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STRUCTURAL ALTERATION OF SPERMATOZOA IN THE PERSONS EMPLOYED IN LEAD ACID BATTERY FACTORY

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Abstract : Lead is one of the industrially heavy metals that caused adverse effects on male reproductive system among battery factory workers, but information on the possible impact of lead on the structural integrity of sperm cell is limited. Thus present study was undertaken to assess the structural details of human spermatozoa of lead acid battery factory workers. Blood and semen samples were collected from total 80 workers (7-15 years exposure) and 40 non-occupationally exposed control subjects. The lead exposed battery factory workers showed lowering ($P<0.001$) of sperm count, density, motility and semen volume along with an increase incidence of sperm abnormality and prolong liquefaction time. Structural alteration of sperm cell was prevalent among the exposed population as evidenced by significantly ($P<0.001$) low sperm viability, low hypoosmotic swelling test (HOST) percentage, high lipid peroxidation of sperm membrane with concomitant alterations of seminal plasma total and dehydro ascorbate level. Sharp depressions, membrane folding and granularity at sperm head surfaces were observed by scanning electron microscopy (SEM). Both blood lead and semen lead was significantly ($P<0.001$) higher among the factory workers. Thus it appears plausible that lead may reduce the antioxidant level in seminal plasma and enhance the lipid peroxidative changes in sperm membrane leading to concomitant structural damage of sperm cell surface in the workers employed in lead acid battery factories.

Key words : sperm morphology lipid peroxidation vitamin C blood lead semen lead battery factory workers

INTRODUCTION

Lead caused alteration of male reproductive system in experimental animals and exposed factory workers including high blood lead level, reduced semen quality and quantity of sperm cell along with high percentage of abnormality

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(1–3). Lead exposure and moderate lead absorption produces alteration in fertility with decreased production in spermatozoa in the battery factory workers probably due to the direct toxic effect of lead on germinal epithelium of testis during spermatogenesis (4–5). The reduction in fecundity was reported among 1349 battery factory workers in Denmark where exposure level was more than 10 years (6). Reduction in sperm motility, count, density and low antioxidant profile along with increase incidence of sperm abnormality and sperm membrane lipid peroxidation was prevalent after occupational lead exposure (7–9). The action of seminal antioxidant in spermatozoal lipid peroxidation is essential for the maintenance of native structure, so complete loss of antioxidant action may leading to spermatozoal denaturation during lipid peroxidation (10).

Interference of inorganic lead to the hypothalamic-pituitary-gonadal axis has been the subject of great controversy since lead may have an indirect or secondary effect on the endocrine axis (11). The extent of damage including depression of testicular and endocrine function after moderate exposure of lead was reported by Cullen et. al. (12). Reported evidence of lead induced effect on men with blood lead concentration of more than 40 µg/dl including altered testosterone concentration, decreased libido, reduction in sperm count, alive spermatozoa and sperm activity, chromosomal damage, abnormal prostatic function and finally infertility (13). But the reports of Telisman et. al. (14) revealed significant decrease in human semen quality after moderate exposure to lead and cadmium, but no relevant effects was detected on the reproductive hormone levels. Earlier reports also supported the above findings (15, 16). In the light of ongoing development of this research it is now necessary to accumulate more findings in this subject. Therefore, this study was undertaken to assess the structural details of human sperm of lead acid battery factory workers. However, this study is the first of its kind in India, which deals with the structural alteration of spermatozoa in battery factory workers, routinely exposed to lead fumes and lead dusts.

METHODS

Prior to study, the clearance was obtained from the ethical committee (ICMR, Govt. of India). 40 non-occupationally exposed control subjects (group I) and 80 exposed workers of active reproductive age (31–45 years), 55 ± 5 kg weight and 160 ± 5 cm height were randomly selected from the lead acid battery factories in Kolkata. They were divided into two groups depending on the duration of exposure: (1) low exposed group with 7–10 years exposure for 8 hours/day (Group II : $n = 30$) and (2) high exposed group for 8 hours/day exposure over a period of more than 10–15 years (Group III : $n = 50$). Using interview technique as a tool for data collection, detail information of the subjects were recorded on the pre designed proforma that includes age, socio-economic status, working schedule, duration of work, use of protective devices, smoking and other addiction history, marital status and number of children, use of contraceptive devices, history of disease of the individual subjects and his family.

Collection of biological sample

Semen samples were collected from the subjects of the three groups in a clean, dry, sterilized, wide mouth, well stopper glass vial by masturbation after 2–5 days of abstinence (17). One part of semen was stored at –20°C in lead free storage vial for measurement of lead content. 2 ml of morning, fasting, venous blood was collected aseptically and stored at –20°C in lead free EDTA vial for metal analysis.

1. Morphological analysis of spermatozoa: Morphological analysis is essential for evaluating the fertility potential of the subjects after lead exposure. Sperm density, total count, motility and morphology were measured following WHO recommended protocol (17). Whole liquefying semen was mixed with eosin and smear was made to observe the viability of spermatozoa by dye exclusion vital staining technique, which indicates structural integrity of sperm head membrane as dead cells became red after penetration of eosin through the damage sperm head membrane (18). Hypoosmotic swelling test (HOST) indicated structural integrity and compliance of sperm tail membrane as water entre the live cells through intact membrane in an attempt to reach the osmotic equilibrium when exposed to hypoosmotic medium. Sperm cells showing coiled tail after incubating at 37°C for 30 minutes in the hypoosmotic solution were considered as HOST positive (19). All the morphological parameters were measured at 400x magnification (Model: Ch20i, Olympus, India).

2. Biochemical analysis: After liquefaction, whole semen was centrifuged at 800xg for 10 minutes and sperms were separated from seminal plasma. Sperm membrane lipid peroxidation was determined at 530 nm (Model: DU 64, Beckman Spectrophotometer, USA) by measuring the amount of malonaldehyde-thiobarbiturate complex (20). Total and dehydro ascorbate concentration were measured spectrophotometrically at 540 nm by DNPH-thiourea reagent in concentrated acid medium with and without bromination of the protein free seminal plasma respectively (21).

3. Scanning electron microscopy (SEM) of spermatozoa: Based on morphological data, selected samples were fixed by glutaraldehyde and subsequently washed with phosphate buffered saline at pH 7.4 followed by dehydration in graded ethanol series. Air-dried samples were then gold-coated using sputtering technique (22) and images were acquired at 15000x, 5000x, 7500x and 10000x magnifications (Model: JSM 5200, Jeol, Japan).

4. Lead analysis: Blood and semen samples were digested for 20 minutes with 65% suprapure nitric acid and 30% hydrogen peroxide in the digestion chamber of Ethos D Microwave Labstation with terminal 20 operating system (Milestone srl, Italy) followed by solution in distilled water. Then absorbance was taken at 283.3 nm using atomic absorption spectrophotometer (Model: GBC AVANTA AAS, software version 1.33, Australia) attached with GF 3000 graphite furnace to note the body burden of lead after exposure at work place, essential for exploring the in-depth mechanism of action (23).

The data obtained from control and exposed groups were compared and one-way ANOVA and Scheffe's 'F' test were carried

out for level of significance following the computer based statistical software SPSS, version 10.0 for Windows (SPSS Inc., USA).

RESULTS

According to questionnaire all the workers were married. The average marriage life was 4 years and the couples never used any contraceptive measures throughout the period. All the subjects belonged to low socio-economic status according to modified Kuppuswamy's socio-economic classification (24). There was no reproductive disease history among the working and control population, but 40% workers were issueless and visited to reproductive clinics for fertility problem. Smoking (out of 83% smokers, 50% used bidi and rest 33% used cigarette), alcohol consumption (100%) and use of gutkha/ panparag (17%) were predominant among the battery factory workers (group II and III) as compared to the control subjects (group I: out of 15% smokers, 7% used bidi and 8% used cigarette; 2% used alcohol and 1.5% used gutkha/panparag occasionally).

Prolonged liquefaction and reduced semen volume was predominant in both the exposed groups with respect to control and in between the two exposed groups ($P<0.001$). Control and low exposed subjects were normospermic and high exposed subjects were hypospermic in nature as per the reference value (25). There was no sign of hyperspermia after lead exposure. Seminal viscosity was significantly decreased in group III compared with group I ($P<0.001$), but not in group II (Table I).

TABLE I: Effects of lead on human semen quality of lead acid battery factory workers.

Parameters	*Control (group I)*	*Low exposed (group II)*	*High exposed (group III)*
Liquefaction time (minutes)	15±0.88 (10)	24.35±0.56^{a1} (20)	33.76±0.97$^{a1\ b1}$ (25)
Seminal viscosity (mm)	2.46±0.15 (15)	2.04±0.18^{aNS} (15)	1.53±0.20$^{a1\ bNS}$ (20)
Seminal volume (ml)	4.65±0.16 (20)	2.61±0.10^{a1} (30)	1.36±0.06$^{a1\ b1}$ (30)
Sperm density (million/ml)	137±7.20 (30)	74.70±2.44^{a1} (40)	28.97±1.94$^{a1\ b1}$ (30)
Sperm count (million)	391.50±12.75 (22)	177.72±10.27^{a1} (25)	54.63±4.81$^{a1\ b1}$ (30)
Gross sperm motility (%)	79±2.20 (15)	59.91±1.05^{a1} (34)	31.08±1.76$^{a1\ b1}$ (49)
Gross sperm abnormality (%)	33.75±1.09 (20)	44.54±0.57^{a1} (26)	60.04±1.53$^{a1\ b1}$ (26)
Total head abnormality (%)	44.61±1.28 (16)	54.90±0.68^{a1} (23)	67.37±1.23$^{a1\ b1}$ (21)
Total mid piece abnormality (%)	23.05±0.62 (32)	33.53±0.72^{a1} (17)	45.45±1.39$^{a1\ b1}$ (10)
Total tail abnormality (%)	39.76±1.04 (18)	50.76±0.70^{a1} (28)	65.20±1.18$^{a1\ b1}$ (13)

$^{a1}P<0.001$, ^{aNS}Nonsignificant at $P<0.05$ (compared with group I); $^{b1}P<0.001$, ^{bNS}Nonsignificant at $P<0.05$ (compared with group II). Number in the parenthesis indicates sample size of individual parameter in each group.

Reduced sperm density, count and gross motility was observed among the working group II and III with respect to control group I and in between the two exposed groups (P<0.001). Increased incidence of gross sperm abnormality in group II and III was also observed (P<0.001). The percentage value of low exposed group was slightly higher than the reference value, but high exposed group was teratospermic (25). The control group value was within the normal range. The present study also showed that total sperm head, mid piece and tail abnormality was increased significantly in group II and III workers with respect to group I and in between the two exposed groups (P<0.001) (Table I).

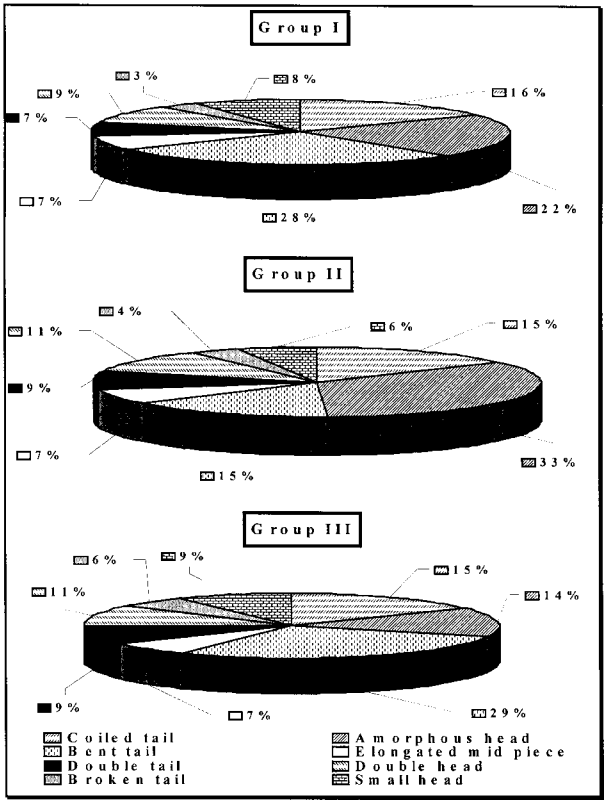


Fig. 1 : Percentage (%) of detail sperm abnormality of lead exposed workers (group II and III) and control subjects (group I). Significant at P<0.001.

Fig. 1 represented the percentage of detail sperm abnormality of lead exposed workers of group II (low exposed) and III (high exposed) as well as non-occupationally exposed control subjects of group I. The variations of amorphous head, double head and coiled tail were well documented in both the exposed groups as compared to control, whereas broken tail and small head abnormality were comparatively not varied much.

Sperm viability and HOST percentage was significantly reduced in both the exposed

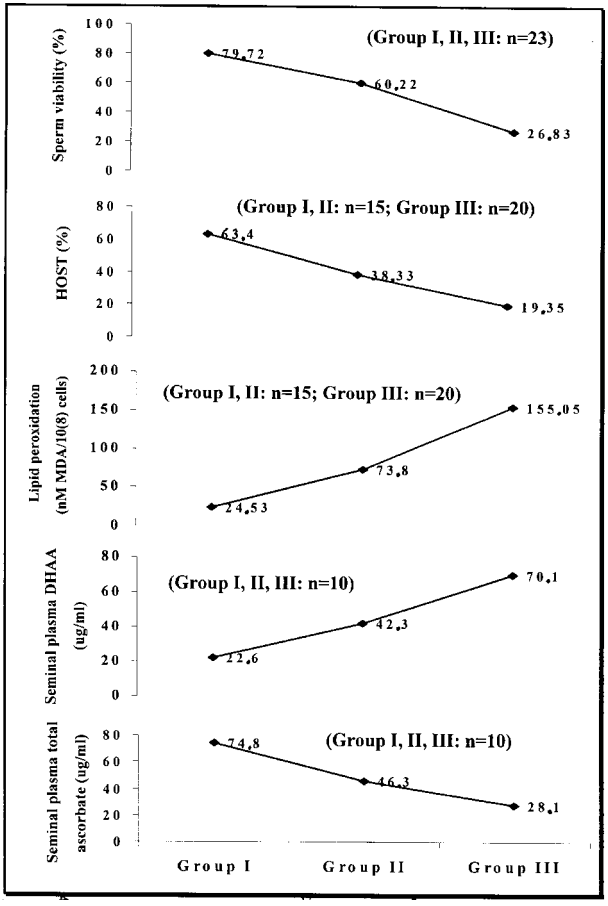


Fig. 2 : Lead induced changes of human sperm membrane integrity in lead acid battery factory workers. n = sample size of individual parameter in each group. Significant at P<0.001.

groups and in between the two exposed groups (P<0.001). Lipid peroxidation of sperm membrane showed significant high value among group II and III workers in comparison to the control subjects of group I (P<0.001), indicating loss of sperm membrane integrity after occupational lead exposure. This finding was supported by significant low level of seminal plasma total ascorbate with concomitant high value of dehydro ascorbate concentration in seminal plasma of the same comparable groups (P<0.001) (Fig. 2).

SEM of spermatozoa exhibited sharp depression and development of granularity at sperm head surface of the exposed groups compared to the smooth membrane surface

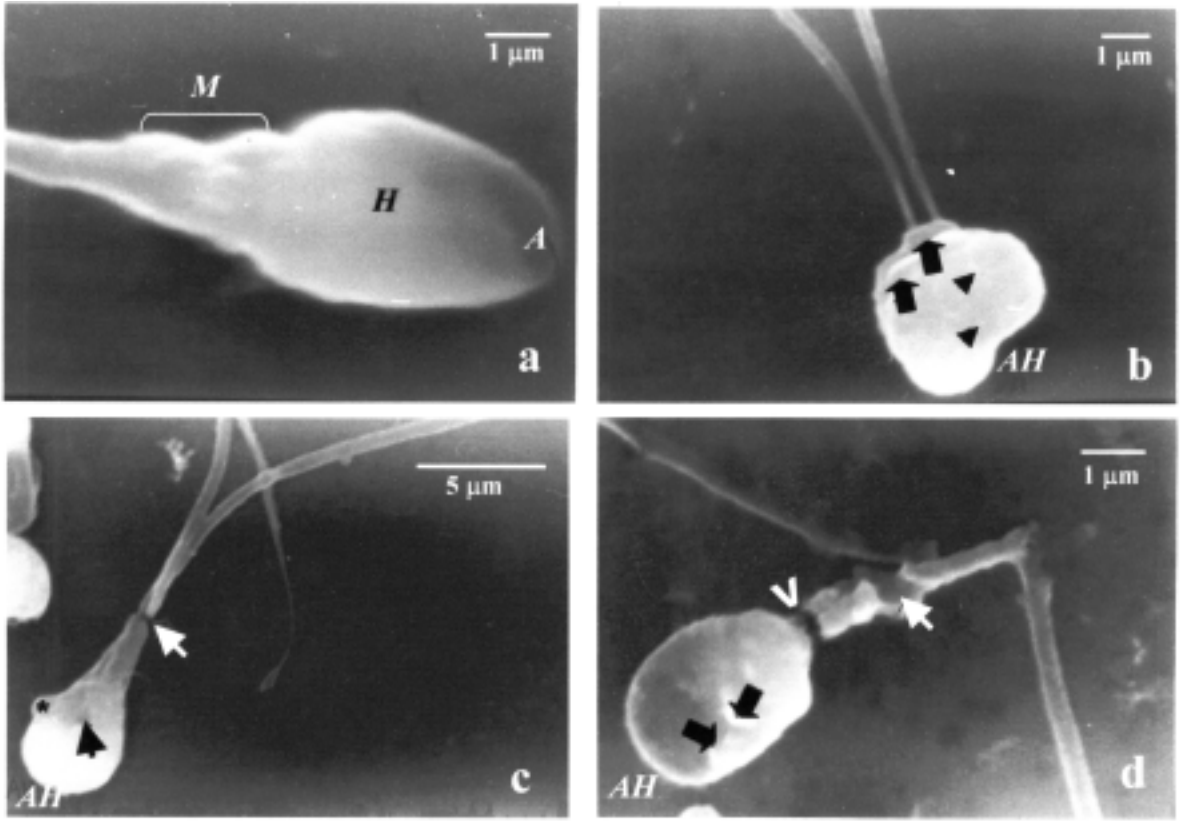
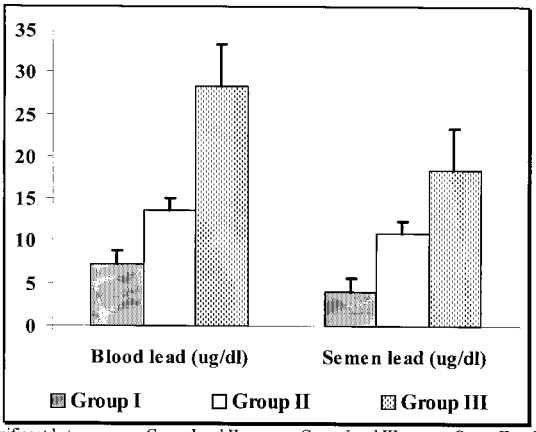


Fig. 3 : SEM of control and lead exposed human spermatozoa. (a) Control human sperm (group I) showing normal oval shaped head (H) with smooth surface and clear acrosome (A), mid piece (M) and tail. The average length of sperm head = 3.7 µ and average breadth = 2.6 µ. Mid piece was approximately 1.7 µ long and 1.3 µ wide x15000. (b) Exposed human sperm of battery factory worker (group II) showing amorphous head (AH) and double tail. Small granules (↑) and membrane folding (⇑) was appeared on sperm head surface where acrosome region was not clearly visible. The average length of sperm head = 3.7 µ and average breadth = 3.5 µ. Mid piece was absent x7500. (c) Exposed human sperm of battery factory worker (group II) showing amorphous asymmetrical (*) head (AH) containing sharp depression (↑) on the surface. The average length of sperm head = 4.2 µ and average breadth = 3.6 µ. Elongated mid piece (approximately 2.6 µ long and 1.2 µ wide) and partial breaking of tail (⇑) from mid piece was observed x5000. (d) Exposed human sperm of battery factory worker (group III) showing double tail with typical structure (↑) and tail became separated (∧) from head that cannot be explainable. Mid piece was absent. Amorphous head (AH) exhibited surface granularity (⇑) and invisible acrosome region. The average length of sperm head = 3.9 µ and average breadth = 2.6 µ x10000.



Significant between	Group I and II	Group I and III	Group II and III
Blood lead (μg/dl) →	P<0.001	P<0.001	P<0.001
Semen lead (μg/dl) →	P<0.001	P<0.001	P<0.001

Fig. 4 : Lead content in whole blood and semen after exposure, n = 10 (sample size of individual parameter in each group).

of the control (Fig. 3), hence confirmed the previous viability experiment that revealed lead induced degenerative changes on sperm head membrane surface in the exposed workers of lead acid battery factories.

Lead concentration in whole blood and semen was increased significantly in both the exposed groups and also between the two exposed groups (P<0.001). The increment of blood lead was 1.9 fold in group II and 3.9 fold in group III with respect to group I, whereas for semen lead, 2.7 fold and 4.6 fold were the increment pattern of the two exposed groups (group II and III) respectively (Fig. 4).

DISCUSSION

The present study exhibited deterioration in normal sperm morphology, count and motile sperm percentage in the lead acid battery factory workers compared to the non-occupationally exposed matched control subjects of the same socio-economic status. Morphological analysis of spermatozoa revealed low sperm count after occupational lead exposure, which indicated low cellular turnover that might be due to cellular regression. This observation was supported by the concomitant decrease of sperm nucleic acids and protein content and increased activity of lysosomal acid phosphatase in battery factory workers (7) and in printing press workers (26). Further, the diminution of sperm density, total count, sperm protein and nucleic acids content were in accordance with the view that increased percentage of hyploidy (<n DNA) at sub-Gl phase due to fragmentation/ breakage of sperm head nuclear DNA in respect of duration of lead exposure (communicated with International journal). These workers suffered from heavy lead exposure during their work (27), which caused adverse effects on sperm morphology and count (3, 15, 28–29). Morphological changes of spermatozoa were also depended on the duration and nature of exposure (2, 14).

Present study showed significant decrease in gross sperm motility, indicating low cellular activity after occupational lead exposure (1, 3, 4). This study was in corroboration with the previous observation of Roy Chowdhury et. al. by showing sharp diminution of sperm ATPase activity with concomitant alteration of seminal fructose content (30) leading to low sperm motility (5, 7). The probable explanation: fructose was utilized by ATP hydrolysis during spermatozoal motility (31), which became disturbed after lead exposure probably due to inhibition of ATPase by lead (26, 30). Our study also revealed that low sperm motility

was associated with low antioxidant level with concomitant rise in the rate of malonaldehyde production in both the exposed groups, suggesting the susceptibility of spermatozoa to lipid peroxidation after occupational lead exposure. This finding showed similarities with the earlier study of Rhemrev et. al. (32). Earlier study also reported that 56.6% chronically lead exposed battery factory workers have elevated malonaldehyde level, hence higher lipid peroxidation (33).

As cellular viability depends on the intact membrane structure (19, 34), therefore diminution of sperm vitality and HOST percentage in both the exposed groups along with high lipid peroxidation of sperm membrane as well as corresponding antioxidant profile strongly suggested the probable loss of sperm membrane integrity and development of degenerative changes on sperm surfaces depending on the extent of lead exposure at work place. Therefore, our finding was in corroboration with the previous observation (10, 30). Our present observation was further confirmed by SEM images where granular texture, depressions and membrane folding at the exposed sperm head surfaces compared to the smooth membrane surface of the control one, exhibited different degree of damage along with different types of strikingly abnormal sperm morphology. Thus the finer structure of human sperm head surface might be varied after occupational lead exposure (30).

Low volume of ejaculate, low viscosity of semen and prolonged liquefaction time in the exposed population of lead acid battery factories were also noticed from the present study. 95% of ejaculatory volume comes from the two accessory sex glands viz. seminal vesicle and prostate and alteration of normal liquefaction indicating deficiency of liquefying agent due to low prostatic secretary activity (25). Thus our result indicated probable dysfunction of accessory gland seminal vesicle and prostate after toxic insult of lead at work place.

Deterioration of liquefaction time, seminal volume and viscosity, sperm density, count, viability and morphology, sperm activity and membrane integrity in the present study were in accordance with the view that the lead content in whole blood and semen was increased in respect of duration of lead exposure at work place leading to reduced fertility, as obtained from the questionnaire. Xuezhi (35) showed that lead exposure caused prolonged liquefaction time, low volume and count, increased incidence of non viable spermatozoa and retarded sperm activity in male workers with more than 40 µg/dl of blood lead level, which is similar to the observation of Apostoli et. al. (1). Semen lead was higher in infertile men than the fertile group and low lead content in semen was the indicator of low industrial exposure (36). Moderate lead exposure also caused reduction in sperm quality and quantity among the battery factory workers (12).

Thus, the presence of lead in the semen of the exposed workers indicated that lead might directly cross the blood testis barrier and exerted its effect on sperm morphology, membrane integrity and motile activity leading to reduce fertility (37, 38, 39). In the present study, 40% lead exposed workers were infertile and few workers have an

experience of recurrent abortion, which was supported by earlier observations (38, 39). As the normal cell membrane is the prerequisite for the proper function of the cell, therefore in conclusion it can be pointed out that subtle membrane defect of morphologically abnormal spermatozoa due to lead induced high lipid peroxidative damage of the membrane with low antioxidant support, may ultimately caused the reduction in fertility among lead exposed battery factory workers (38, 39). Additionally the habit of consumption of alcohol, gutkha and smoking etc. may act as the contributing factors for the alteration of semen quality and fertility among them (40, 41).

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