperm hyperactivation and induced an early onset of sperm capacitation via various cell-signaling molecules such as cAMP, Ca^{2+} and protein kinases in hamsters [42]. Targeted disruption of the sperm-specific catalytic subunit, i.e., Ca^{2+} of protein kinase- A (PKA), led to hypo-tyrosine phosphorylation of sperm proteins accompanied by a lack of hyperactivation in mice spermatozoa [43].

In mammals, fertilization requires the release of spermatozoa into female reproductive tract. After ejaculation, to become fully fertilization competent, mammalian sperm must undergo a combination of sequential maturation process in female reproductive tract. Austin [44], demonstrated independently that sperm acquire fertilization capacity only after residing in the female reproductive tract for a finite period of time in a process known as sperm capacitation. Capacitation include variations in sperm intracellular ions concentrations, plasma membrane fluidity as a result of changes in localization of membrane antigens and removal of cholesterol [45]. In particular, capacitation has been associated with a cAMP/PKA-dependent increase in protein tyrosine phosphorylation [46]. Capacitation involves modifications occurring both in the head (i.e., preparation for the acrosome reaction) and the tail (i.e., motility changes such as hyperactivation) which renders sperm to penetrate the egg following acrosome reaction (exocytosis of acrosomal contents). The physiological event of mammalian sperm capacitation had been recognized for a long time, but the molecular players regulating capacitation are still poorly understood. Interestingly, the process of capacitation can occur in-vitro in most species and the conditions required for sperm capacitation in-vitro include a balanced salt solution containing appropriate electrolytes concentrations (e.g., Na^+, K^+, Cl^-, $HCO3^-$, Mg^{2+}, Ca^{2+}, and PO_4^{3-}), metabolic energy sources (e.g., glucose, pyruvate and lactate) which support the high ATP consumption needed for motility and serum albumin as a a cholesterol acceptor. The important mediators of signal transduction pathways leading to capacitation include cAMP, Ca^{2+}, HCO^{3-}, inositol triphosphate (IP3), protein kinase A (PKA), protein tyrosine kinase (PTK), phospholipase-C (PLC).

Ca^{2+} is shown to play a very important role in sperm capacitation and acrosome reaction by influencing the activity of sperm adenylate cyclase and PLC [47]. Pentoxifylline (cAMP phosphodiesterase inhibitor) causes hyperactivated motility of hamster spermatozoa via increasing sperm cAMP level [48]. Inhibition of Protein kinase – A (PKA) activity led to an inhibition of cAMP dependent protein tyrosine phosphorylation in mice [46] and in hamster [49]. Mice that lack the sperm-specific PKA catalytic subunit Cα2, was infertile despite normal mating behavior, and their sperm shows defects in motility and capacitation-associated events such as the increased tyrosine phosphorylation [43]. This indicates that sperm capacitation and protein tyrosine phosphorylation are regulated through a PKA pathway, invoking an important role for tyrosine phosphorylation in sperm capacitation. Time dependent increase in protein tyrosine phosphorylation during capacitation has also been observed in cauda epididymis sperm [46]. As mature spermatozoa lack de novo gene expression, acquisition of fertilization competence is invariably dependent on post-translational modifications especially phosphorylation of pre-existing structural and intracellular proteins of spermatozoa during capacitation. AKAP4

was the first tyrosine phosphorylated protein identified in the humans [50], mouse [46] and hamster species [51]. The lack of AKAP4 gene expression results in loss of progressive sperm motility, leading to male infertility [52]. Similarly, tyrosine phosphorylated form of AKAP-3 recruits PKA to the sperm flagellum changing protein phosphorylation status and increasing sperm motility [53]. Phosphorylated AKAPs appears to interact with PKA and facilitate flagellar protein phosphorylation in a localization-specific manner. Chaperone protein VCP also undergoes tyrosine phosphorylation. VCP is important for membrane fusion, possibly involved in acrosome reaction [32, 33]. Dihydrolipoamide dehydrogenase (DHLD) [37], phospholipid hydroperoxide glutathione peroxidase (PHGPx) [38] and pyruvate dehydrogenase A2 (PDHA2) are among the metabolic-mitochondrial enzymes that are tyrosine phosphorylated and are localized to sperm flagellum; the inhibition of DHLD leads to decrease in sperm hyperactivation [54]. Calcium-binding tyrosine phosphorylation-regulated protein (CABYARa) and the Calcium/calmodulin-dependent protein kinase IV (CaMKIV) are other tyrosine phosphorylated proteins in humans and involved in calcium regulated protein tyrosine phosphorylation of sperm proteins [55, 56]. Thorough understanding of capacitation and molecular characterization of functionally important phosphorylated sperm proteins is required to benefit reproductive strategies, agriculture.

Sperm signaling pathways also required an optimal level of sperm-generated reactive oxygen species (ROS) for protein tyrosine phosphorylation [42]. The signaling pathway involving protein tyrosine phosphorylation is distinctly associated with hyperactivated motility during sperm capacitation in mice [46], humans [57], and hamsters [34, 35]. The number of Sertoli cells determine the number of sperms produced in adulthood, because each Sertoli cell can support only a finite number of germ cells that develop into sperm [58]. Cadmium (Cd) is reported to cross the blood-testis barrier and induce excessive oxidative stress in Sertoli cells leading to necrosis in mice spermatozoa [59]. Cd exposure led to halt the process of spermatogenesis and normal testicular development by inhibiting the synthesis of testosterone in adult mice [60]. Consequently, Cd caused remarkable drop in weight of testes and epididymis, sperm concentration, motility, and synchronously an elevation in dead and abnormal sperm [61]. Disruption of spermatogenesis in men at any stage of cell differentiation can decrease the total sperm count, increase the abnormal sperm count, impair the stability of sperm chromatin or damage sperm DNA [62], lowered epididymis sperm count, and testicular weight, aberrant chromosome numbers rather than the normal [63], chromosomes break, and lowered testosterone levels in male [64, 65]. Metal's accumulation in epididymis, prostate, and seminal fluid may impair progressive sperm motility [66, 67] and thus reproductive efficiency. Therefore, in this chapter we have discussed the effect of different heavy metals that effect male reproduction with special focus on sperm capacitation via a modification in tyrosine signaling mechanisms [68–71].

2. Effect of mercuric chloride on tyrosine phosphorylation

Reproductive toxicity of mercury has been described in several animal studies in which sperm motility, epididymal sperm count and normal sperm morphology decreased among rats, mice, fish, monkeys and humans after mercury exposure [72–75]. Evidence is usually limited to animal data or to in-vitro studies [76, 77]. The clinical and epidemiological findings are scarce and controversial, and often difficult to interpret because of multiple exposures to different agents and latency of effects. Human studies are few and contradictory too [78]. Seminal fluid mercury concentrations are correlated with abnormal sperm morphology and abnormal

sperm motility [79]. Furthermore, infertile, and sub-fertile men have higher mercury levels than the fertile men [80] and tubular atrophy and Sertoli-cell-only syndrome has been observed among infertile patients that have been exposed to mercury [81]. Kushawaha et al. [39, 40] reported that in-vitro exposure of mercuric chloride (0.031 μg/mL) leads to significant increase in spontaneous acrosome reaction, intracellular Ca^{2+} and cAMP levels, and capacitation failure may be due to inhibition of 55, 70, and 80 kDa tyrosine phosphorylation of protein. Proteins of 80 and 105 kDa are the main substrates for enzymes and are important in acrosome reactions [82–84]. Sperm capacitation is a sequential process which involves several signaling pathways and ultrastructural changes such as modifications in membrane lipid composition, increased permeability to ions [85, 86] and phosphorylation of proteins on tyrosine (Tyr), serine (Ser) and threonine (Thr) residues [82, 87–89]. The cAMP/PKA-dependent increase in tyrosine phosphorylation of two fibrous sheath proteins, p80 and p105 related to A-kinase anchoring proteins (AKAPs), is one of the prominent events associated with capacitation [89, 90]. Martinez et al. [91] investigated the effects and underlying mechanisms of chronic mercury exposure at low levels on male reproductive system of rats. Three-month-old male Wistar rats were exposed to 4.6 μg/kg to 0.07 μg/kg/day subsequent dose of $HgCl_2$ for 60 days and they found that mercury treatment decreased daily sperm production, count, motility, and increased head and tail morphologic abnormalities. Moreover, mercury treatment decreased luteinizing hormone levels, increased lipid peroxidation in testis and decreased antioxidant enzymes activities (superoxide dismutase and catalase) in reproductive organs. According to the findings of in-vitro study by Arabi [92], $HgCl_2$ at 50 to 550 μM concentration affected the sperm membrane and DNA integrity, viability, and acrosomal status of normal bull spermatozoa. They recorded a sharp increase in lipid peroxidation/LPO rate; highest was at 550 μM mercury concentration, indicating the deleterious effect of mercury on sperm membrane intactness. There was also a strong negative correlation between LPO rate and % viable spermatozoa. Comet assay study revealed that mercury is capable of inducing DNA breaks in sperm nuclei. The correlation between LPO rate and % DNA breaks was 0.984 [92, 93]. Oxidative stress seemed to be the potential mechanism involved in mercury - induced male reproductive toxicity. Kinematic patterns of goldfish *Carassiusauratus* spermatozoa after mercury exposure (100 to 368 μM) studied by Van Look et al. [94]. They reported that sperm flagellar length was significantly shortened after instant exposure mercuric chloride, while curvilinear velocity (VCL) and the percentage of motile sperm were significantly decreased at mercuric chloride concentration of 1 and 10 mg/l (3.68 and 36.8 μM), respectively. After 24 h exposure to 0.001 mg/l (0.0037 μM) $HgCl_2$, flagellar length was significantly reduced in 38% of the spermatozoa. Following exposure to 0·1 mg/l (0·37 μM) mercuric chloride for 24 h, however, majority of the spermatozoa (98%) had significantly shortened flagella and increased sperm head length, width and area. Sperm motility was also significantly decreased at 0.1 mg/1(0.37 μM) mercuric chloride, probably due to significantly reduced flagellar length at this concentration. Several animal studies indicate that mercury is a male reproductive toxicant, but human studies are few and contradictory. Vergilio et al. [95] investigated the toxic effects of mercury chloride (1 μM - 30 μM) on testes and sperms of tropical fish (Gymnotuscarapo) and showed decrease in the sperm count (36.8%) after 20 μM/24 h treatment and subsequent decrease (48.7%) was observed after 20 μM/96 h. Hg (20 μM) also altered the sperm morphology in 24 h and 96 h where sperm head abnormalities were present.

Mocevic et al. [96] examined semen characteristics and serum levels of reproductive hormones in relation to environmental exposure to mercury. Blood and semen samples were collected from 529 male partners of pregnant women living in

Greenland, Poland and Ukraine between May 2002 and February 2004 [97]. Total content of mercury in whole blood was 9.2 ng/ml in Greenland (0.2–385.8 ng/ml), 1.0 ng/ml in Poland (0.2–6.4 ng/ml21) and 1.0 ng/ml in Ukraine (0.2–4.9 ng/ml). They found a significantly positive association between blood levels of mercury and serum concentration of inhibin B in men from Greenland (β = 50.074, 95% confidence interval (CI) = 50.021 to 0.126) and in an analysis including men from all three regions (β = 50.067, 95% CI = 50.024 to 0.110). The association may be due to beneficial effects of polyunsaturated fatty acids (PUFAs), which are contained in seafood and fish. No significant association (P < .0.05) was found between blood concentrations of mercury and any of the other measured semen characteristics (semen volume, total sperm count, sperm concentration, morphology and motility) and reproductive hormones (free androgen index (FAI), follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone and LH_3 testosterone) in any region. These findings did not provide evidence that environmental mercury exposure in Greenlandic and European men with median whole blood concentration up to 10 ng/ml had adverse effects on biomarkers of male reproductive health. Overall, studies have found that mercury accumulates in testes, inhibits enzymes necessary for sperm production, affects DNA in sperm, causes aberrant number of chromosomes in cells, and induces chromosomes breaks; all of which can cause infertility, spontaneous abortion, or birth defects. From the foregoing scientific data it is apparent that mercury is a metal of great global concern and has the potential to alter reproductive functions in males thus, still further investigation on protein phosphorylation during capacitation are warranted.

3. Effect of cadmium (Cd) on tyrosine phosphorylation

Cd possesses oxidation state +2 just like mercury and calcium with half-life of 15–30 years with low execration rate. It can accumulate into the non-smoking population via fumes, dust, contaminated food and water and it is widely use in cancer drugs [98, 99]. Tobacco plant absorbs Cd specially into leaves which is then used in smoking [100]. Apart from this 0.5 mg of Cd is radially reported into per kg fertilizer which is then accumulate into the fruits, vegetables and grains [101, 102]. Cd is reported to accumulate in various tissues via bloodstream [103]. Ca shows a high affinity toward sulfhydryl (–SH and GHS) and disulphide groups (-S-S) of the proteins and result in increased production of ROS [104]. Epigenetic changes like DNA methylation are reported to associated with the in-vivo Cd exposure in three-month-old rats. Short time exposure of Cd for 24 h–1 week induces hypomethylation, while longer times (8–10 weeks) induce hypermethylation [105]. In-vivo orally administered Cd (1, 2 or 4 mg kg^{-1}) to 3–7-days postpartum rats for 30 min did not showed any effect on sperm motility, but significantly decrease the rate of fertilization and embryo development indicating that Cd affects the epigenetic factors [106, 107]. Cd is also reported to induced germ cell apoptosis, loss of daily sperm production, and decreased sperm motility might be responsible for the decline of male fertility [108, 109] specifically spontaneous acrosome reaction in mouse [109–111], rats [112], ram [113], rabbit [114] and sheep [115, 116] sperms. Research indicates that oxidative stress and apoptosis are the major players which affects the in the post-translation modifications like phosphorylation and methylation [117, 118]. Ca^{2+}/calmodulin-dependent kinase II (CaMK-II) which is sensitive to concentration of intracellular calcium and calmodulin, are involve in apoptotic pathway [119–121] and responsible for phosphorylation of serine/thrionine residue of tyrosine kinase [119]. Wang et al. [122] reported that 10 μM Cd inhibited

the sperm motility, GAPDH activity, AMPK activity and ATP production, and induced tyrosine phosphorylation of 55–57KDa proteins. These results suggest that Cd-induced tyrosine phosphorylation of 55–57KDa proteins particularly localized in the middle piece of sperm that may inhibit or interfere with mitochondria and ultimately affect the motility of sperm. Exposure of adult rats to 2 mg/kg Cd for 24 hr. induced the ROS and catalase activity and also inhibit the TGF- β3 response and p38 MAPK phosphorylation [123, 124]. Role of tyrosine-phosphorylated dihydrolipoamide dehydrogenase (DLD) was reported in capacitation, hyperactivation and acrosome reaction in hamster [37, 125] after Cd exposure of 1.2 mg/kg BW that induce tyrosine phosphorylation of DLD leads to lower the dehydrogenase activity, and thus affect the mitochondria and sperm motility. Only few studies are reported the effect of Cd during sperm capacitation. As capacitation process involve the influx of Ca^{2+} ions, thereby in presence of Cd which is also having similar charge as Ca, may mimic or replace the Ca entry by competitive binding and, thus affecting the capacitation process. More research is warranted to find out the molecular mechanism of Cd toxicity on capacitation in different species with different doses.

4. Effect of arsenic on tyrosine phosphorylation

Arsenic is mainly present in four forms namely arsenate (As(V)), arsenite (As (III)), MMA (monomethylarsonic acid), and DMA (dimethylarsenic acid) [126]. Trace quantities of arsenic were found in drinking water of rats, hamsters, goats, chickens and humans [127]. Arsenic-induced male infertility is reported to cause abnormal sperms, decreased sperm count, and decreased sperm motility in both humans and animals [128–130]. Exposure of the cells to arsenic increased total cellular tyrosine phosphorylation of 110–120, 90, 70, 56, and 40 kDa proteins [131]. Arsenic-induced tyrosine-phosphorylation in EGFR [132]. It is not known how arsenic induces the activation of EGFR either by the conformational changes or by dimerization of EGFR, which results in the activation of EGFR [133]. It was proposed that arsenic might activate EGFR through generation of ROS that, in turn, triggered the conformational changes in the receptor [134, 135]. The arsenic-induced activation of EGFR recruits Sh-c and phosphorylates its tyrosine residues, which results in enhancement of the interactions between Sh-c and Grb2. Signals are then relayed to the downstream signaling proteins [132]. Inhibition of EGFR kinase blocked arsenic-induced activation of MAPKs [136]. Arsenic may activate with the vicinal sulfhydryl groups of the Src molecule, (2) direct interactions with extracellular matrix proteins to induce integrin rearrangements, or (3) the generation of ROS [137, 138]. Biscardi and colleagues found that Src was able to phosphorylate EGFR at two unique tyrosine residues, distinct from the autophosphorylation sites, to activate EGFR in association with the activation of other cell signaling proteins [139, 140]. Arsenic induces Src and that activates downstream proteins e.g., MAPKs via EGFR-dependent and EGFR-independent pathways [138, 141]. Shim et al. [142] reported that arsenic inhibits Ca^{2+} influx into antigen-activated mast cells and inhibit tyrosine phosphorylation. These results indicate that the target of arsenic is upstream of the Ca^{2+} influx which is a major pathway of sperm capacitation as well. Thus, further detailed studies are warranted to find out the effects of arsenic on sperm capacitation mechanism.

Six months exposure to sodium arsenite (1, 5, or 25 mg/L) reduced Voltage-dependent anion channel protein 3 (VDAC3), which leads to impaired capacitation and fertilization process in male rats [143, 144]. cAMP activates the serine/threonine Kinase and cAMP-dependent protein kinase catalytic subunit alpha (PRKACA), which in turn activates tyrosine through phosphorylation. Blocking of

PRKACA altered the tyrosine phosphorylation at the protein level which results in impairment of capacitation of sperm [143, 144]. Arsenic exposure on the proteome and metabolome in rat testis leads to 36 up-regulated and 34 down-regulated proteins and 13 metabolites (8 high and 5 low). Theses altered proteins were related to spermatogenesis, fertilization, fertility, and mating behavior which may be mediated by the ERK/AKT/NF-κB-dependent signaling pathway [143, 144]. However, these studies indicate the toxic effect of arsenic, but arsenic-induced male reproductive toxicity, particularly effect on capacitation and tyrosine phosphorylation mechanisms are still far from being completely understood.

5. Effect of lead (Pb) on tyrosine phosphorylation

It is well known that there has been a worldwide decrease in human male fertility in recent years. One of the main factors affecting this is environmental pollution. Lead is one of the major heavy metal contaminants that threatens the health of animals and human beings at global level. It is a naturally occurring element and widely used in acid batteries, paints, smelters, and paper printing. It accumulates into human and animal blood, bone and soft tissues with a half-life of 35 days in blood and 20–30 years in bone via contaminated food, and drinking water [145]. Pb has also been reported to accumulate in the epididymus and some glands [146, 147] and is considered a male reproductive toxicant [148]. The mechanism of toxicity of Pb is still not very clear. Pb mainly targets events of spermatogenesis and spermatozoa function via free radical generation, apoptosis, motility, and DNA fragmentation, and ultimately declines the rate of fertilization [149]. Recently Hassan et al. [150] reported that exposure of 20 mg PbAc/kg bwt, orally in rats for 45 days resulted in significant decrease in testis weight, spermatozoa count, testosterone levels, and antioxidant enzymes levels. Histological study indicated that Pb -exposed group was devoid of germ cells and maturation arrest with the formation of giant primary spermatocytes. Some studies reported that Pb has the ability to displace zinc and results in alteration in Ca^{2+} mediated process [151].

Capacitation is highly Ca^{2+} dependent process which means lead exposure could inhibit or induce the capacitation. Only few studies are reported about the effect of Pb on tyrosine phosphorylation during capacitation. Yuanqiao et al. [152] reported that 10–100 μM lead acetate dose-dependently inhibited total and progressive motility measures, capacitation and progesterone-induced acrosome reaction in humans. It also decreased the intracellular concentrations of cyclic adenosine monophosphate (cAMP) and intracellular calcium (Ca^{2+})i, and reduced the tyrosine phosphorylation of sperm proteins, all of which are thought to be key factors in regulation of capacitation. These findings suggest that lead inhibits human sperm functions by reducing the levels of sperm intracellular cAMP, (Ca^{2+})i and tyrosine phosphorylation of sperm proteins *in-vitro*. Voltage-dependent Ca^{2+} channels, known as Catsper, are mainly involved in regulation of capacitation by mediating Ca^{2+} influx [153]. Therefore, it can be postulated that Pb exposure decreases intracellular Ca^{2+} by inhibiting progesterone -induced acrosome reaction via voltage-dependent channels. Further concentration and time dependent studies are warranted to explicate the effects of Pb on sperm capacitation and tyrosine signaling mechanism.

6. Conclusions

Heavy metals affect tyrosine phosphorylation during capacitation of spermatozoa and lead to male infertility. Alteration in tyrosine signaling might be a result of

various stress conditions which are produced by heavy metals in cells like oxidative stress, apoptosis, mitochondrial damage, calcium influx and change in osmolarity of cells. Particularly Hg, Pb, As and Cd inhibit or induce tyrosine phosphorylation of sperm proteins. There are several factors including animal species and strains, gender, age, stress, genetic disorders, nutritional status, smoking, alcohol consumption, use of medicines, and concomitant exposure to other chemicals or even physical factors which will influence both the metabolism and the dose–response relationships including reproduction that affects biological processes specifically signaling mechanism. Therefore, extensive research is warranted focusing on tyrosine phosphorylation signaling during sperm capacitation using large sample size or population with minimum dose which are reported in human blood after exposure of lead, mercury, arsenic and cadmium. It is now generally accepted that the mammalian testes are very sensitive to heavy metals, and these induce changes in the testicular biochemical functions via ROS and DNA damage that ultimately affect the fertilizing ability particularly capacitation in spermatozoa.

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References

[1] Qiu, Yao-Wen. "Bioaccumulation of heavy metals both in wild and mariculture food chains in Daya Bay, South China." *Estuarine, Coastal and Shelf Science* 163 (2015): 7-14.

[2] Zhang, Tao, Jujun Ruan, Bo Zhang, Shaoyou Lu, Chuanzi Gao, Lifei Huang, Xueyuan Bai, Lei Xie, Mingwei Gui, and Rong-liang Qiu. "Heavy metals in human urine, foods and drinking water from an e-waste dismantling area: Identification of exposure sources and metal-induced health risk." *Ecotoxicology and environmental safety* 169 (2019): 707-713.

[3] Cobbina, Samuel J., Yao Chen, Zhaoxiang Zhou, Xueshan Wu, Ting Zhao, Zhen Zhang, Weiwei Feng et al. "Toxicity assessment due to sub-chronic exposure to individual and mixtures of four toxic heavy metals." Journal of Hazardous Materials 294 (2015): 109-120.

[4] Toth, G., T. Hermann, M. R. Da Silva, and L. Montanarella. "Heavy metals in agricultural soils of the European Union with implications for food safety." Environment international 88 (2016): 299-309.

[5] Clarkson, Thomas W., and Laszlo Magos. "The toxicology of mercury and its chemical compounds." Critical reviews in toxicology 36, no. 8 (2006): 609-662.

[6] World Health Organization. *Health guidelines for the use of wastewater in agriculture and aquaculture: report of a WHO scientific group [meeting held in Geneva from 18 to 23 November 1987]*. World Health Organization, 1989.

[7] Wirth, Julia J., and Renee S. Mijal. "Adverse effects of low level heavy metal exposure on male reproductive function." Systems biology in reproductive medicine 56, no. 2 (2010): 147-167.

[8] Henkel, Ralf. "Environmental contamination and testicular function." In *Bioenvironmental Issues Affecting Men's Reproductive and Sexual Health*, pp. 191-208. Academic Press, 2018.

[9] Iavicoli, Ivo, Luca Fontana, and Antonio Bergamaschi. "The effects of metals as endocrine disruptors." Journal of Toxicology and Environmental Health, Part B 12, no. 3 (2009): 206-223.

[10] Jensen, Tina Kold, Jens Peter Bonde, and Michael Joffe. "The influence of occupational exposure on male reproductive function." *Occupational Medicine* 56, no. 8 (2006): 544-553.

[11] Schrag, S. Don, and Robert L. Dixon. "Occupational exposures associated with male reproductive dysfunction." *Annual review of pharmacology and toxicology* 25, no. 1 (1985): 567-592.

[12] Wijesekara, G. U. S., D. M. S. Fernando, S. Wijerathna, and N. Bandara. "Environmental and occupational exposures as a cause of male infertility." (2016).

[13] Lauwerys, Robert, Harry Roels, Pierre Genet, Guy Toussaint, André Bouckaert, and Serge De Cooman. "Fertility of male workers exposed to mercury vapor or to manganese dust: a questionnaire study." *American Journal of Industrial Medicine* 7, no. 2 (1978): 171-176.

[14] Choy, C.M., Yeung, Q.S., Briton-Jones, C.M., Cheung, C.K., Lam, C.W. and Haines, C.J. Relationship between semen parameters and mercury concentrations in blood and in seminal fluid from subfertile males in Hong Kong. Fertil.Steril, no. 78(2002b):426-428.

[15] Hutchinson, Lee M., Benett M. Trinh, Rachel K. Palmer, Christopher A. Preziosi, Jonathan H. Pelletier, Hannah M. Nelson, and Julie A. Gosse. "Inorganic arsenite inhibits IgE receptor-mediated degranulation of

mast cells." Journal of Applied Toxicology 31, no. 3 (2011): 231-241.

[16] Abernathy, Charles O., Yung-Pin Liu, David Longfellow, H. Vasken Aposhian, Barbara Beck, Bruce Fowler, Robert Goyer et al. "Arsenic: health effects, mechanisms of actions, and research issues." Environmental health perspectives 107, no. 7 (1999): 593-597.

[17] Paul, Somnath, Nandana Das, Pritha Bhattacharjee, Mayukh Banerjee, Jayanta K. Das, Nilendu Sarma, Ajoy Sarkar et al. "Arsenic-induced toxicity and carcinogenicity: a two-wave cross-sectional study in arsenicosis individuals in West Bengal, India." *Journal of exposure science & environmental epidemiology* 23, no. 2 (2013): 156-162.

[18] Smith, Allan H., Elena O. Lingas, and Mahfuzar Rahman. "Contamination of drinking-water by arsenic in Bangladesh: a public health emergency." Bulletin of the World Health Organization 78 (2000): 1093-1103.

[19] Xia, Yajuan, Timothy J. Wade, Kegong Wu, Yanhong Li, Zhixiong Ning, X. Chris Le, Binfei Chen, Yong Feng, Judy L. Mumford, and Xingzhou He. "Well water arsenic exposure, arsenic induced skin-lesions and self-reported morbidity in Inner Mongolia." *International journal of environmental research and public health* 6, no. 3 (2009): 1010-1025.

[20] Bernhoft, Robin A. "Cadmium toxicity and treatment." *The Scientific World Journal* 2013 (2013).

[21] Waalkes, Michael P., Larry K. Keefer, and Bhalchandra A. Diwan. "Induction of proliferative lesions of the uterus, testes, and liver in Swiss mice given repeated injections of sodium arsenate: possible estrogenic mode of action." Toxicology and applied pharmacology 166, no. 1 (2000): 24-35.

[22] Yang, Hong, and Yan Shu. "Cadmium transporters in the kidney and cadmium-induced nephrotoxicity." International journal of molecular sciences 16, no. 1 (2015): 1484-1494

[23] Gozin, Alexia, Elisabeth Franzini, Valérie Andrieu, Lydie Da Costa, Emmanuelle Rollet-Labelle, and Catherine Pasquier. "Reactive oxygen species activate focal adhesion kinase, paxillin and p130cas tyrosine phosphorylation in endothelial cells." Free Radical Biology and Medicine 25, no. 9 (1998): 1021-1032.

[24] Visconti, Pablo E. "Understanding the molecular basis of sperm capacitation through kinase design." *Proceedings of the National Academy of Sciences* 106, no. 3 (2009): 667-668.

[25] Chen, Ming Hui, Risto Kerkelä, and Thomas Force. "Mechanisms of cardiac dysfunction associated with tyrosine kinase inhibitor cancer therapeutics." *Circulation* 118, no. 1 (2008): 84-95.

[26] Kolibaba, Kathryn S., and Brian J. Druker. "Protein tyrosine kinases and cancer." *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer* 1333, no. 3 (1997): F217-F248.

[27] Zhong, Zhenyu, Elsa Sanchez-Lopez, and Michael Karin. "Autophagy, inflammation, and immunity: a troika governing cancer and its treatment." Cell 166, no. 2 (2016): 288-298.

[28] Kemble, David J. A biochemical study on the regulation of the Src and FGFR family of protein tyrosine kinases. University of Rhode Island, 2009.

[29] Siveen, K.S., Prabhu, K.S., Achkar, I.W., Kuttikrishnan, S., Shyam, S., Khan, A.Q., Merhi, M., Dermime, S. and Uddin, S., 2018. Role of non-receptor tyrosine kinases in hematological malignances and its targeting by natural products. Molecular cancer, 17(1), pp.1-21.

[30] Blume-Jensen, Peter, and Tony Hunter. "Oncogenic kinase signalling." Nature 411, no. 6835 (2001): 355-365.

[31] Dubois, Marie-Françoise, and Olivier Bensaude. "MAP kinase activation during heat shock in quiescent and exponentially growing mammalian cells." FEBS letters 324, no. 2 (1993): 191-195.

[32] Ficarro, Scott, Olga Chertihin, V. Anne Westbrook, Forest White, Friederike Jayes, Petr Kalab, Jarrod A. Marto et al. "Phosphoproteome analysis of capacitated human sperm: evidence of tyrosine phosphorylation of a kinase-anchoring protein 3 and valosin-containing protein/p97 during capacitation." *Journal of Biological Chemistry* 278, no. 13 (2003a): 11579-11589.

[33] Ficarro, Scott, Olga Chertihin, V. Anne Westbrook, Forest White, Friederike Jayes, Petr Kalab, Jarrod A. Marto et al. "Phosphoproteome analysis of capacitated human sperm: evidence of tyrosine phosphorylation of a kinase-anchoring protein 3 and valosin-containing protein/p97 during capacitation." *Journal of Biological Chemistry* 278, no. 13 (2003b): 11579-11589.

[34] Jha, Kula Nand, and S. Shivaji. "Identification of the major tyrosine phosphorylated protein of capacitated hamster spermatozoa as a homologue of mammalian sperm a kinase anchoring protein." *Molecular Reproduction and Development* 61, no. 2 (2002a): 258-270.

[35] Jha, Kula Nand, and S. Shivaji. "Identification of the major tyrosine phosphorylated protein of capacitated hamster spermatozoa as a homologue of mammalian sperm a kinase anchoring protein." *Molecular Reproduction and Development* 61, no. 2 (2002b): 258-270.

[36] Kota, Janaiah, Chalonda R. Handy, Amanda M. Haidet, Chrystal L. Montgomery, Amy Eagle, Louise R. Rodino-Klapac, Danielle Tucker et al. "Follistatin gene delivery enhances muscle growth and strength in nonhuman primates." *Science translational medicine* 1, no. 6 (2009): 6ra15-6ra15.

[37] Mitra, Kasturi, and S. Shivaji. "Novel tyrosine-phosphorylated post-pyruvate metabolic enzyme, dihydrolipoamide dehydrogenase, involved in capacitation of hamster spermatozoa." *Biology of reproduction* 70, no. 4 (2004): 887-899.

[38] NagDas, Subir K., Virginia P. Winfrey, and Gary E. Olson. "Tyrosine phosphorylation generates multiple isoforms of the mitochondrial capsule protein, phospholipid hydroperoxide glutathione peroxidase (PHGPx), during hamster sperm capacitation." Biology of reproduction 72, no. 1 (2005): 164-171.

[39] Kushawaha, Bhawna, Rajkumar Singh Yadav, Dilip Kumar Swain, Pradeep K. Rai, and Satish Kumar Garg. "Mercury-induced inhibition of tyrosine phosphorylation of sperm proteins and altered functional dynamics of buck spermatozoa: An in vitro study." *Biological trace element research* (2020): 1-15.

[40] Kushawaha, Bhawna, Rajkumar Singh Yadav, Dilip Kumar Swain, Priyambada Kumari, Akhilesh Kumar, Brijesh Yadav, Mukul Anand, Sarvajeet Yadav, Dipty Singh, and Satish Kumar Garg. "Collapsed mitochondrial cristae in goat spermatozoa due to mercury result in lethality and compromised motility along with altered kinematic patterns." *Scientific reports* 11 (2021).

[41] Visconti, Pablo E., Linda R. Johnson, Maria Oyaski, Miguel Fornés, Stuart B. Moss, George L. Gerton, and Gregory S. Kopf. "Regulation, localization, and anchoring of protein kinase A subunits during mouse sperm capacitation." Developmental biology 192, no. 2 (1997): 351-363.

[42] Seshagiri, P. B., H. S. Lalitha, A. Mishra, and G. V. Sireesha. "Embryo-endometrial proteases during early mammalian development." (2003).

[43] Nolan, Michael A., Donner F. Babcock, Gunther Wennemuth, William Brown, Kimberly A. Burton, and G. Stanley McKnight. "Sperm-specific protein kinase A catalytic subunit Cα2 orchestrates cAMP signaling for male fertility." *Proceedings of the National Academy of Sciences* 101, no. 37 (2004): 13483-13488.

[44] Austin, C. R. "Observations on the penetration of the sperm into the mammalian egg." Australian journal of biological sciences 4, no. 4 (1951): 581-596.

[45] Yanagimachi, R. "Fertility of mammalian spermatozoa: its development and relativity." Zygote 2, no. 4 (1994): 371-372.

[46] Visconti, Pablo E., Janice L. Bailey, Grace D. Moore, Dieyun Pan, Patricia Olds-Clarke, and Gregory S. Kopf. "Capacitation of mouse spermatozoa. I. Correlation between the capacitation state and protein tyrosine phosphorylation." Development 121, no. 4 (1995): 1129-1137.

[47] Wertheimer, Eva V., Ana M. Salicioni, Weimin Liu, Claudia L. Trevino, Julio Chavez, Enrique O. Hernández-González, Alberto Darszon, and Pablo E. Visconti. "Chloride is essential for capacitation and for the capacitation-associated increase in tyrosine phosphorylation." Journal of Biological Chemistry 283, no. 51 (2008): 35539-35550.

[48] Jayaprakash, D., K. Santhosh Kumar, S. Shivaji, and P. B. Seshagiri. "Pentoxifylline induces hyperactivation and acrosome reaction in spermatozoa of golden hamsters: changes in motility kinematics." *Human reproduction (Oxford, England)* 12, no. 10 (1997): 2192-2199.

[49] Ain, Rupasri, K. Uma Devi, S. Shivaji, and P. B. Seshagiri. "Pentoxifylline-stimulated capacitation and acrosome reaction in hamster spermatozoa: involvement of intracellular signalling molecules." *Molecular human reproduction* 5, no. 7 (1999): 618-626.

[50] Carrera, Alfonso, Jiri Moos, Xiao Ping Ning, George L. Gerton, Jan Tesarik, Gregory S. Kopf, and Stuart B. Moss. "Regulation of protein tyrosine phosphorylation in human sperm by a calcium/calmodulin-dependent mechanism: identification of A kinase anchor proteins as major substrates for tyrosine phosphorylation." Developmental biology 180, no. 1 (1996): 284-296.

[51] Lefievre, Linda, Kula N. Jha, E. V. E. de LAMIRANDE, Pablo E. Visconti, and Claude Gagnon. "Activation of protein kinase A during human sperm capacitation and acrosome reaction." *Journal of andrology* 23, no. 5 (2002): 709-716.

[52] Miki, Kiyoshi, William D. Willis, Paula R. Brown, Eugenia H. Goulding, Kerry D. Fulcher, and Edward M. Eddy. "Targeted disruption of the Akap4 gene causes defects in sperm flagellum and motility." Developmental biology 248, no. 2 (2002): 331-342.

[53] Luconi, M., I. Porazzi, P. Ferruzzi, S. Marchiani, G. Forti, and E. Baldi. "Tyrosine phosphorylation of the a kinase anchoring protein 3 (AKAP3) and soluble adenylate cyclase are involved in the increase of human sperm motility by bicarbonate." Biology of reproduction 72, no. 1 (2005): 22-32.

[54] Kumar, Vivek, Nandini Rangaraj, and Sisinthy Shivaji. "Activity of pyruvate dehydrogenase A (PDHA) in hamster spermatozoa correlates positively with hyperactivation and is associated with sperm capacitation." Biology of reproduction 75, no. 5 (2006): 767-777.

[55] Hansen Naaby, Soren, Arabinda Mandal, Michael J. Wolkowicz, Buer Sen, V. Anne Westbrook, Jagathpala Shetty, Scott A. Coonrod et al. "CABYR, a novel calcium-binding tyrosine

phosphorylation-regulated fibrous sheath protein involved in capacitation." *Developmental biology* 242, no. 2 (2002): 236-254.

[56] Marín-Briggiler, Clara I., Kula N. Jha, Olga Chertihin, Mariano G. Buffone, John C. Herr, Mónica H. Vazquez-Levin, and Pablo E. Visconti. "Evidence of the presence of calcium/calmodulin-dependent protein kinase IV in human sperm and its involvement in motility regulation." Journal of Cell Science 118, no. 9 (2005): 2013-2022.

[57] Leclerc, Pierre, Eve de Lamirande, and Claude Gagnon. "Cyclic adenosine 3′, 5′ monophosphate-dependent regulation of protein tyrosine phosphorylation in relation to human sperm capacitation and motility." Biology of reproduction 55, no. 3 (1996): 684-692.

[58] Sharpe, Richard M., Chris McKinnell, Catrina Kivlin, and Jane S. Fisher. "Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood." REPRODUCTION CAMBRIDGE- 125, no. 6 (2003): 769-784.

[59] Thompson, Jennifer, and John Bannigan. "Cadmium: toxic effects on the reproductive system and the embryo." Reproductive toxicology 25, no. 3 (2008): 304-315.

[60] Ji, Yan-Li, Hua Wang, Ping Liu, Qun Wang, Xian-Feng Zhao, Xiu-Hong Meng, Tao Yu et al. "Pubertal cadmium exposure impairs testicular development and spermatogenesis via disrupting testicular testosterone synthesis in adult mice." Reproductive Toxicology 29, no. 2 (2010): 176-183.

[61] El-Demerdash, Fatma M., Mokhtar I. Yousef, Fatma S. Kedwany, and Hoda H. Baghdadi. "Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and β-carotene." Food and chemical toxicology 42, no. 10 (2004): 1563-1571.

[62] Boujbiha, Mohamed Ali, Khaled Hamden, Fadhel Guermazi, Ali Bouslama, Asma Omezzine, Abdelaziz Kammoun, and Abdelfattah El Feki. "Testicular toxicity in mercuric chloride treated rats: association with oxidative stress." *Reproductive toxicology* 28, no. 1 (2009): 81-89.

[63] Huang, Kai, Han Li, Bin Zhang, Tongzhang Zheng, Yuanyuan Li, Aifen Zhou, Xiaofu Du et al. "Prenatal cadmium exposure and preterm low birth weight in China." Journal of exposure science & environmental epidemiology 27, no. 5 (2017): 491-496.

[64] Lee, I. P., and R. L. Dixon. "Factors influencing reproduction and genetic toxic effects on male gonads." Environmental health perspectives 24 (1978): 117-127.

[65] Toft, Gunnar, and Louis J. Guillette Jr. "Decreased sperm count and sexual behavior in mosquitofish exposed to water from a pesticide-contaminated lake." Ecotoxicology and environmental safety 60, no. 1 (2005): 15-20.

[66] Marchlewicz, M., M. Protasowicki, L. Rozewicka, M. Piasecka, and M. Laszczyńska. "Effect of long-term exposure to lead on testis and epididymis in rats." Folia histochemica et cytobiologica 31, no. 2 (1993): 55-62.

[67] Wang, Yi-Xin, Peng Wang, Wei Feng, Chong Liu, Pan Yang, Ying-Jun Chen, Li Sun et al. "Relationships between seminal plasma metals/metalloids and semen quality, sperm apoptosis and DNA integrity." Environmental pollution 224 (2017): 224-234.

[68] Si, Yuming, and Makoto Okuno. "Role of tyrosine phosphorylation of flagellar proteins in hamster sperm hyperactivation." Biology of reproduction 61, no. 1 (1999): 240-246.

[69] Smith, Allan H., and Craig M. Steinmaus. "Health effects of arsenic and chromium in drinking water: recent human findings." Annual review of public health 30 (2009): 107-122.

[70] Tóth, G., T. Hermann, M. R. Da Silva, and L. Montanarella. "Heavy metals in agricultural soils of the European Union with implications for food safety." Environment international 88 (2016): 299-309.

[71] Zhang, Jingcheng, Pengxiang Qu, Chuan Zhou, Xin Liu, Xiaonan Ma, Mengyun Wang, Yongsheng Wang, Jianmin Su, Jun Liu, and Yong Zhang. "MicroRNA-125b is a key epigenetic regulatory factor that promotes nuclear transfer reprogramming." Journal of Biological Chemistry 292, no. 38 (2017): 15916-15926.

[72] Ghaffari, Mohammad Ali, and Behrooz Motlagh. "In vitro effect of lead, silver, tin, mercury, indium and bismuth on human sperm creatine kinase activity: a presumable mechanism for men infertility." *Iranian biomedical journal* 15, no. 1-2 (2011): 38.

[73] Hayati, Alfiah, Erika Wulansari, Dhea Sanggita Armando, Ari Sofiyanti, Muhammad Hilman Fu'adil Amin, and Manikya Pramudya. "Effects of in vitro exposure of mercury on sperm quality and fertility of tropical fish Cyprinus carpio L." *The Egyptian Journal of Aquatic* Research 45, no. 2 (2019): 189-195.

[74] Mohamed, Mostafa K., Thomas M. Burbacher, and N. Karle Mottet. "Effects of methyl mercury on testicular functions in Macaca fascicularis monkeys." *Pharmacology & toxicology* 60, no. 1 (1987): 29-36.

[75] Orisakwe, Orish E., Onyenmechi J. Afonne, Eddy Nwobodo, Lasbrey Asomugha, and Chudi E. Dioka. "Low-dose mercury induces testicular damage protected by zinc in mice." European Journal of Obstetrics & Gynecology and Reproductive Biology 95, no. 1 (2001): 92-96.

[76] Massanyi, Peter, Martin Massanyi, Roberto Madeddu, Robert Stawarz, and Norbert Lukac. "Effects of Cadmium, Lead, and Mercury on the Structure and Function of Reproductive Organs." Toxics 8, no. 4 (2020): 94.

[77] Siu, Erica R., Dolores D. Mruk, Catarina S. Porto, and C. Yan Cheng. "Cadmium-induced testicular injury." Toxicology and applied pharmacology 238, no. 3 (2009): 240-249.

[78] Henriques, Magda Carvalho, Susana Loureiro, Margarida Fardilha, and Maria Teresa Herdeiro. "Exposure to mercury and human reproductive health: A systematic review." Reproductive toxicology 85 (2019): 93-103.

[79] Choy, Christine MY, Christopher WK Lam, Lorena TF Cheung, Christine M. Briton-Jones, L. P. Cheung, and Christopher J. Haines. "Infertility, blood mercury concentrations and dietary seafood consumption: a case–control study." *BJOG: An International Journal of Obstetrics & Gynaecology* 109, no. 10 (2002): 1121-1125.

[80] Dickman, M. D., and K. M. C. Leung. "Mercury and organochlorine exposure from fish consumption in Hong Kong." Chemosphere 37, no. 5 (1998): 991-1015.

[81] Keck, Christoph, Martin Bergmann, Erik Ernst, Cornelia Müller, Sabine Kliesch, and Eberhard Nieschlag. "Autometallographic detection of mercury in testicular tissue of an infertile man exposed to mercury vapor." Reproductive Toxicology 7, no. 5 (1993): 469-475.

[82] Baldi, Elisabetta, Michaela Luconi, Lorella Bonaccorsi, Monica Muratori, and Gianni Forti. "Intracellular events and signaling pathways involved in sperm acquisition of fertilizing capacity and acrosome reaction." Front Biosci 5, no. 1 (2000): 110-123.

[83] De Lamirande, E., and C. Gagnon. "The extracellular signal-regulated kinase (ERK) pathway is involved in human sperm function and modulated by the superoxide anion." Molecular human reproduction 8, no. 2 (2002): 124-135.

[84] Fu, Jieli, Yuhua Li, Lirui Wang, Linqing Zhen, Qiangzhen Yang, Peifei Li, and Xinhong Li. "Bovine serum albumin and skim-milk improve boar sperm motility by enhancing energy metabolism and protein modifications during liquid storage at 17 C." Theriogenology 102 (2017): 87-97.

[85] Demarco, Ignacio A., Felipe Espinosa, Jennifer Edwards, Julian Sosnik, Jose Luis De la Vega-Beltran, Joel W. Hockensmith, Gregory S. Kopf, Alberto Darszon, and Pablo E. Visconti. "Involvement of a Na+/HCO 3– cotransporter in mouse sperm capacitation." *Journal of Biological Chemistry* 278, no. 9 (2003): 7001-7009.

[86] Krasznai, Zoltán, Masaaki Morisawa, Zoárd Tibor Krasznai, Sachiko Morisawa, Kazuo Inaba, Zsuzsa Kassai Bazsáné, Bálint Rubovszky, Béla Bodnár, Antal Borsos, and Teréz Márián. "Gadolinium, a mechano-sensitive channel blocker, inhibits osmosis-initiated motility of sea-and freshwater fish sperm, but does not affect human or ascidian sperm motility." *Cell motility and the cytoskeleton* 55, no. 4 (2003): 232-243.

[87] Grasa, Patricia, Carmen Colas, Margarita Gallego, Luís Monteagudo, Teresa Muino-Blanco, and José Alvaro Cebrián-Pérez. "Changes in content and localization of proteins phosphorylated at tyrosine, serine and threonine residues during ram sperm capacitation and acrosome reaction." *Reproduction* 137, no. 4 (2009): 655.

[88] Liguori, L., E. De Lamirande, A. Minelli, and C. Gagnon. "Various protein kinases regulate human sperm acrosome reaction and the associated phosphorylation of Tyr residues and of the Thr-Glu-Tyr motif." Molecular human reproduction 11, no. 3 (2004): 211-221.

[89] Urner, Françoise, and Denny Sakkas. "Protein phosphorylation in mammalian spermatozoa." Reproduction 125, no. 1 (2003): 17-26.

[90] Visconti, P. E., V. A. Westbrook, O. Chertihin, I. Demarco, S. Sleight, and A. B. Diekman. "Novel signaling pathways involved in sperm acquisition of fertilizing capacity." Journal of reproductive immunology 53, no. 1-2 (2002): 133-150.

[91] Martinez, Caroline S., Alyne G. Escobar, João Guilherme D. Torres, Daniela S. Brum, Francielli W. Santos, María J. Alonso, Mercedes Salaices et al. "Chronic exposure to low doses of mercury impairs sperm quality and induces oxidative stress in rats." *Journal of Toxicology and Environmental Health, Part A* 77, no. 1-3 (2014): 143-154.

[92] Arabi, M. "The role of mercury in the etiology of sperm dysfunction in holstein bulls." Asian-australasian journal of animal sciences 19, no. 3 (2006): 335-340.

[93] Arabi, M., and M. S. Heydarnejad. "In vitro mercury exposure on spermatozoa from normospermic individuals." Pakistan Journal of Biological Sciences 10, no. 15 (2007): 2448-2453.

[94] Van Look, K. J. W., and D. E. Kime. "Automated sperm morphology analysis in fishes: the effect of mercury on goldfish sperm." Journal of Fish Biology 63, no. 4 (2003): 1020-1033.

[95] Vergílio, C. S., R. V. Moreira, C. E. V. Carvalho, and E. J. T. Melo. "Effects of in vitro exposure to mercury on male gonads and sperm structure of the tropical fish tuvira G ymnotus carapo (L.)." *Journal of fish diseases* 37, no. 6 (2014): 543-551.

[96] Mocevic, Emina, Ina O. Specht, Jacob L. Marott, Aleksander

Giwercman, Bo AG Jönsson, Gunnar Toft, Thomas Lundh, and Jens Peter Bonde. "Environmental mercury exposure, semen quality and reproductive hormones in Greenlandic Inuit and European men: a cross-sectional study." Asian journal of andrology 15, no. 1 (2013): 97.

[97] Chan, Celia HY, Ernest HY Ng, Cecilia LW Chan, and Timothy HY Chan. "Effectiveness of psychosocial group intervention for reducing anxiety in women undergoing in vitro fertilization: a randomized controlled study." Fertility and sterility 85, no. 2 (2006): 339-346

[98] Batlle-Bayer, Laura, Alba Bala, Elodie Lemaire, Jaume Albertí, Isabel García-Herrero, Rubén Aldaco, and Pere Fullana-i-Palmer. "An energy-and nutrient-corrected functional unit to compare LCAs of diets." Science of The Total Environment 671 (2019): 175-179.

[99] Jarup, Lars. "Cadmium overload and toxicity." Nephrology Dialysis Transplantation 17, no. suppl_2 (2002): 35-39

[100] Dias, Maria Celeste, Cristina Monteiro, José Moutinho-Pereira, Carlos Correia, Berta Gonçalves, and Conceição Santos. "Cadmium toxicity affects photosynthesis and plant growth at different levels." *Acta physiologiae plantarum* 35, no. 4 (2013): 1281-1289.

[101] Chaney, Rufus L. "Food safety issues for mineral and organic fertilizers." Advances in Agronomy 117 (2012): 51-116.

[102] Singh, Priyanka, Husna Siddiqui, Fareen Sami, Yamshi Arif, Andrzej Bajguz, and Shamsul Hayat. "Cadmium: A Threatening Agent for Plants." In Plant Responses to Soil Pollution, pp. 59-88. Springer, Singapore, 2020.

[103] Satarug, Soisungwan. "Cadmium sources and toxicity." (2019): 25.

[104] Matovic, Vesna, Aleksandra Buha, Danijela Đukić-Ćosić, and Zorica Bulat. "Insight into the oxidative stress induced by lead and/or cadmium in blood, liver and kidneys." Food and Chemical Toxicology 78 (2015): 130-140.

[105] Takiguchi, Masufumi, William E. Achanzar, Wei Qu, Guying Li, and Michael P. Waalkes. "Effects of cadmium on DNA-(Cytosine-5) methyltransferase activity and DNA methylation status during cadmium-induced cellular transformation." Experimental cell research 286, no. 2 (2003): 355-365.

[106] Geng, Hui-Xia, and Lai Wang. "Cadmium: Toxic effects on placental and embryonic development." Environmental toxicology and pharmacology 67 (2019): 102-107.

[107] Zhu, Qiqi, Xiaoheng Li, and Ren-Shan Ge. "Toxicological effects of cadmium on mammalian testis." Frontiers in Genetics 11 (2020): 527.

[108] Meeker, John D., Mary G. Rossano, Bridget Protas, Michael P. Diamond, Elizabeth Puscheck, Douglas Daly, Nigel Paneth, and Julia J. Wirth. "Cadmium, lead, and other metals in relation to semen quality: human evidence for molybdenum as a male reproductive toxicant." Environmental health perspectives 116, no. 11 (2008): 1473-1479.

[109] Oliveira, Helena, Marcello Spanò, Conceição Santos, and Maria de Lourdes Pereira. "Adverse effects of cadmium exposure on mouse sperm." Reproductive Toxicology 28, no. 4 (2009): 550-555.

[110] Rana, S. V. S. "Perspectives in endocrine toxicity of heavy metals—a review." Biological trace element research 160, no. 1 (2014): 1-14.

[111] Shojaeepour, Saeedeh, Fariba Sharififar, Tahereh Haghpanah, Maryam Iranpour, Masoud Imani, and Shahriar Dabiri. "Panax ginseng ameliorate toxic effects of cadmium on germ cell apoptosis, sperm quality, and oxidative stress in male Wistar rats." Toxin Reviews (2021): 1-13.

[112] Obembe, Olawale O., and Yunus Raji. "Effects of aqueous extract of Moringa oleifera seed on cadmium-induced reproductive toxicity in male Wistar rats." African health sciences 18, no. 3 (2018): 653-663.

[113] Leoni, Giovanni, Luisa Bogliolo, Gianni Deiana, Fiammetta Berlinguer, Irma Rosati, Pier Paolo Pintus, Sergio Ledda, and Salvatore Naitana. "Influence of cadmium exposure on in vitro ovine gamete dysfunction." Reproductive toxicology 16, no. 4 (2002): 371-377.

[114] Roychowdhury, A., and A. K. Gautam. "Alteration of human sperm and other seminal constituents after lead exposure." Indian journal of physiology and allied sciences, no. 49 (1995): 68-73.

[115] Heidari, Amir Hassan, Mohammad Javad Zamiri, Mohammad Naser Nazem, Mohammad Reza Jafarzadeh Shirazi, Amir Akhlaghi, and Zarbakht Ansari Pirsaraei. "Detrimental effects of long-term exposure to heavy metals on histology, size and trace elements of testes and sperm parameters in Kermani Sheep." *Ecotoxicology and Environmental Safety* 207 (2021): 111563.

[116] Saleh, Ramadan A., and ASHOK AGARWAL HCLD. "Oxidative stress and male infertility: from research bench to clinical practice." Journal of andrology 23, no. 6 (2002): 737-752.

[117] Kiziler, Ali Riza, Birsen Aydemir, Ilhan Onaran, Bulent Alici, Hamdi Ozkara, Tevfik Gulyasar, and Mehmet Can Akyolcu. "High levels of cadmium and lead in seminal fluid and blood of smoking men are associated with high oxidative stress and damage in infertile subjects." *Biological trace element research* 120, no. 1 (2007): 82-91.

[118] Li, Jin-Long, Rui Gao, Shu Li, Jin-Tao Wang, Zhao-Xin Tang, and Shi-Wen Xu. "Testicular toxicity induced by dietary cadmium in cocks and ameliorative effect by selenium." Biometals 23, no. 4 (2010): 695-705.

[119] Choong, Grace, Ying Liu, and Douglas M. Templeton. "Interplay of calcium and cadmium in mediating cadmium toxicity." Chemico-biological interactions 211 (2014): 54-65.

[120] Templeton, Douglas M., and Ying Liu. "Multiple roles of cadmium in cell death and survival." Chemico-biological interactions 188, no. 2 (2010): 267-275.

[121] Zhao, Li-lin, Yan-fei Ru, Miao Liu, Jia-nan Tang, Ju-fen Zheng, Bin Wu, Yi-hua Gu, and Hui-juan Shi. "Reproductive effects of cadmium on sperm function and early embryonic development in vitro." PloS one 12, no. 11 (2017): e0186727.

[122] Wang, Xinghao, Ruijuan Qu, Jiaoqin Liu, Zhongbo Wei, Liansheng Wang, Shaogui Yang, Qingguo Huang, and Zunyao Wang. "Effect of different carbon nanotubes on cadmium toxicity to Daphnia magna: The role of catalyst impurities and adsorption capacity." Environmental Pollution 208 (2016): 732-738.

[123] Nair, Ambily Ravindran, Olivier DeGheselle, Karen Smeets, Emmy Van Kerkhove, and Ann Cuypers. "Cadmium-induced pathologies: where is the oxidative balance lost (or not)?." *International journal of molecular sciences* 14, no. 3 (2013): 6116-6143.

[124] Rinaldi, Mariagrazia, Antonio Micali, Herbert Marini, Elena B. Adamo, Domenico Puzzolo, Antonina Pisani, Vincenzo Trichilo, Domenica Altavilla, Francesco Squadrito, and Letteria Minutoli. "Cadmium, organ toxicity and therapeutic approaches: a review on brain, kidney and testis damage." *Current medicinal chemistry* 24, no. 35 (2017): 3879-3893

[125] Li, Xinhong, Lirui Wang, Yuhua Li, Na Zhao, Linqing Zhen, Jieli Fu, and Qiangzhen Yang. "Calcium regulates motility and protein phosphorylation by changing cAMP and ATP concentrations

in boar sperm in vitro." Animal reproduction science 172 (2016): 39-51.

[126] Kitchin KT, Wallace K. 2008. Evidence against the nuclear in situ binding of arsenicals—oxidative stress theory of arsenic carcinogenesis. Toxicol Appl Pharmacol232(2):252-25718671993.

[127] Mazumder, D.N.G. (2008) Chronic arsenic toxicity and human health. Indian J Med Res 128:436-447.

[128] Centeno, J.A., Mullick, F.G., Martinez, L., Page, N.P., Gibb, H., Longfellow, D. (2002) Pathology related to chronic arsenic exposure. Environ Health Perspect 110:883-886.

[129] Pant, N., Murthy, R.C. and Srivastava, S.P. (2004) Male reproductive toxicity of sodium arsenite in mice. Hum Exp Toxicol 23:399-403.

[130] Sarkar, M., Biswas, N.M. and Ghosh, D. Effect of sodium arsenite on testicular 5-3,17-HSD activities in albino rats: Dose and duration dependent responses. Medical Science and Research, no 19(1991):789-790.

[131] Hossain, Khaled, Anwarul A. Akhand, Masashi Kato, Jun Du, Kozue Takeda, Jianghong Wu, Kei Takeuchi, Wei Liu, Haruhiko Suzuki, and Izumi Nakashima. "Arsenite induces apoptosis of murine T lymphocytes through membrane raft-linked signaling for activation of c-Jun amino-terminal kinase." The Journal of Immunology 165, no. 8 (2000): 4290-4297.

[132] Chen, Wei, Jennifer L. Martindale, Nikki J. Holbrook, and Yusen Liu. "Tumor promoter arsenite activates extracellular signal-regulated kinase through a signaling pathway mediated by epidermal growth factor receptor and Shc." Molecular and Cellular Biology 18, no. 9 (1998): 5178.

[133] Wu, Weidong, Lee M. Graves, Ilona Jaspers, Robert B. Devlin, William Reed, and James M. Samet. "Activation of the EGF receptor signaling pathway in human airway epithelial cells exposed to metals." American Journal of Physiology-Lung Cellular and Molecular Physiology 277, no. 5 (1999): L924-L931.

[134] Hu, Yuxin, Jin Li, Bin Lou, Ruirui Wu, Gang Wang, Chunwei Lu, Huihui Wang, Jingbo Pi, and Yuanyuan Xu. "The role of reactive oxygen species in arsenic toxicity." Biomolecules 10, no. 2 (2020): 240.

[135] Suc, Isabelle, Olivier Meilhac, Isabelle Lajoie-mazenc, Jean Vandaele, Günther Jurgens, Robert Salvayre, and Anne Nègre-salvayre. "Activation of EGF receptor by oxidized LDL." The FASEB Journal 12, no. 9 (1998): 665-671.

[136] Wu, Weidong, Ilona Jaspers, Wenli Zhang, Lee M. Graves, and James M. Samet. "Role of Ras in metal-induced EGF receptor signaling and NF-κB activation in human airway epithelial cells." American Journal of Physiology-Lung Cellular and Molecular Physiology 282, no. 5 (2002): L1040-L1048.

[137] Kodigepalli, Karthik M., Punashi S. Dutta, Kyle A. Bauckman, and Meera Nanjundan. "SnoN/SkiL expression is modulated via arsenic trioxide-induced activation of the PI3K/AKT pathway in ovarian cancer cells." FEBS letters 587, no. 1 (2013): 5-16.

[138] Simeonova, Petia P., Shiyi Wang, Tracy Hulderman, and Michael I. Luster. "c-Src-dependent activation of the epidermal growth factor receptor and mitogen-activated protein kinase pathway by arsenic: Role in carcinogenesis." Journal of Biological Chemistry 277, no. 4 (2002): 2945-2950.

[139] Biscardi, Jacqueline S., Ming-Chei Maa, David A. Tice, Michael E. Cox, Tzeng-Horne Leu, and Sarah J. Parsons. "c-Src-mediated phosphorylation of the epidermal growth factor receptor on Tyr845 and Tyr1101 is associated with

modulation of receptor function." Journal of Biological Chemistry 274, no. 12 (1999): 8335-8343.

[140] Tice, D.A., Biscardi, J.S., Nickles, A.L. and Parsons, S.J., 1999. Mechanism of biological synergy between cellular Src and epidermal growth factor receptor. Proceedings of the National Academy of Sciences, *96*(4), pp.1415-1420.

[141] Renu, Kaviyarasi, Harishkumar Madhyastha, Radha Madhyastha, Masugi Maruyama, Sathishkumar Vinayagam, and Abilash Valsala Gopalakrishnan. "Review on molecular and biochemical insights of arsenic-mediated male reproductive toxicity." Life sciences 212 (2018): 37-58.

[142] Shim, Juyoung, Rachel H. Kennedy, Lisa M. Weatherly, Lee M. Hutchinson, Jonathan H. Pelletier, Hina N. Hashmi, Kayla Blais, Alejandro Velez, and Julie A. Gosse. "Arsenic inhibits mast cell degranulation via suppression of early tyrosine phosphorylation events." Journal of Applied Toxicology 36, no. 11 (2016): 1446-1459.

[143] Huang, Qingyu, Lianzhong Luo, Ambreen Alamdar, Jie Zhang, Liangpo Liu, Meiping Tian, Syed Ali Musstjab Akber Shah Eqani, and Heqing Shen. "Integrated proteomics and metabolomics analysis of rat testis: mechanism of arsenic-induced male reproductive toxicity." Scientific reports 6, no. 1 (2016a): 1-12.

[144] Q. Huang, L. Luo, A. Alamdar, J. Zhang, L. Liu, M. Tian, S.A.M.A.S. Eqani, H. Shen, Integrated proteomics and metabolomics analysis of rat testis: mechanism of arsenic-induced male reproductive toxicity, Sci. Rep. 6 (2016b) 32518, https://doi. org/10.1038/srep32518.

[145] Gavaghan, H., 2002. Lead, unsafe at any level. Bulletin of the World Health Organization, *80*, pp.82-82.

[146] Li, Na, Yu-hua Hou, Dan-dan Ma, Wei-xin Jing, Hans-Uwe Dahms, and Lan Wang. "Lead accumulation, oxidative damage and histopathological alteration in testes and accessory glands of freshwater crab, Sinopotamon henanense, induced by acute lead exposure." Ecotoxicology and environmental safety 117 (2015): 20-27.

[147] Oldereid, N. B., Y. Thomassen, A. Attramadal, B. Olaisen, and K. Purvis. "Concentrations of lead, cadmium and zinc in the tissues of reproductive organs of men." Reproduction 99, no. 2 (1993): 421-425.

[148] Rahman, Zeeshanur, and Ved Pal Singh. "The relative impact of toxic heavy metals (THMs) (arsenic (As), cadmium (Cd), chromium (Cr) (VI), mercury (Hg), and lead (Pb)) on the total environment: an overview." *Environmental monitoring and assessment* 191, no. 7 (2019): 1-21.

[149] Gandhi, Jason, Rafael J. Hernandez, Andrew Chen, Noel L. Smith, Yefim R. Sheynkin, Gargi Joshi, and Sardar Ali Khan. "Impaired hypothalamic-pituitary-testicular axis activity, spermatogenesis, and sperm function promote infertility in males with lead poisoning." *Zygote* 25, no. 2 (2017): 103-110.

[150] Hassan, Eman, Khaled Kahilo, Tarek Kamal, Marwa Hassan, and Mohamed Saleh Elgawish. "The protective effect of epigallocatechin-3-gallate on testicular oxidative stress in lead-induced toxicity mediated by Cyp19 gene/estradiol level." Toxicology 422 (2019): 76-83.

[151] Bridges, Christy C., and Rudolfs K. Zalups. "Molecular and ionic mimicry and the transport of toxic metals." Toxicology and applied pharmacology 204, no. 3 (2005): 274-308.

[152] He, Yuanqiao, Qianxing Zou, Houyang Chen, Shiqi Weng, Tao Luo, and Xuhui Zeng. "Lead inhibits human sperm functions by reducing the levels of intracellular calcium, cAMP, and

tyrosine phosphorylation." The Tohoku journal of experimental medicine 238, no. 4 (2016): 295-303.

[153] Tamburrino, L., S. Marchiani, E. Vicini, B. Muciaccia, M. Cambi, S. Pellegrini, G. Forti, M. Muratori, and E. Baldi. "Quantification of CatSper1 expression in human spermatozoa and relation to functional parameters." Human Reproduction 30, no. 7 (2015): 1532-1544.