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# Effect of Heavy Metals on Tyrosine Kinases Signaling during Sperm Capacitation

*Bhawna Kushwaha, Rohit Beniwal, Aradhana Mohanty, Ajay Kumar Singh, Raj Kumar Yadav and Satish Kumar Garg*

## 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  $\text{Ca}^{2+}$  and  $\text{HCO}_3^-$ -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  $\text{Ca}^{++}$  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, carcinogenicity, 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.,  $Na^+$ ,  $K^+$ ,  $Cl^-$ ,  $HCO_3^-$ ,  $Mg^{2+}$ ,  $Ca^{2+}$ , 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 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  $C\alpha 2$ , 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  $\mu\text{g}/\text{mL}$ ) leads to significant increase in spontaneous acrosome reaction, intracellular  $\text{Ca}^{2+}$  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  $\mu\text{g}/\text{kg}$  to 0.07  $\mu\text{g}/\text{kg}/\text{day}$  subsequent dose of  $\text{HgCl}_2$  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],  $\text{HgCl}_2$  at 50 to 550  $\mu\text{M}$  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  $\mu\text{M}$  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 *Carassius auratus* spermatozoa after mercury exposure (100 to 368  $\mu\text{M}$ ) 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  $\text{mg}/\text{l}$  (3.68 and 36.8  $\mu\text{M}$ ), respectively. After 24 h exposure to 0.001  $\text{mg}/\text{l}$  (0.0037  $\mu\text{M}$ )  $\text{HgCl}_2$ , flagellar length was significantly reduced in 38% of the spermatozoa. Following exposure to 0.1  $\text{mg}/\text{l}$  (0.37  $\mu\text{M}$ ) 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  $\text{mg}/\text{l}$  (0.37  $\mu\text{M}$ ) 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  $\mu\text{M}$  - 30  $\mu\text{M}$ ) on testes and sperms of tropical fish (*Gymnotus carapo*) and showed decrease in the sperm count (36.8%) after 20  $\mu\text{M}/24$  h treatment and subsequent decrease (48.7%) was observed after 20  $\mu\text{M}/96$  h. Hg (20  $\mu\text{M}$ ) 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/ml) 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 ( $\beta = 50.074$ , 95% confidence interval (CI) = 50.021 to 0.126) and in an analysis including men from all three regions ( $\beta = 50.067$ , 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 ( $P < .05$ ) 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<sub>3</sub> 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 excretion rate. It can accumulate into the non-smoking population via fumes, dust, contaminated food and water and it is widely used 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]. Cd 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<sup>-1</sup>) 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<sup>2+</sup>/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/threonine residue of tyrosine kinase [119]. Wang et al. [122] reported that 10  $\mu$ M 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- $\beta$ 3 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). These altered proteins were related to spermatogenesis, fertilization, fertility, and mating behavior which may be mediated by the ERK/AKT/NF- $\kappa$ B-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  $\text{Ca}^{2+}$  mediated process [151].

Capacitation is highly  $\text{Ca}^{2+}$  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  $\mu\text{M}$  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 ( $\text{Ca}^{2+}$ )<sub>i</sub>, 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, ( $\text{Ca}^{2+}$ )<sub>i</sub> and tyrosine phosphorylation of sperm proteins *in-vitro*. Voltage-dependent  $\text{Ca}^{2+}$  channels, known as Catsper, are mainly involved in regulation of capacitation by mediating  $\text{Ca}^{2+}$  influx [153]. Therefore, it can be postulated that Pb exposure decreases intracellular  $\text{Ca}^{2+}$  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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