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Research article

EFFECTS OF MATERNAL LEAD ACETATE EXPOSURE DURING LACTATION ON POSTNATAL DEVELOPMENT OF OVARIES IN OFFSPRING OF SWISS ALBINO MICE.

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ABSTRACT: The course of human development from conception to adulthood is extremely complex. The developing organism is particularly vulnerable to toxic insult because of rapid cell division and differentiation and severely affected during gestation and lactation. The aim of the present study was to evaluate lead toxicity on the female reproductive system during neonatal period. A total of 30 female mice randomly divided into two equal groups; control and treatment group. During experimental period, lactating female mice was given lead acetate (0.5ml/Day) from day 1 to day 21 of lactation. At 7, 14 and 21 days after birth, the ovaries weights and diameters of different developing follicles were measured. Following tissue processing, 5 μm sections were stained with haematoxylin eosin and evaluated with quantitative techniques. Ovarian parameters in different groups were compared by one-way ANOVA. The results indicated that exposure of lead caused histological alteration in developing ovaries of mice and significantly ($P< 0.05$) decreased Ovaries weights and diameters of different developing ovarian follicles. Studies conducted on females revealed that lead suppresses the development of various follicles during fetal and neonatal life. It appears that lead interferes during specific events of ovarian developmental stages, which may create higher sensitivity for dysfunction in reproductive system during adulthood.
The present investigation evaluates the relative influences of prenatal and postnatal exposure of lead acetate on growth and ovarian histology in female offspring during postnatal development.
Keywords: Development, Histology, Lactation, Lead Toxicity, Swiss Mice

INTRODUCTION

The humans are exposed to various types of environmental contaminants at different stages of their life span, majority of them are harmful. Exposure of heavy metals during early age of life has been associated with adverse effects on development of gonads. 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 as gonadal cell proliferation [1] or alterations in the distribution of the toxicant to gonadal cells [2, 3]. The oldest harmful agent known to mankind is lead. Lead is of public health concern due to their toxic effects on various developing organs, persistence in pregnant and breast feeding mothers [4]. It is well known that lead passes through the placenta from mother to fetus and accumulates in fetal tissues during gestation [5] and can be obtained through the milk during lactation [6]. From high to low doses of lead exposure, there are different responses of lead including reduced fertility, spontaneous abortions, low birth weight, impairment in folliculogenesis, and even damage to the ovaries are also reported [7]. Animals have shown that low levels of lead accumulation in the ovaries could impede folliculogenesis [8]. A low Pb concentration in the mouse ovary caused dysfunction of folliculogenesis, with fewer primordial follicles and an increase in atretic antral follicles [9]. Oral administration of lead in high doses to reduction in the number of ovarian follicles revealing a strong correlation between blood lead level and atresia of ovarian follicles of albino mice [10]. Wiebe et al.,[11] found that lead exposure may significantly alter steroid production and gonadotrophin binding in the ovaries of adult rats. The coordinated development of follicles within the ovary is under the control of hormones and growth factors [12]. Once the lead is in the bloodstream, it is distributed into soft and hard tissues [13].

Milk is the most important food source for newborn, however, also is a pathway of maternal excretion of toxic elements such as lead, and these toxins impact most severely on the newborn at a time of rapid development of the central nervous system [14], since neonates absorb 40-50 times more lead than adults [15]. It appears from the evidences that the neonatal period is a critical stage in the process of sexual development and maturation in primates [16]. Keeping the above information in mind, present study was undertaken to investigate the effects of maternal lead exposure during lactation on postpartum development of ovary in offspring mice.

MATERIALS AND METHODS

Animals

A total of 60 pregnant female Swiss mice were used for experiments. The mice were obtained from Veterinari College, Mhow (M.P.). The animals were housed in stainless steel cages under standard animal house conditions. They received standard pellet food and distilled water was available *ad libitum*.

Treatments

After childbirth, all lactating females and their pups divided into two equal (control and treated) groups (N= 15 in each group). At parturition (day 0 lactation) the lactating females of treated group were administered with 0.5ml/day 0f 0.5ppm aqueous lead acetate for a period of 7, 14 and 21 days of lactation.

Measurements of Body and Ovarian Weights of Pups

Body and ovarian tissue weights of the control and treated pups of mice were measured at the end of 7, 14 and 21 days with an automatic balance (AND GX-600, Japan).

Histological Studies

At the termination of experiments mice were anesthetized with diethyl ether and ovaries were removed and quickly excised out and fixed in aqueous Bouins's fluid. After 24 hours, the ovaries were washed, dehydrated in graded series of alcohol, cleared in xylene and embedded in paraffin wax. The serial sections cut at 5 μm serial paraffin sections were prepared and stained with hematoxylin-eosin. Appropriate sections were observed under a binocular research microscope. Histopathological changes were noted in control and experimental animals.

For measuring the diameter of ovarian follicles in each developmental stage, 45 microscopic fields were randomly chosen in each mice. Then, using an ocular micrometer of light microscopy (Olympus EH), at a magnification of ×10, the largest and smallest diameters of each ovarian follicle were measured and the mean was calculated. To avoid counting the same follicle more than once, only individual follicles having an oocyte with a nucleus were evaluated, and we measured the size of the follicles in which the oocyte was present with an ocular micrometer.

Statistical analysis

Ovarian parameters in different groups were compared by one-way ANOVA and Tukey's test was used as a *post hoc* test. Differences were considered to be significant when $P< 0.05$, $P< 0.01$ and $P< 0.001$.

RESULTS

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

Days 7 post partum

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. The ovaries of control female infants exhibited large number of oogonia and primary follicles with single layer of squamous epithelium and a large eccentric nucleus (Fig.1). 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 oogonia and primordial follicles were less in number. Few primordial follicles were surrounded by atrophied cells. Animals showed decreased number of follicles that enter in the growing phase (Fig.2). The thickness of peritoneal covering and diameter of oogonia and primordial follicles were not significantly defer from control (Table 1)

Days 14 post partum

The ovary of a fourteen day old mouse showing normal histological structure of surface epithelium, cortex and medulla with many developing follicles including oogonia, primary and secondary, follicles. (Fig 3).

While in treated group the ovaries showed more drastic changes. The primary follicles exhibited degenerative changes characterized by pyknotic nuclei. Some of these were clumped together while others had big vacuoles. The secondary follicles appeared enlarged and were surrounded by atrophied double layered granulosa cells. The vacuolization in cytoplasm increased due to lead intoxication (Fig. 4). The diameter of all the developing follicles were significantly ($P< 0.05$, $P< 0.01$) reduced in comparison to control (Table 2).

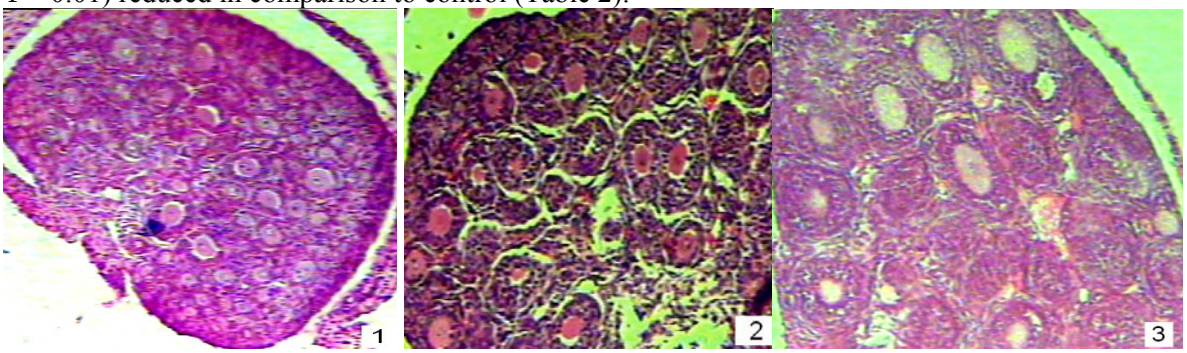

Fig 1: Histological section of ovarian follicles in ovary of mice at 7 days post partum in control group, illustrates normal structure of oogonia and primary follicels.

Fig 2: Histological section of mice ovary at 7 days post partum in lead treated group showing less number of primary follicles and some of them were surrounded by atrophied cells.

Fig 3: Histological section of ovarian follicles in ovary of mice at 14 days post partum in control group, showing normal histological structure of cortex and medulla with many developing follicles.

Table 1: Effect of lead acetate on Body Weight (g), Ovary Weight (mg) and Diameter of Developing Follicles (mμ) on 7 days post partum period of mice.

S.No.	Parameters	Control	Treated with LA
1.	Body Weight	3.45±1.14	3.33±2.17^{NS}
2.	Ovary Weight	0.003±0.005	0.003±0.001 ^{NS}
3.	Oogonia	7.291±0.008	7.195±0.005 ^{NS}
4.	Primary Follicles	12.00±0.013	12.010±0.016 ^{NS}

All values are expressed in ±SEM Significant level, NS = Non significant, * = (P<0.05), ** = (P<0.01) * (P<0.001)**

Table 2: Effect of lead acetate on Body Weight (g), Ovary Weight (mg) and Diameter of Developing Follicles (mμ) on 14 days post partum period of mice.

S.No.	Parameters	Control	Treated with LA
1.	Body Weight	5.89±2.25	5.70±1.59 ^{NS}
2.	Ovary Weight	0.008±0.009	0.005±0.007 **
3.	Oogonia	8.690±0.0006	6.897±0.009 ^{NS}
4.	Primary Follicles	14.00±0.002	11.150±0.001*
5.	Secandary Follicles	18.089±0.010	15.068±0.08**

All values are expressed in ±SEM Significant level, NS = Non significant, * = (P<0.05), ** = (P<0.01) * (P<0.001)**

Days 21 post partum

The following 21 days reveals the later phases of development of the primary follicles takes on a very distinctive appearance, which differs markedly from the ovary in infancy. Follicle development has progressively established so that simultaneously small, medium and large follicles are very clearly seen and in proper architecture. There is marked differentiation between medullary and cortex region. (Fig.5). On 21day ovary of lead treated mice showed severe damaging pattern in its size, shape and structure including germinal epithelium, cortex and inner medullary region. In a few cases the immature follicles were highly damaged and were surrounded by atrophied follicular cells with karyohypertrophy. The cells of theca and granulose layers of growing follicles were disorganized and swollen. The compactness of different cells among layers were reduced i.e. they were loosely packed in comparison to control. The structure of germinal epithelium was irregular. Different types of follicles were present although very less number of primary follicles was present at the periphery just under the germinal epithelium but there structure was not intact. 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.6). The diameter of different developing follicles were significantly ($P \leq 0.001$) reduced as comparison to control (Table 3).

Table 3: Effect of lead acetate on Body Weight (g), Ovary Weight (mg) and Diameter of Developing Follicles (mμ) on 14 days post partum period of mice.

S.No.	Parameters	Control	Treated with LA
1.	Body Weight	9.69±5.89	6.89±7.99*
2.	Ovary Weight	0.010±0.089	0.006±0.009***
3.	Oogonia	9.720±0.008	6.490±0.003**
4.	Primary Follicles	19.065±0.005	14.015±0.003***
5.	Secandary Follicles	23.00±0.004	16.020±0.006***
6.	Growing Follicles	26.04±0.17	19.080±0.009***

All values are expressed in ±SEM Significant level, NS = Non significant, * = (P<0.05), ** = (P<0.01) * (P<0.001)**

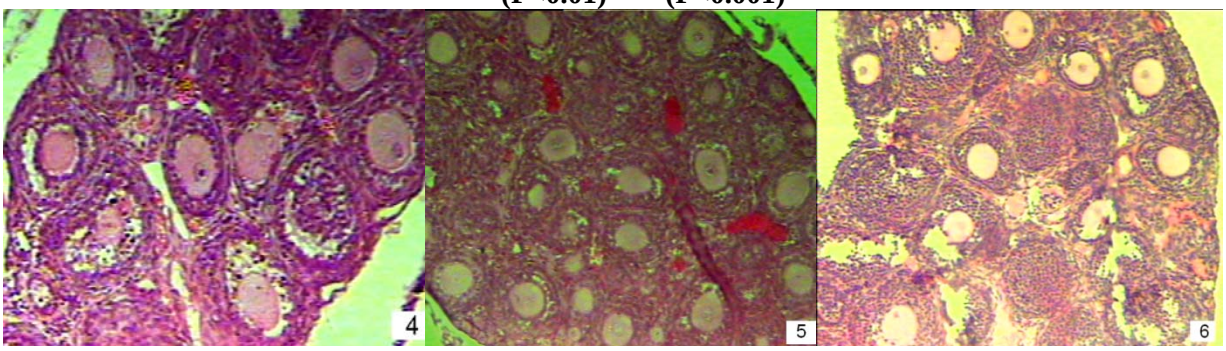

Fig 4: Histological section of mice ovary at 14 days post partum in lead treated group showing degenerative changes in developing follicles, characterized by pyknotic nuclei. Some of these were clumped together while others had big vacuoles.

Fig 5: Histological section of ovarian follicles in ovary of mice at 21 days post partum in control group, illustrates normal appearance of different developing follicles encircled by theca and granulosa cells.

Fig 6: Histological section of mice ovary at 21 days post partum in lead treated group shows severe damage in various ovary components. In a few cases the immature follicles were highly damaged and were surrounded by atrophied follicular cells with karyohypertrophy. The cells of theca and granulose layers of growing follicles were disorganized and swollen.

DISCUSSION

The ovarian follicle is the fundamental 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 [17]. The development of both the mammalian oocyte and the somatic cell compartments of the ovarian follicle are highly coordinated; this coordination ensures that the ovulated oocyte is ready to undergo fertilization and subsequent embryogenesis. Disruption of this synchrony results in oocyte developmental failure. Increasing evidence demonstrated that lead could be transported from dams to pups through milk [18,19] and disrupt the folliculogenesis in the developing ovaries of pups. In our study, in the control group from 1 to 21 days post partum, the diameter of follicles decreased and very less number of follicles developed in growing phase but lead produced increasing damage in the ovary during these days. The reduction in the number of developing follicles from 7 to 21 days of postnatal life was increased markedly. In consistent to our results Shabaka et al., [20] also studied lead acetate induced reduction of germ cell number in the developing gonads. In the present investigation, it was noted that the post partum development of ovaries in female sucking pups exhibited remarkable deformation when exposed to lead acetate through dam's milk. The developing follicles were surrounded by atrophied follicular cells layers. They were also disorganized and some follicles exhibited degenerative changes characterized by pyknotic nuclei. Some of these were clumped together while others had big vacuoles. Bires et al., [21] also observed histological changes in the number of ovarian follicles and the increase occurrence of primary atretic follicles indicated alterations in the membrane structures and organelles of oocytes and in the follicular cells of stratum granulosum. Taupeau et al., (22) reported lead accumulation in low concentration in the ovary caused dysfunction of folliculogenesis with fewer primordial follicles and an increase in atretic antral follicles. Our results are also in conformations with the above findings. Many studies have suggested that not only the high lead dose can damage the ovary but even the low doses has provoked an inhibition in the folliculogenesis leading to the dysfunctions of this process [23] Thus, maternal lead exposure during lactation can impair ovarian development in female offspring is of great interest. The present results showed that the ovarian weight and diameter of developing ovarian follicles were significantly decreased in pups whose mothers were exposed to lead during lactation.

In summary, the present results allow us to reach the following conclusions. First, lead could be transported from dams to pups through milk; second, neonatal lead exposure through milk persistently disrupts ovarian development. Moreover, the histological modification in the basic precursor of gonads during the post partum development causes reduced fertility in adulthood in female. This might be characterized to abnormal development or dysfunction of ovarian follicles and the all cells which are responsible for providing normal environment of the follicles proliferation and maturation in females

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