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Developing Gonads and Lead Exposure

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Abstract:

Reproductive toxicants can impair reproduction by acting on the development of the male and female. Reproductive toxicants can produce their adverse effects on reproductive axis by several direct and indirect mechanisms. There has been growing concern about human reproductive disruption by xenobiotics including drugs, occupational, and environmental exposures of toxicant. The present work is carried out to study the lead toxicity in experimental Swiss albino male and female mice. Lead acetate was given (640mg/kg/BW) to pregnant Swiss mice during gestation and lactation period. The results indicated that exposure of lead caused a significant alteration in development of gonads at different dose levels of lead acetate. Lead acetate alters the histology of developing testes as well as ovary. In present investigation it is observed that lead acetate given in high dose caused decreased body weight of developing pups in experimental groups as compared to control pups. Microscopic examination revealed that lead induced apparent damage and reduction in the number of seminiferous tubules and primordial germ cells. Studies conducted on females revealed mostly miscarriages, premature delivery and infant mortality. Lead suppresses the development of primordial follicles during foetal and neonatal life. Changes in the number of primordial, primary and secondary follicles were also observed. We can conclude from our findings that lead acetate administrated during gestation and lactation adversely affects developing testis as well as ovary of Swiss mice.

Keywords: Gonads, Postnatal, Development, Lead, Swiss mice.

1.0 Introduction:

Recently, there has been substantial interest in the potential adverse effects of exposure to environmental hazardous chemicals on male and female reproduction. Lead as an industrial pollutant and immunotoxicant, has the potential to adversely affect human and animal health. It induces a broad range of physiological, biochemical and neurological dysfunctions in human (Nordberg, *et al.,* 2007). The clinical assessment of reproductive toxicity can be an extremely difficult task. Age dependent alterations in developing reproductive tract sensitivity to other toxicants including metals may also occur. Possible mechanisms for altered resistance of the pre-pubertal gonad may reside in a decreased rate of gonadal cell proliferation (Mattison, 1981) or alterations in the distribution of the toxicant to gonadal cells (Janson, 1975; Setchell and Sharp, 1981**).** Wide M. (1980; 1983) has evidence suggesting that in addition to effects on progesterone secretion, lead also alters uterine estrogen receptors which may have further impact on the initiation and maintenance of pregnancy. Reproductive consequences of lead exposure are widespread (Patrick, 2006), affecting almost all aspects of reproduction (Zheng, *et al.,* 2003). The ovarian follicle is the functional unit of the ovary. It contains the oocyte that may eventually ovulate, undergo fertilization and form an embryo. It also provides the steroid and protein hormones required for maintenance of the ovarian cycle, the secondary sex characteristics and preparation of the uterus for implantation (J.K.Findle, *et al.,* 2009). Bires, *et al.,* (1995) noted histological changes in the number of ovarian follicles and increase occurrence of primary atretic follicles indicated alterations in the membrane structures and organelles of oocytes and in the follicular cells of stratum granulosum. The strain differences also account for major variations in the assessment of ovarian histology with regard to the types and number of follicles (Mayers, *et al.,* 2004). Histological examples of atretic primordial follicles in postnatal or adult ovaries are very limited (Perez, *et al.,* 1999; Depalo, *et al.,* 2003).

2.0 Materials and Methods:

2.1 Animals:

Swiss albino mice weighing 25 – 30 gm and aged 7 - 9 weeks old were obtained from the Animal House of Department of Zoology University College of Science (MLS University, Udaipur, INDIA). Animals were housed in polyvinyl chloride cages (290 × 320 × 390 mm) and maintained under standard laboratory conditions. The animals had free access to food (mice feed) and water. The maintenance and handling of the animals were done according to the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals, Ministry of Environment and Forests, Government of India. The experimental protocols were approved by the Institutional Animal Ethical Committee of the University NO.CS/Res/07/759.

2.2 Experimental Protocol:

Female and male mice in the ratio of 4:1 were kept in cages and the day on which sperm was detected in the vaginal smear was counted as day 1 of pregnancy. Group l (control group) mothers were gavaged with equal volume of distilled water. Lead acetate (640mg/kg/BW) was administrated orally on every day from GD 10 to PND 21 considered as Group 2. The pups from each of the groups were randomly selected and were sacrificed on PND 7 and 21 days. Body weight was monitored on weekly basis for the mothers as well as for the postnatal pups until they were sacrificed.

3.0 Results and Discussion:

All the mice in control group were remained active and healthy with normal feeding behaviour and body weight. Lead acetate exposed groups of male and female were lethargic and more irritable and lost their body weight as compare with control group. Results of the present study indicated dose dependent significant decrease in body weight after administration of lead acetate, as compared to controls. Histhopathological alterations in the various components in developing testis and the ovaries are as follows:

3.1 Testis:

On 7th PND

In present investigation control testis of 1st week showed well developed seminiferous tubules and regular distribution of cells. Sertoli cells were located near the basement membrane of the seminiferous epithelium and spermatogonia cells were also located and arranged in layers with round shape and surrounded by single germ layer. At day 7 PND there was distinct lumen visible in the centre of the tubules **(Fig.1)**. The regular shape of seminiferous tubules and their arrangements were altered by lead acetate at 1st week of the developing period. Lead acetate reduced the germ cells and spermatogonia and disarranged their distribution. Spermatogonia which were embedded at the side margins of sertoli cells got detached from these cells and scattered all around and inside the developing seminiferous tubules. In lead treated testis this lumen was totally obliterated by cell debris **(Fig.2)**.

3.2 OVARY:

On 7th PND

Histological section of control ovary showed normal structure of germinal epithelium, cortex and inner medullary region. The stroma in the ovary was mainly concentrated in the centre of the organ with evident surface epithelium. The primordial follicles showing a single layer of squamous epithelium and a large eccentric nucleus were found in adequate number mainly seen at periphery of the ovary. Smaller follicles were found at cortical region where as larger follicles were seen at central medullary region. Primary follicles surrounded by a single layer of cuboidal follicular cells with zona pellucida present between the oocyte and the adjacent follicular cells known as granulosa cells **(Fig.3)**. In lead exposed female pups the ovary appeared compact with irregular shape. The stroma in the lead treated ovary was not very clear and the surface epithelium was not intact. The primordial follicles were very less in number and their structure was altered by lead acetate. Few primary follicles were seen with scattered cuboidal cells and distorted nucleus. Animals showed decreased number of follicles that enter in the growing phase **(Fig.4)**.

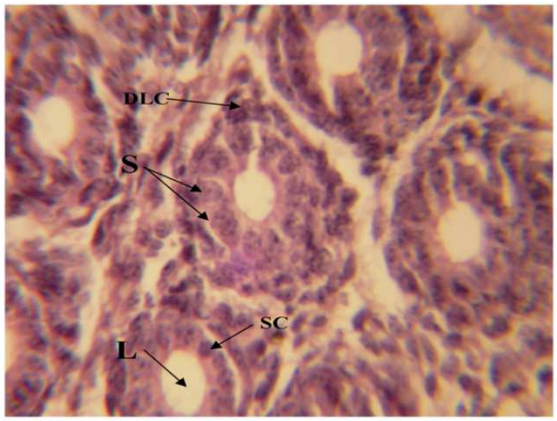

Fig. 1. Photomicrograph of control mice testis showing normal development and distribution of seminiferous tubules at PND 7 testis. Eosin and Haematoxylin stain. 450X. (L = Lumen, S = Spermatogonia, DLC = Developing Leydig cells, SC = Sertoli cells)

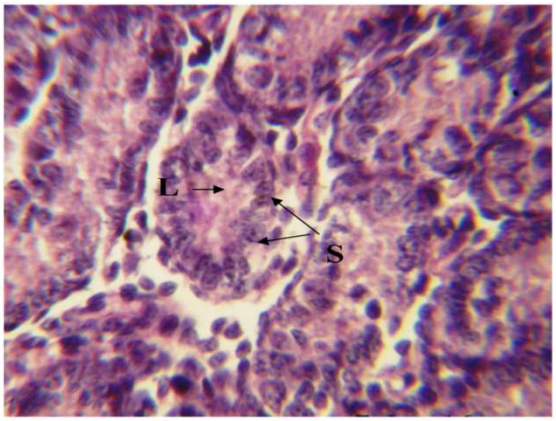

Fig. 2. Photomicrograph of PND 7day treated mice testis showing altered shape and size of seminiferous tubules. Many of spermatogonial cells are clumped. Lumen is totally obliterated by cells debris. Eosin and Haematoxylin stain. 450X. (L = Lumen, S = Spermatogonia

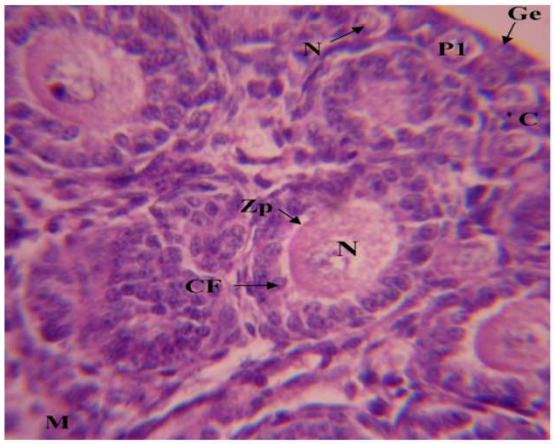

Fig.3: Histological sections of ovarian follicles in ovary of mice at PND7 in control group, illustrates normal structure of germinal epithelium (Ge), Cortex (C) and inner medullary (M) region. The structure of primordial follicles (P1) with large eccentric nucleus (N) mainly found at periphery and primary follicles having single layer of cuboidal follicular cells (CF) with zona pellucida (Zp).

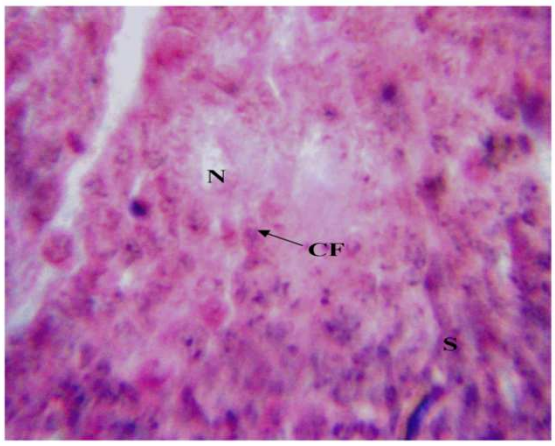

Fig.4: Histological sections of mice ovary at PND7 in lead group showing undifferentiated stroma and less number of primordial follicles. Primordial follicles lacking squamous follicular cells (SF) and all the cuboidal follicular cells (CF) are scattered and nucleus (N) is distorted

3.3 Testis:

On 21st PND

On 21st day, in control testis the seminiferous tubules were arranged in regular manner. The spermatogonia, intermediate spermatogonia and primary spermatocytes in various phases of development were observed. Sertoli cells were clearly detected near the seminiferous epithelium. Sometimes they appeared near the periphery of the tubules. Their cytoplasm was well stained **(Fig.5)**. High dose of Lead acetate affected the developing seminiferous tubules and changed their shape and size and decreased the size of tubules. In treated group the hexagonal shape of tubules and large gaps were detected between tubules in comparison to control. Sertoli cells became shrinked and the lumen of seminiferous tubules obliterated by cell debris. The precursors of developing Leydig cells dispersed and they were rarely seen in groups **(Fig.6)**.

3.4 OVARY:

On 21st PND

The control ovary on 21st day showed normal structure of surface epithelium, and well developed stroma with cortical and medullary regions and small, medium and large follicles were seen. Primary follicles were seen along with cuboidal granulosa cells arranged in a single layer surrounding the oocyte. Numerous secondary and tertiary follicles were found along with zona pellucida, oocytes showing proper nucleus and multiple layers of granulosa cells were also visible. Preantral and antral follicles were also apparent

with a fluid filled cavity antrum present among the granulosa cells. Graffian follicle was very large in size, granulosa cells were in its original number, shape and position, and Thecal cells were present in normal state. Atretic follicles were in normal range **(Fig.7)**. On 21day ovary of lead treated pups showed severe damaging pattern in its size, shape and structure including germinal epithelium, cortex and inner medullary region. The structure of germinal epithelium was irregular. Distribution of follicles were present in cortical and medullary region and the inter follicular spaces were devoid of stromal cells. Different types of follicles were present although very less number of primordial follicles were present at the periphery just under the germinal epithelium but there structure was not intact. In all types of developing follicles viz. primary, secondary and others, all the granulosa cells aggregate together so distinct layers of granulosa cells were not visible. There was shrinkage in granulosa cells and substantially the regular structure is completely lost. In most of follicles oocyte was not intact and in few follicles it was completely destroyed. There was an increase in number of atretic follicles as compared to other follicles **(Fig.8)**.

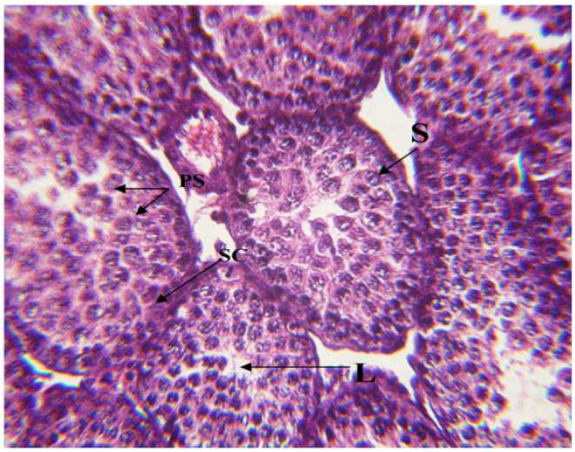

Fig. 5. Photomicrograph of transverse section of 21day control mice testis showing developing spermatogonia with various stages in seminiferous tubules. Large number of sertoli cells present in the centre of the seminiferous tubules. Developing Leydig cells are visible in between the seminiferous tubules. Eosin and Haematoxylin stain. 450X. (L = Lumen, S = Spermatogonia, PS = Primary Spermatocytes, SC = Sertoli cells)

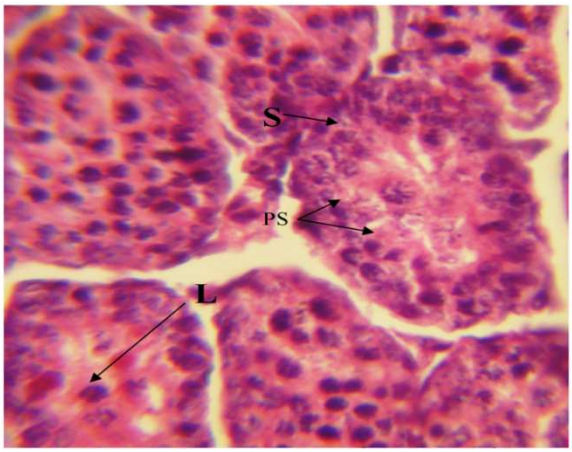

Fig. 6. Photomicrograph of transverse section of 21day treated mice testis showing reduction in the number of spermatogonia. Their regular arrangement in layers is disturbed and they are scattered in the seminiferous tubules. Lumen is obliterated by cellular debris. Eosin and Haematoxylin stain. 450X. (L = Lumen, S = Spermatogonia, PS = Primary Spermatocytes)

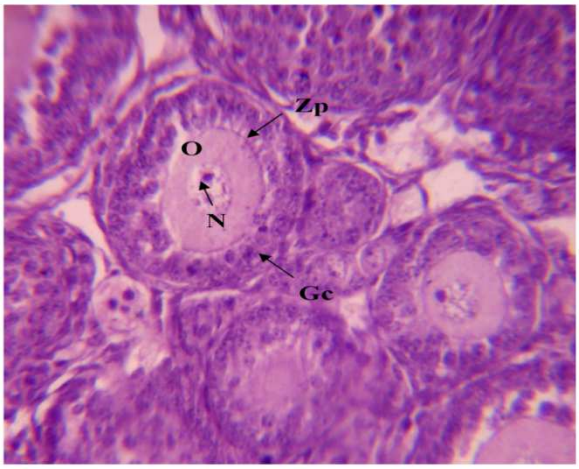

Fig.7: Histological sections of ovarian follicles in mice ovary at PND21 in control group illustrates different types of developing follicles with zona pellucida and oocyte showing proper nucleus (N) and multiple layers of granulosa cells.

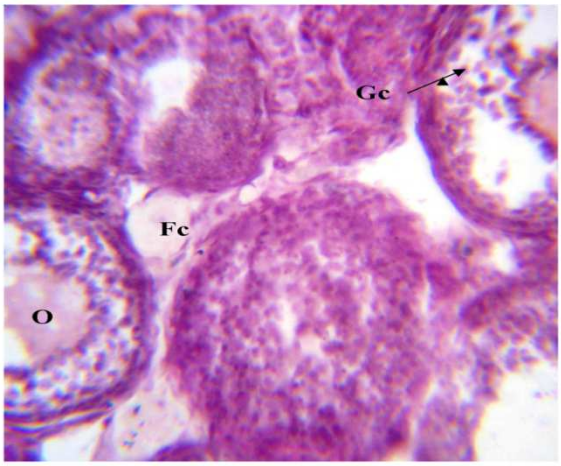

Fig.8: Histological sections of mice ovary at PND21 in lead group animal shows severe damage in various ovary components. All the cuboidal follicular cells are scattered and distorted including shrinked granulosa cells and distorted oocytes (O), in which nucleus is not visible. Interfollicular cells (Fc) are devoid of stromal cells (St) with hollow spaces.

In the present study lead induced histhopathological alterations in the various components in developing testis and the ovary were studied. Daily doses of lead acetate caused significant decrease in the average body weight and significant modifications in the histology of the mice testis. Banu, *et al.,* (2007) reported that lead acetate given in high dose cause dose-dependent significant decrease in body weight of both animals. The microscopical examinations of the ovary in the present study revealed the apparent damage and reduction in the number of primordial follicles, distorted structure of developing follicles and marked increase in number of atretic follicles. The present study on developing testis showed that Lead altered the regular shape of seminiferous tubules and their arrangements. Lead reduced the germ cells and spermatogonia and disarranged their distribution in seminiferous tubules. The developing seminiferous tubules and their shape and size also decreased by lead acetate. In treated group the hexagonal shape of tubules and large gaps were detected between tubules than in control.

The histopathological results revealed a degeneration of germinal epithelium with sloughing of germ cells into the lumen of seminiferous tubules by Yasser Said El-Sayed and Mahmoud Shaban El-Neweshy (2010). Atrophication of seminiferous tubules and the number of Leydig cells appeared to be lower in Pb-treated group (Guang Shan, *et al.,* 2009). Many studies previously by other researchers have also shown the reduction of germinal epithelium which was seems to be due to damage of germinal cells (Batra, *et al.,* 2001; 2004). Loss of germinal epithelium cells was also observed by Adhikari, *et al*., (2001). Isabel Corpas, *et al.,* (2001) reported that effects were reflected in the reduction of the thickness of epithelium and of seminiferous tubule diameter (STD) as a consequence of the action of lead in the reduction in numbers of prospermatogonia and spermatocytes. It is known that the phase of spermatogenesis are negatively affected by several factors including pollutants such as lead reported by McMichael, *et al.,* (1986). Batarseh, *et al*., (1986) reported that Lead acetate may inhibit spermatogenesis by a disturbance of metabolic activity of the Sertoli cells.

Lead induces decreased sperm count, motility and increased morphological abnormalities in animals (Hsu, *et al.,* 1998; Hsu, *et al.,* 1997). Study with male CF-1 mice indicated the significant decrease in epididymal sperm count at low dose of lead exposure (0.25% via drinking water). Decreased motility and increased incidence of teratospermia at higher dose of lead exposure (0.50%) along with inhibition of post-meiotic cells mainly pachytene spermatocytes were noted. The sperm analysis from those subjects revealed the morphological abnormalities of sperms (mainly, the tail abnormality) and significant decreased in certain key seminal constituents like, fructose and succinic dehydrogenase which indicated the male reproductive functional impairment in system exposed to lead (Chowdhury, *et al.,* 1984; Chowdhury, *et al.,* 1986). The microscopical examinations of the ovary in the present study revealed the apparent damage and reduction in the number of primordial follicles, distorted structure of developing follicles and marked increase in number of atretic follicles.

Many studies suggest that lead causes direct damage to the ovaries, resulting in ovarian follicular cyst and fewer corpora lutea at high lead concentrations (Hilderbrand, *et al.,* 1973). Lead exposed mice have atretic antral follicles, with detached granulosa cells, pycnotic nuclei in the granulosa cells and hypertrophic theca layer. The present study correlates with these findings. There were significant differences between control and treated groups, in the thickness of theca and granulosa layers. Compaction of ovary, structure of primary follicles and decreased number of growing follicles were also observed in the ovaries at postnatal day 7 due to exposure during gestation, and this exposure continuously causing damage until 21 day of lactation. Since bone is the major storage compartment in the body, specific conditions like pregnancy and lactation cause accelerated bone demineralization that could lead to acute lead poisoning (Gardella C, 2001). Postnatal exposure to lead during the critical period for hypothalamic programming has been demonstrated to alter the development and function of the reproductive system in female rodents.

Our study finds that ovaries of newborn mice contain several numbers of primordial follicles with single layer of squamous follicular cells, germ cells and stroma. Ovarian follicles successively develop and are destroyed in postnatal life and the total number of follicles is reduced in later stages. Experimental studies on animals have shown that low levels of lead accumulation in the ovaries could impede folliculogenesis. Lefevre B. (2001) and Junaid M, *et al*., (1997) reported that exposure to high lead concentrations caused considerable damage to mouse ovaries. Histological examples of atretic primordial follicles in postnatal or adult

ovaries are very limited (Perez, et al., 1999; Depalo, *et al.,* 2003). The strain differences also account for major variations in the assessment of ovarian histology with regard to the types and numbers of follicles (Myers, *et al.,* 2004). Depletion of primordial follicles in the postnatal mouse ovary is well documented. Lead toxicity is associated with interfering female hormonal regulation and decrease progesterone regulation. Since progesterone is a key hormone for establishing and maintaining pregnancy; it plays a role as an abortifacient. However the actual mechanism of lead induced progesterone inhibition is not clear; the suspected hypothalamic pituitary secretion might be responsible for the luteal phase of the cycle. Result revealed that small and medium follicles were significantly affected; therefore lead seems to affect the follicular development and maturation.

Lead was observed as being able to pass through the placenta of pregnant mice and reached and accumulated within the tissues of fetus. Similar results were found by Danielesson, *et al.,* (1983) proved that the lead is capable of reaching embryonic and fetal tissues at different periods of gestation in the mouse. Additionally, lead accumulated in many organs and body fluids especially the gonads and seminal fluid in addition to the testicular tissue and caused harmful effects on the performance of reproduction reported by Silbergeld (1983).

4.0 Conclusions:

This brief overview of gonadal development toxicity indicates that reproductive toxin produce adverse effects in a wide variety of ways and at multiple sites in the developing reproductive system of male and female offspring's.

From our findings we can conclude that lead passed through placenta during gestation and through milk during lactation. After reaching in embryonic and fetal tissue it create hindrance in the developing gonads of offspring's in lead exposed mothers. As the exposure point and duration was same for male and female pups, both sexes were affected during development. The histopathological modification in the basic precursor of gonads during the postnatal development causes reduced fertility in adulthood in both sexes. This might be characterized to abnormal development or dysfunction of sertoli cells and the all cells which are responsible for providing normal environment of the germ cell proliferation and maturation in males and females.

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