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Arsenic Disrupts Dynamics Of Nexin -Dynein Regulatory Complex In Mice Spermatozoa Causing Infertility

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Abstract
Arsenic is one of the naturally present and widely distributed toxicants in ecosystem. It is also produced by anthropogenic activities. As it is present in water bodies mainly, it can easily enter the food chain and finally reach human body.It has very severe carcinogenic and teratogenic effects on physiological systems. Current study focuses on the effect of arsenic on reproductive system. Ourelectron microscopic studies clearly demonstrate this effect. Nexin-dynein regulatory complex is a component of flagellum of the mammalian spermatozoa. It is mainly involved in regulating motility of spermatozoa.Arsenic accumulation effects testicular tissues.Human population inhabiting the Arsenic-hit area of Gangetic zone of Bihar shows disruption in the motility apparatus of sperm leading to high frequency of asthenospermia. Similar trends were observed from the data of experimental mice exposed to arsenic.Sperm-tail ultrastructure shows clear abnormalities in arrangement of microtubular components of nexin - dynein regulatory complex.

Key Words:*Sodium Arsenite, Swiss Albino Mice, Spermatozoa, Nexin-Dynein,*

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I. INTRODUCTION:

Arsenic (As) is a ubiquitous metalloid present naturally as well as produced anthropogenically in the environment. It is a natural pollutant, toxin and carcinogen. It is generally found in rocks of the earth's crust in varying concentrations. Natural leaching from rocks results in dissolution of As in groundwater, however, the cause of elevated As contamination of groundwater is not known. Natural leaching of arsenic from rocks contributes to ground water arsenic contamination. In ground water, As is present in various forms such as H3AsO3, H2AsO3, HAsO3, H3AsO4, H2AsO4 and HAsO4.Arsenite and arsenate are the predominant forms found in groundwater; however, arsenite is more toxic than arsenate. The concentration of As in groundwater varies with geographical location. One of the toxic forms of As, H3AsO3, is more prominent in West Bengal (India) and Bangladesh, however HAsO4 and H2AsO4 are more prominent in Arizona (USA) and Korea respectively[ATSDR, 2019].

A wide range of As related human illnesses have been reported across various parts of the world. Rivers originating from mountains and rainwater can easily carry dissolved minerals, pesticides, fertilizers and chemicals with them which finally seep into the underground water. Human beings are more vulnerable to As toxicity in groundwater as opposed to surface or shallow water due to their biochemical changes. The effect of arsenic on the entire biological system specially on human health is widely noted from Ganga-Brahmaputra region of India. Cities and villages on the banks of river Ganga are severely affected from the arsenic poisoning.

Arsenic is a non-essential element for human beings. According to the World Health Organization (WHO), the safe level of As in blood is less than 1 ppb. The level of toxicity depends on the chemical form of As in the environment. The incidence of As related health issues is steadily rising, particularly in the Ganges-Brahmaputra region of northern India.

Infertility is global concern nowadays and is very common in young couples due to various reasons. Among the males, abnormalities in testicular development, descent of the testes as well as abnormal spermatozoa formation leads to infertility. Sperm motility is also one of the main reasons of male infertility. Therefore, it becomes necessary to study the complexity in the formation of main parts of the spermatozoa which help in their locomotion. Mice models are one of the most important tools in investigating the causes

ofmale infertility. Arsenic exposure for a long period even in low concentrations can damage many tissues and organs leading severe abnormalities in liver, kidney, heart, skin, ovary and testes[GrazielaDomingues, 2018]. In this study we are investigating the effect of As on male reproductive system.

The motility of the spermatozoa andthe fertility in animals, is dependent on the structural and functional integrity of the sperm flagellum or tail. The central component of sperm flagellum is the axoneme that is composed of the '9+2' microtubule arrangement, dynein arms, radial spokes, and the Nexin-Dynein Regulatory Complex (N-DRC). The N-DRC is localized between doublet microtubules and recently, it has been reported that a component of the N-DRC, is essential for proper sperm motility and male fertility in mice [Akane Morohoshi,2020].Arsenic exposure in experimental rats has shown to produce steroidogenic dysfunction leading to impairment of spermatogenesis. [Mónica Ferreira, 2012]. The present study was designed to investigate the duration-dependent effect of arsenic exposure on thespermatozoa count, morphology and ultrastructure, histopathology of testes, as well as the hormonal levels in Swiss albino mice.

II. MATERIAL AND METHODS:

Experimental Animals: Swiss albino malemice,*Musmusculus***,** of 8-10 weeks of age and weighing 20-25gwere purchased from Animal House division of CSIR-Central Drug Research Institute, Lucknow. The mice were reared in the animal house of S.S. Hospital and Research Institute, Patna in the Quarantine Premises for 14 days. The mice were kept in polypropylene cages (5 mice/ cage). The beddings were of paddy husk .They were kept at room temp. 28±2 ˚C and humidity 50±5% in a controlled light,12 hours light and 12 hours dark. Animal were maintained in ideal condition as per the ethical guidelines of the CPCSEA(CPCSEA Regd. no. 1840/PO/ReBi/S/S/CPCSEA), Govt. of India and Institutional Animal Ethics Committee (IAEC).Animals were given *ad libitum* access to food & water and were visually inspected daily to check for loose pellets and their behavioral activity and health status.

Mice grouping and dosing: After14 days of acclimatization, mice weighing 28-32gm ±4 gm were assigned for experimental purpose. Mice were divided in 6 experimental groups (5 mice per group).Group 1 served as the normal control group while the rest of the mice groups served as the arsenic treated groups.Sodium arsenite was dissolved in distilled water and administered every day to the arsenic treated mice groups by oral gavage method. The dosage was standardized to 2mg/kg body weight of mice. Arsenic treated mice were divided into 5 groups according to the duration of the As treatment as 2 weeks, 4 weeks, 8 weeks, 12 weeks and 16 weeks treated mice.

HistopathologicalStudies:

Light Microscopic Study: Histological experiments were performed according to the respective duration of treatment dosage. Experimental mice of every group were euthanized and the testes were dissected.The Testis was dissected and fixed in 10% formalin and embedded in paraffin. 4-5 µm thick sections were cut and stained with Haemotoxylin and Eosin. The sections were examined under light microscope.

Electron Microscopic Study: The sectionsof tissues (testis and cauda epididymis) were dissected and fixed in 2% glutaraldehyde with post-fixation in 1% osmic acid. Tissue processing was performed according to the standard methods. The ultra-thin sections were observed under the Transmission Electron Microscope (MORGAGINI-268D, Fei Company, Netherland).

Scanning electron microscopy: The sperm from 3 males per species were plated on poly-Dlysine-coated cover glasses (diameter of 12 mm). Then, the cover glasses were submerged into fixation solution (2.5% glutaraldehyde in 0.1 M HEPES buffer, pH = 7.5) to fix the sperm overnight at 4 °C. Next, the sperm were post-fixed in 1% osmium tetroxide in 0.1 M HEPES buffer (pH = 7.5) and were sequentially dehydrated in 30, 50, 70, 80, 95, 100, and 100% ethanol solutions for 10 min each. Sperm were then processed and subsequently examined in the scanning electron microscope (Quanta 250, FEI Co., Ltd., USA)

Statistical Analysis: Data obtained from the experiments were correlated and analyzed by one way ANOVA and values of $P<0.05$ were considered as statistically significant.

III. RESULTS AND DISCUSSION:

Our findings show remarkable histopathological and ultrastructural changes in the testes and spermatozoa of the mice due to arsenic exposure.

The histological sections of testes of the treated mice show prominent duration dependent damage on arsenic exposure when compared to the control group.Control mice seminiferous tubule show spermatogonia, spermatocytes, spermatids and developing spermatozoa in normal stages of spermatogenesis [Plate 1, fig

1].Marked abnormalities were found in the seminiferous epithelium as well as the spermatogenic cells of the seminiferous tubules of the arsenic treated mice. 2 and 4 weeks As exposed mice seminiferous tubule show disruption in spermatogenesis and thinning of seminiferous epithelium. Obliteration of tubular lumen with spermatocytes and spermatids moving towards centre can be seen [Plate 1, fig 2 and 3]. 8 and 12 weeks As exposed mice seminiferous tubules show obliteration in lumen with apoptotic testicular cells and irregular seminiferous epithelium, disintegration of peritubular membrane, with disorganization and sloughing of immature germ cells [Plate 1, fig 4 and 5]. 16 weeks arsenic exposed mice seminiferous tubules demonstrate very severe degeneration in the seminiferous tubules with marked damage to seminiferous epithelium, decreased spermatogenic material and autolysis of testicular cells. The inter-tubular spaces increased with marked loss of Leydig cells. Vacuolization of seminiferous epithelium, loss of germ cells population along with Leydig cell atrophy with some sections showing complete degeneration of testicular cells was also observed [Plate 1, fig 6].

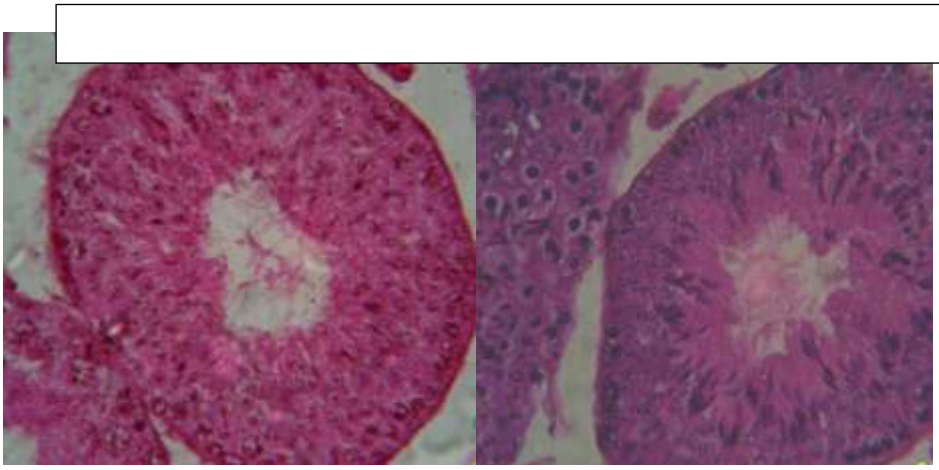


Fig.1. Transverse section of testis of control mice showing development of normal spermatogonia, spermatocytes and spermatids

Fig. 2. 2 weeks As exposure interferes with normal stages of spermatogenesis causing degenerationof seminiferous epithelium.

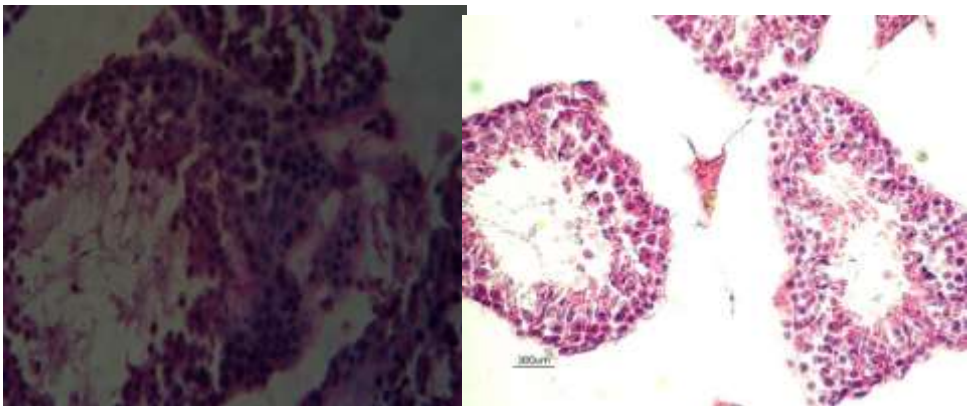


Fig.3. 4 weeks As exposure causes further distortion in shape of seminiferous tubules. Inter-tubular space is obliterated.

Fig.4. 8 weeks As exposure showing loss of seminiferous epithelium and distorted spermatocytes. Spermatids and developing spermatozoa arevery few in number.

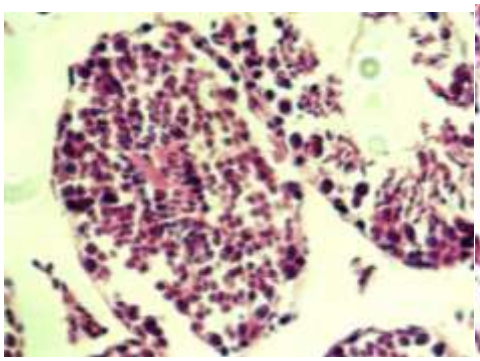


Fig. 5. 12 weeks As exposure causing obliteration of lumen. Seminiferous epithelium is irregular.

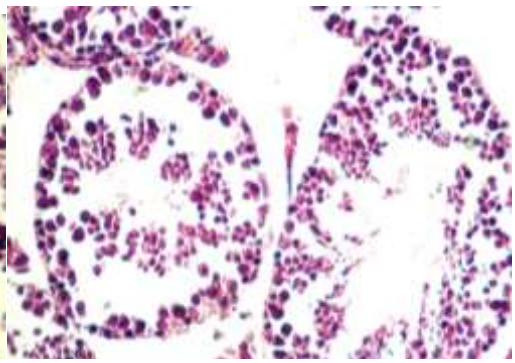


Fig. 6. 16 weeks As exposure shows complete disruption of spermatogenesis. Testicular cells are fewer in number and show apoptotic features.

The ultrastructural study revealed significant damage in the microtubular architecture of the arsenic exposed mice spermatozoa as compared to the control group. The electron micrographs of control mice spermatozoon showed normal acrosome, homogenous chromatin with regular plasma and nuclear membranes. The sperm tail axoneme is composed of the '9+2' microtubule arrangement, dynein arms, radial spokes, and normal Nexin-Dynein Regulatory Complex (N-DRC) is also observed [Plate 2, fig 1]. Electron micrographs of testes of mice after administration of NaAsO2 at 2mg/gm body wt. show varying degree of degeneration in the sperm organelles including the Nexin-Dynein complex [Plate 2, fig2, 3, 4, 5a and b, 6a and b]. In the 12 weeks As exposed mice spermatozoa, some cells show complete detachment of the neck. The outer sheath of microtubules both A+B are swollen and show fusion Dyenin&Nexin are not clear In both the 12 and 14 weeks As exposed mice spermatozoa, the 9+2 microtubular structure is disturbed and the inner pair of microtubular structure show degeneration.14 weeks of As exposure showed degeneration of central hub, dynein and nexin arm along with malformed or rudimentary axoneme. Rudiments of mitochondria along with dissolved protofilament of microtubule were observed [Plate 2, fig 6a and 6b]. The deleterious effects on the cytoskeletal elements of the spermatozoa tail were directly proportional to the duration for which the mouse was exposed to sodium arsenite.

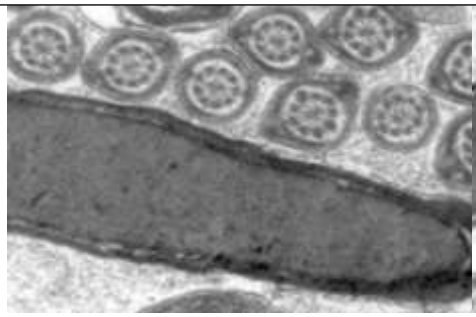


Fig.1. Microtubules are normal as seen in cross section of sperm. During spermiogenesis one spermatid showing elongation with normal nuclear membrane and plasma membrane Prominent Dynenin and Nexinbond .

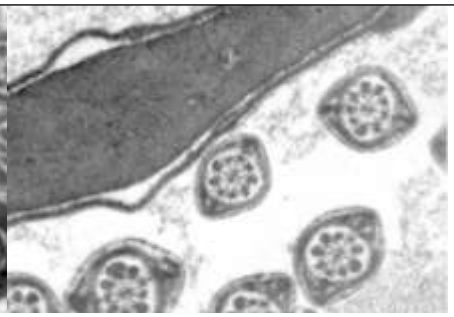


Fig.2. 2 weeks As treated sample: Developing spermatozoa showing bulging out of plasma membrane on proximal part. One microtubules' section at hand corner show hook like structure on fibrous sheath. Such abnormalities also noticeable in growing stage, on other tubules also in two weeks As administered mice's testes. Dynein arm showing degeneration but in others present but nexin bond in most of the microtubules get degenerated.

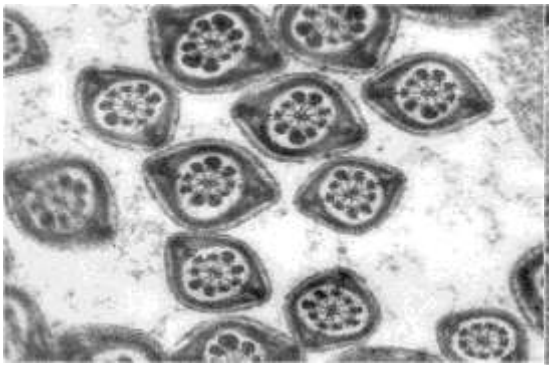


Fig.3. 4 Weeks treated sample, Growing dense appendages on the fibrous sheath are significant in 4 weeks treated mice's testes. But plasma membrane at tail portion are normal.

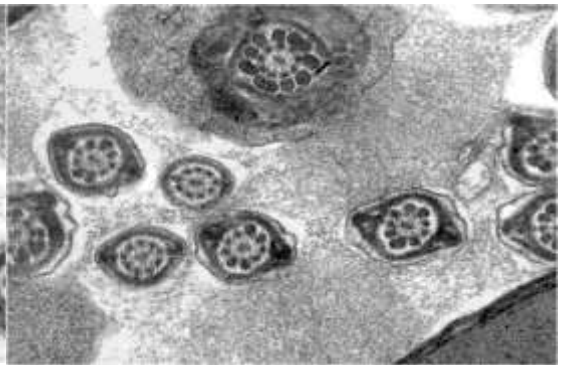


Fig.4. 8 Weeks treated sample, the effect of As administration after 8 weeks on the middle piece and tail piece showing many plasma membrane in cross section of both pieces of tail. The C.S. of middle piece show abnormality on outer fibrous sheath in two blocks. Note the Gap between Nuclear membrane & outer sheath of microtubules has increased tail section of spermatozoa.

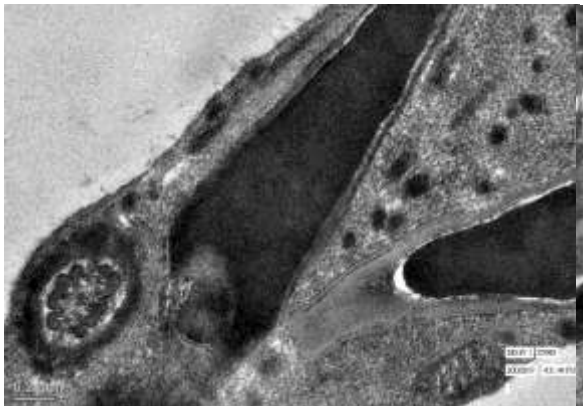


Fig.5a. 12 weeks treated samples. Note the broken double plasma membrane on acrosome region. Neck of the completely detached due to arsenic toxicity. The section of tail portion showing thick outer dense fibrous sheath.The 9+2 microtubular structure is disturbed the inner pair of microtubular structure show degeneration. The outer sheath of microtubules both A+B are swollen and show fusion Dyenin&Nexin are not clear.

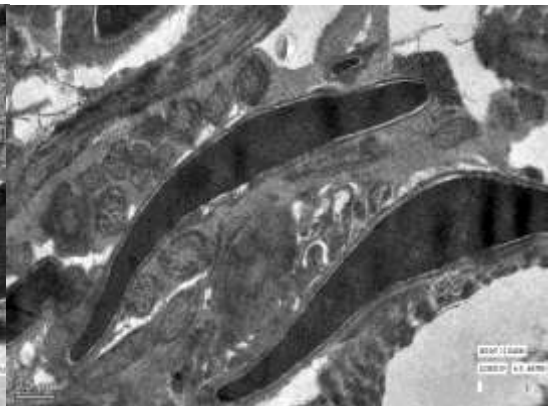


Fig.5b. 12 weeks treated samples: Microtubules show disintegration. The dynamic polymers of tubules show degeneration as well as fusion. In the middle microtubules does not show paired structure. Note the fusion of plasma membrane of sperm with sertoli cell component.

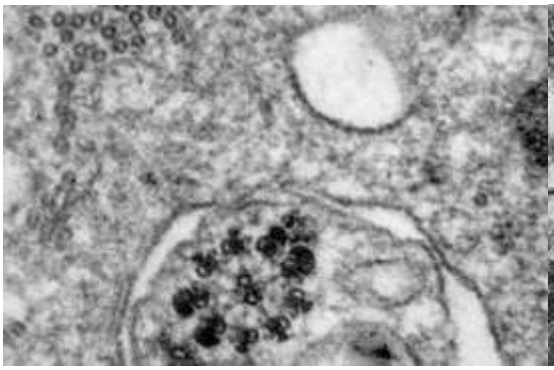


Fig.6a.16 weeks treated samples: Naked microtubule without any fibrous sheath and plasma membrane can be seen. Few outer dense sheath are swollen. Tubulin dimers showing disintegration.

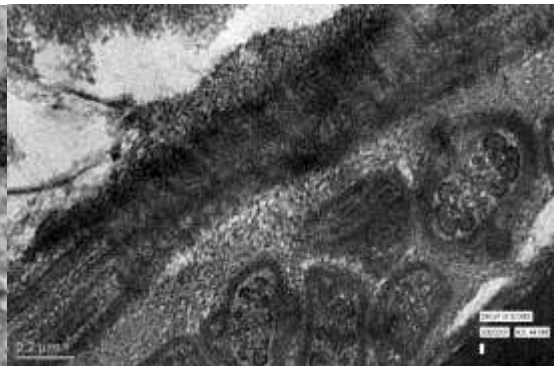
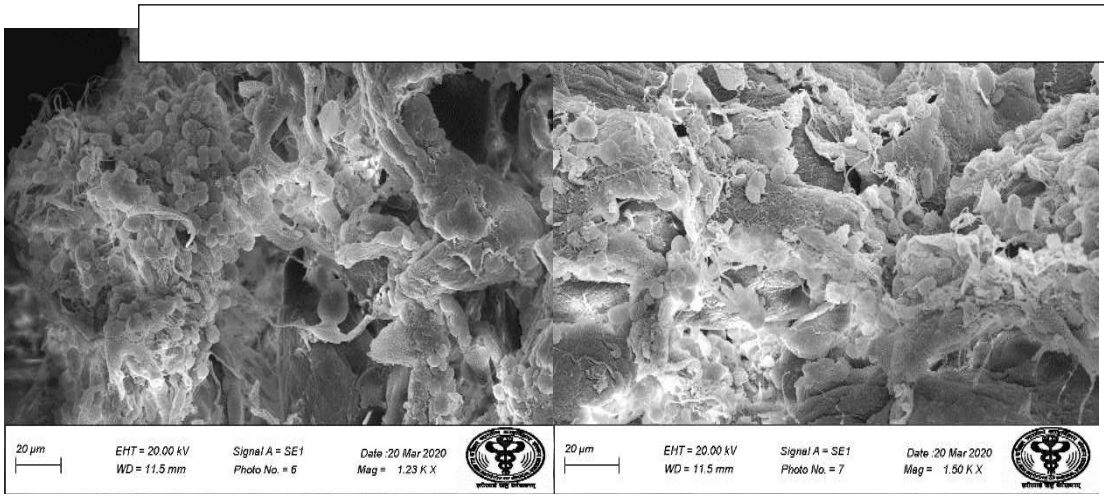


Fig.6b. 16 weeks treated samples: Fusion of polymers of tubulin. No Dyenin –Nexin bond outer circumferancial are irregular structured microtubulin .

The Scanning electron micrographs of the experimental mice show that as compared to the normal hook shaped spermatozoa in normal mice, arsenic exposure causes the fusion of spermatozoa head; tail thickening or its absence was also noted across various experimental groups; acrosome appears as a dot in some or is completely absent in other spermatozoa, the midpiece is seen to be obliterated in the groups exposed to longer durations [Plate 3, fig 1-4]. It was difficult to draw any significant conclusion based only on scanning electron micrographs, but in combination with the light and transmission electron micrographs study, the deleterious effects of arsenic on the sperm morphology can be clearly seen, especially in the tail region made of the cytoskeletal elements.



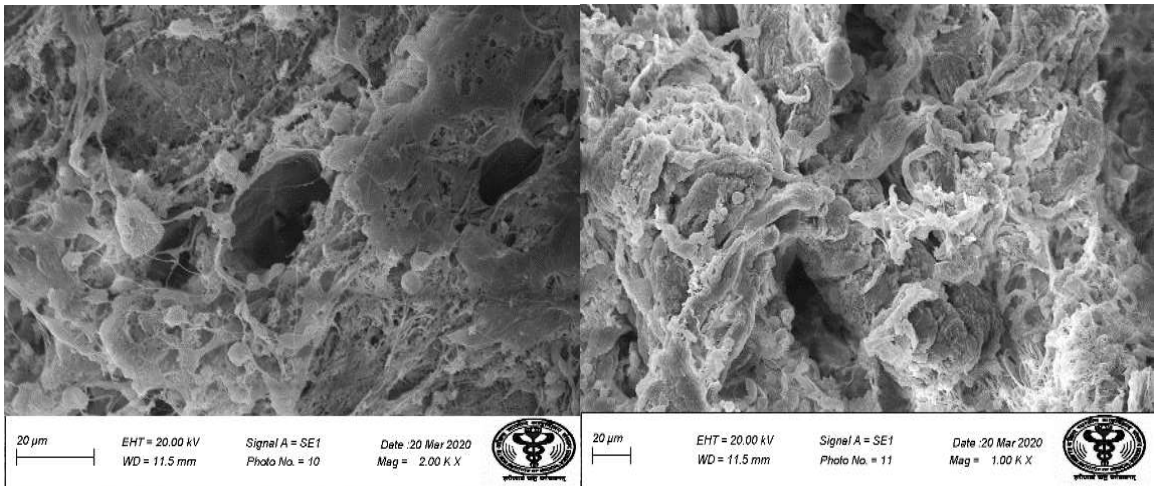


Fig3. 12 weeks treated samples: Giant spatula shaped infertile spermatozoa present. Almost all spermatozoa are fully round head and matured but no normal spermatozoa can be seen. Only two on the right hand corner spermatozoa which are showing fusion but fully developed acrosome are

Roy Chowdhury [2004] indicated that heavy metals like lead, mercury and chromium causes cytotoxic effect in the male reproductive function. In today's scenario, ingestion of contaminated drinking water is the major route for human exposure to arsenic. Arsenicals are widespread in the environment as a result of natural and anthropogenic occurrence[ATSDR, 2019]. The present work demonstrates that arsenic causes toxic effects at the histopathological and ultrastructural levels in the testes of male mice. Arsenic exposure affects the spermatogenesis as well as the sperm head and tail structure.Similar results were seen by *Han et al*., [2020] in adult male mice where arsenic influenced spermatogenesis by disorganizing the elongation of spermatids. Our results are in concurrence with our earlier work which showed that sodium arsenite exposure can lead to decreased sperm motility, increased abnormal sperm morphology and decreased sperm viability. [Nath *et al*, 2019.] Sodium arsenite exposure has been found to cause reduction in daily sperm production, in number of spermatids in the testis, and in sperm in the epididymal caput/corpus regions [Ana Cláudia Ferreira Souza *et al.*, 2016]. Toxic effects of arsenic trioxide on spermatogonia are associated with oxidative stress, mitochondrial dysfunction, autophagy and metabolomic alterations [Chen *et al.,* 2019] Correlating the various parameters of the present work as well as related researches, the deleterious impact of arsenic exposure on the male reproduction is severe, which can impact the fertility significantly.

IV. CONCLUSION:

In conclusion, we propose that the arsenic exposure exerts a primarily disintegrating effect on the testicular cells, spermatozoa membranes and tail. The deleterious effect of arsenic can be seen on all the morphological aspects of the testicular cells and the spermatozoa ultimately leading to necrosis. Additionally, the As exposure causes alteration of chromatin and cytoskeletal components. The ultrastructural studies done in the present work demonstrate that arsenic disrupts dynamics of nexin -dynein regulatory complex in mice spermatozoa. Thus, chronic or prolonged exposure to arsenic can lead to infertility in the males.

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