Embryological developmental changes in the gonads of male mice associated with lead administration

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Abstract:
The biological systems of human in the modern world are increasingly being exposed to lead which exists in the environment. Women at reproductive age and pregnant are more susceptible to the danger of environmental lead pollutant leading to infertility. Prenatal exposure to lead may cause abnormal growth, spontaneous abortion and congenital problems. As there is an increase usage of electrical generators that depend on lead-based gasoline by Iraqi people which lead to increase the air pollution with this toxic substance induced us to detect through this study its possible negative effects on the fate of conception and embryonic development using the mouse as a model. The study aims to asses the effect of low dose concentrations of lead acetate given to pregnant female mice on the development of gonads of male mice embryos at different periods of gestation. Mature mice aging 8-10 weeks (180 mice) weighing 25-27 grams were used. The animals were divided into three major experimental groups (G1, G2, G3) according to the level of the dose (30 animals/group), paralleled with three control groups (C1, C2, C3). Each major group subdivided into three minor groups (10 animals/group) according to different periods for sacrificing during gestation period (day 14, day 17, and day 20). Vaginal smears were taken from all animals daily until metestrus phase and mating was occurred using one male mouse for each female, the first day of pregnancy registered, indicated by presence of vaginal plug. Experimental groups G1, G2 and G3 administrated daily with either 0.1, 0.2 or 0.4 mg/kg body weight of lead acetate respectively dissolved in normal saline, injected intraperitoneally for 14, 17 or 20 days of gestation, while corresponding control animals C1, C2 and C3 were injected with normal saline only with doses and periods similar to that used with experimental animals. Mother’s body weight, weight of uterus, weight and numbers of fetuses in right and left horn, pregnancy outcomes (abortion, and stillbirth), and diameter of fetal testes were recorded after 14, 17, and 20 day post coitum (dpc). Results showed a significant decrease in all the studied parameters of the experimental groups including: mother’s body weights, uterus weights, numbers and weights of fetuses, pregnancy outcomes (abortion and stillbirth) and diameter of fetal testes compared to that of the control groups. Histological study showed significant increase in the diameter of the testis at age 14 days with the low dose, then it become significant decrease at age 17 and 20 days of gestation with disrupting of testicular structure organization, degeneration of germ cells and absence of basal lamina in all the experimental groups. In addition undescending of the testes to its normal position was observed in all experimental groups at 17 and 20 dpc. It remained adjacent to the kidneys at the upper part of the abdominal cavity while it was normally relocating at the base of the abdominal cavity in control groups at 17 dpc and descent into inguinal canal at 20 dpc. It was concluded from these results that lead acetate given to pregnant female mice impaired the gonads of male mice embryos with reduction in mother’s body and uterus weights along with adverse pregnancy outcomes. These negative effects induced us to avoid the dangerous from using this toxic substance and trying to found effective alternative materials through environmental safety centers.

Key words: lead acetate /mice, Embryological developmental of mice / gonads.

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Introduction:

Exposure to chemical substances during early stages of life is of particular concern. Both unborn and newborn babies are thought to be more susceptible to chemical exposure because these periods represent some of the most complex and sensitive in terms of body development [1]. It has been estimated that every year around 100,000 different types of chemicals are produced and used around the world [2]. Lead is a major environmental pollutant that is known to be a poisonous compound for centuries [3], it is naturally occurring as a bluish-gray metal found in small amounts in the earth’s crust [4]. Exposure to lead occurs primarily through drinking water, food, airborne, lead containing particulates and lead-based paints [5]. However, the effect of lead is the same whether it enters the body through breathing or swallowing. Lead can affect almost every organ and system in the body [4], specifically the soft tissues such as kidneys, brain, lungs, muscles, testis, and ovaries [6]. Lead is one of the most significant reproductive toxicants [7], and could possibly have adverse effects on the developing reproductive system through action on the hypothalamic-pituitary axis during fetal life [8]. Prenatal exposure to low lead levels (Maternal BLL of 14µ/dl) may increase risk of reduced birth weight and premature birth [9]. Additionally, prior exposure to lead is associated with spontaneous abortion [10] and [11]. The increased usage of electrical generators by Iraqi people throughout the last fifteen years in addition to the other known lead resources which cause an increase in the average lead concentration in Baghdad air reaching 2.52µ/m³ [12], which is more than the allowable limitations for air quality standards (0.1-0.3µ/m³) that sit by [13], with all the previous material hazardous of lead mixed with fuel of these machines, induced us to conduct this study aiming to: Detect the possible poisoning effect of this substance on the embryonic development of the fetal gonads.

Materials and methods:

All experiments were performed on 180 mature female Swiss-Webster mice, their ages ranged between 8-10 weeks with a body weight (B wt) ranged between 25-27 g., obtained from the colony of the animal house of the Institute of Embryo Research and Infertility Treatment, Al-Nahrain University. They were kept in a room supplied with air conditioner to keep the temperature between 18-24°C, the air of the room was changed continuously by using ventilating fan and the light was controlled with a range of 12 hours of light and 12 hours of darkness. The animals were housed in plastic cages (3 mice/cage) with a wire grid covers measuring 28 x 15 x 14 cm, supported on ventilated racks [14]. Ninety female mice were divided into three major experimental groups (G1, G2 and G3) according to the level of the dose (30 animal/group). Each major group subdivided into three minor experimental groups (10 animals/group) according to different periods for killing during gestation period (day 14, day 17, and day 20). Another 90 animals with the same age divided at the same way as in the previous experimental groups considered as control groups injected intraperitoneally by normal saline only. Vaginal smear were performed to all the adult female mice to detect heat stage for mating. Females in the estrous phase were left with mature healthy males for mating (1 male/1 female). The occurrence of vaginal plug considered as the first day
of pregnancy, the pregnant female was removed into separate cage. Injection of lead acetate (0.1, 0.2, 0.4 mg/kg body weight /day intraperitoneally, were started at the first day of gestation and continued for (14, 17 or 20 days) for the experimental groups G1, G2, G3 respectively while the three parallel control groups were injected normal saline with the same rout and dose as that used in the experimental groups. At day14, 17 and 20 days of gestation the body weight was measured and 10 animals of each group were scarified at each of these intervals an incision was made in area of the abdomen to remove the pregnant uterus. The abdominal cavity were opened, one horn of the uterus below the oviduct were cut and grasp the end firmly with the fine forceps, pull upward and separate the uterus from the mesometrium using the tips of the forceps or scissors. Then pull the uterus down tautly and out to one side, the same was done to the other horn. The intact uterus were removed and place it in a dish containing worm normal saline, washed and weighted by sensitive electrical balance. Number of died and life fetuses in each uterus were recorded. Each fetus were washed and weighted, then fixed in Bouin's solution for 24 hours, ethanol alcohol for routine histological techniques, paraffin sections with 5 micron thickness were prepared and stained with hematoxylen eosin stain for histological study [15]. Morphological differences of male fetal gonads were examined. In this study the morphometric analysis was applied by using BEL micro image analyzer system ver. 2.0, at first the measurements done were at pixel value, to find the Mean ±SD for the different diameter of testes in pixel at x31000.

Results:
I. Effect of different doses of lead on weights and numbers of fetuses:
A significant decrease (P< 0.05) in weights and numbers of fetuses belongs to mothers injected with 0.1, 0.2 or 0.4 mg/kg B wt. of lead acetate at 14 and 17dpc and highly significant reduction (P< 0.01) was recorded at 20 dpc, in comparison with that of control group as shown in Figure (1),(2), (3), (4), (5), (6). No differences were recoded between right and left horns concerning these parameters.

II. Histological study:
A. Changes in testes diameters:
Administration of 0.1 mg/kg b. wt. of lead acetate causes significant increase (P<0.05) in the diameter of the testes at 14, 17, and 20 dpc as compared to that of control group, while with the higher doses of lead (0.2 and 0.4 mg/kg. b. w.) a significant decrease (P< 0.05) in the diameter of the testes at day 14 and 17 and become highly significant at day 20 were recorded as shown in Table (1).

B. Histological Observations:
The histological sections of the xy gonads of the fetuses belong to mothers from control group showed the linkage of the gonads to the mesonephron at the abdominal cavity at day14 of pregnancy. These gonads with the tunica albuginea at their periphery contained numerous, well organized testicular cords in which primordial germ cells (large cells with prominent nuclei) surrounded by peritubular myoid cells were separated by somatic Sertoli cells at the periphery, the interstitium contained steroidogenic Leydig cells precursors, pericyts were observed in close association with endothelial cells of normal gonadal capillaries, (picture 1),
while gonads of the fetuses belong to experimental groups (G1, G2 and G3) showed mild degenerative changes and micros of spermatic cells, no organized testicular cords, peritubular myoid cells or mesenchyme were seen (picture 2), (picture 3), and (picture 4). Fetal gonads of control groups at 17 and 20 days post coitum exhibit well organized testicular cords with Sertoli cells surrounding germ cells, peritubular myoid cells and extensive interstitial tissue, including Leydig cells which have round nuclei. Mitotic activity of primordial germ cells was clearly demonstrated. The characteristic male-specific coelomic vessels, pericytes around developing capillaries were visible in the mesenchyme, (picture 5) and (picture 9).

While the gonads of fetuses from experimental groups (G2, G3) at 17 dpc showed disorganized testicular cords with degenerative germ cells, disrupting of basal lamina with numerous vacules appeared throughout the structure of the testicular cords (picture 7) and (picture 8). All these degenerative features appeared more severely at 20 dpc. (Picture 10), (Picture 11), and (Picture 12). On the other hand, histological section at 17 and 20 dpc of the control animals revealed the descending of the testes to the pelvic cavity at the level of the bladder, (picture 13), then reach the inguinal canal at 20 dpc., while testes of experimental groups, appeared much smaller and undescended to its normal position, but remained adjacent to the kidneys (metanephron) at the upper part of the abdominal cavity (picture 14), (picture 15), and (picture 16).
Figure (4): Changes in number of fetuses in right horn, and left horn associated with the administration of (0.2 mg/kg B wt.)(G2) of lead acetate to pregnant female mice for 14, 17, 20 dpc.

Values are mean ± standard error (SEM), (n=10 animals/group).

**: Highly significant decrease (P< 0.01).
*: significant decrease (P<0.05).

Table (1): Changes in diameter of testes of male mice embryos associated with the administration of (0.1, 0.2, 0.4mg/kg B wt) of lead acetate to their mothers for 14, 17, 20 dpc in (nm) at x 31000,

<table>
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<th>14 days</th>
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<td>Mean</td>
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<tr>
<td>Control</td>
<td>104.62</td>
<td>3.87</td>
<td>131.07</td>
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<tr>
<td>G1</td>
<td>119.45</td>
<td>*</td>
<td>111.78</td>
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<tr>
<td>G2</td>
<td>100.58</td>
<td>*</td>
<td>6.114</td>
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<tr>
<td>G3</td>
<td>57.77</td>
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Figure (6): Changes in number of fetuses in right horn, and left horn associated with the administration of (0.4 mg/kg B wt.)(G3) of lead acetate to pregnant female mice for 14, 17, 20 dpc.

Values are mean ± standard error (SEM), (n=10 animals/group).

**: Highly significant decrease (P< 0.01).
*: significant decrease (P<0.05).

Picture (1): Testes section of male mouse embryo aged 14 dpc (control group). Note the numerous, well organized testicular cords (tc) in which primordial germ cells (pm) (large cells with prominent nuclei) separated by somatic Sertoli cells (Sc) (H&E, 40X).
Picture (2): Testes section of male mouse embryo aged 14 dpc (G1) belongs to mother treated with (0.1 mg/kg B wt) of lead acetate for 14 days. Note mild degenerative changes of primordial cells (dpm), no organized testicular cords (tc), peritubular myoid cells (pt) were seen, with loose interstitial tissue (H&E, 40X).

Picture (3): Testes section of male mouse embryo aged 14 dpc (G2) belongs to mother treated with (0.2 mg/kg B wt) of lead acetate for 14 days. Note disorganized and lack distinct testicular cords (tc), degenerative seen in Primordial germ cells (dpm) which are irregular in shape with less density (H&E, 40X).

Picture (4): Testes section of male mouse embryo aged 14 dpc (G3) belongs to mother treated with (0.4 mg/kg B wt) of lead acetate for 14 days. Note disorganized and lack distinct testicular cords, sever degenerative of primordial germ cells (dpm) which irregular in shape with less density (H&E, 40X).
Picture (5): Testes section of male mouse embryo aged 17 dpc (control group). Note the well organized testicular cords (tc) filled with germ cells (g) and Sertoli cells (Sc) surrounding primordial germ cells (pm) with extensive interstitial tissue (H&E, 40X).

Picture (6): Testes section of male mouse embryo aged 17 dpc (G1) belongs to mother treated with (0.1 mg/kg B wt) of lead acetate for 17 days. Note irregular testicular cords (tc) with prominent degenerative changes in primordial germ cells (dpm). The sections also showed mild vaculation (v) inside testes cords (H&E, 40X).

Picture (7): Testes section of male mouse embryo aged 17dpc (G2) belongs to mother treated with (0.2 mg/kg B wt) of lead acetate for 17 days. Note disorganized and necrosis of testicular cords (tc) degenerative of primordial germ cells (dpm) Numerous vacules (v) appeared throughout the structure of testicular cords (tc). (H&E, 40X).
Picture (8): Testes section of male mouse embryo aged 17 dpc (G3) belongs to mother treated with (0.4 mg/kg B wt) of lead acetate for 17 days. Note, the severely affected primordial germ cells (pm) with typical vaculation (v) (H&E, 40X).

Picture (9): Testes section of male mouse embryo aged 20 dpc (control group). Note the well organized testicular cords (tc) filled with germ cells (g) and Sertoli cells (Sc) surrounding germ cells (g) with extensive interstitial tissue (H&E, 40X).

Figure (10): Testes section of male mouse embryo aged 20 dpc (G1) belongs to mother treated with (0.1 mg/kg B wt) of lead acetate for 20 days. Note degenerative of primordial germ cells (dpm) inside the testes cords (tc) with vaculation (v) (H&E, 40X).
Figure (11): Testes section of male mouse embryo aged 20 dpc (G2) belongs to mother treated with (0.2 mg/kg B wt) of lead acetate for 20 days. Note irregular testicular cords (tc) which severely decreased in number. Primordial germ cells (pm) severely affected with necrosis. Disrupting basal lamina (bl) (H&E, 40X).

Figure (12): Testes section of male mouse embryo aged 20 dpc (G3) belongs to mother treated with (0.4 mg/kg B wt) of lead acetate for 20 days. Note the high reduction in the numbers of Primordial germ cells (pm) which appears with clear degenerating features. No distinctive testicular cords (tc) appear since there is disrupting of basal lamina (bl) (H&E, 40X).

Figure (13): Longitudinal section of mouse embryo aged 20 dpc (control group), showing descending of the testes (t) to the pelvic cavity at the level of the bladder (b) (10X).
Figure (14): Longitudinal section of mouse embryo aged 20 dpc (G1) (Treated with 0.1 mg/kg B wt of lead acetate), showing the persistence of the testes adjacent (t) to the kidneys (k) (metanephron) in the abdominal cavity (10X).

Figure (15): Longitudinal section of mouse embryo aged 20 dpc (G2) (Treated with 0.2 mg/kg b. wt of lead acetate), showing the persistence of the testes adjacent (t) to the kidneys (k) (metanephron) in the abdominal cavity (10X).

Figure (16): Longitudinal section of mouse embryo aged 20dpc (G3) (Treated with 0.4 mg/kg b. wt of lead acetate), showing the persistence of the testes adjacent (t) to the kidneys (k) (metanephron) in the abdominal cavity (10X).
**Discussion:**

The significant reduction in mother's body and uterus weights in all the animals of the experimental groups may be attributed to decrease in feeding habit which indicates that lead has a general inhibitory action on metabolism [16]. Lead most likely interfere with functions performed by essential minerals such as calcium, iron, copper, and zinc, moreover, lead does interrupt several red blood cell enzymes systems, including delta-aminolevulinic dehydretase and ferrochelatase [17]. It may also diminish hemoglobin synthesis and can react with cell membranes [18].

Results of this study revealed that lead is able to cross the placenta of pregnant female mice reaching the embryonic and fetal tissues, this is similar to the results of [19] and [20], which proved that lead is able to pass through the placenta of pregnant female mice, presumably by passive diffusion, and accumulate in embryos tissues over the period of gestation [21], [22], [23] and [24].

The reduced fetal weight agreed with that found by different researches on mice and rats [25], [26] and [27] which proved that infants born to mothers with prenatal occupational exposure had an increased risk of low birth weight. The mechanism by which the fetal body weight reduced may be due to decreased fetal growth because lead has a wide range of biological effects depending upon the level and duration of exposure. Moreover, in the primitive streak stage of developing embryo, the trophoplast cells are in close contact with maternal blood and lead might therefore more easily interfere with the permeability of the trophoplast cell membranes or the membranes of the visceral endodermal cells of the yolk sac, affecting the transport of compounds essential for the nourishment of the embryo [28].

Furthermore, the fetuses have immature metabolism and is not able to detoxify substances very efficiently, the only routes of excretion is via diffusion or active transport back to the maternal circulation or elimination into the amniotic fluid [29]. The reduction in fetus weight may also attributed to the replacement of vital minerals such as calcium, potassium by lead and binding with the red blood may reduce oxygen carrying capacity of the cells, and making red blood cells to destroy more rapidly resulting the impairment of hemoglobin synthesis in heamopeotic tissues [30]. Furthermore lead distribution in the body fluids was shown to interfere with Na/K ATPase pump and with attaches to the red blood cells membrane [24], this may cause increased permeability of the cells and damage or even death of the cells [31]. Moreover the accumulation of lead in vulnerable tissues causes toxic manifestation and the low elimination rate is responsible for the causes of accumulation [32].

In experimental groups G1, G2 and G3, the significant decrease in fetuses number may be related mainly to the high percentage of failed implantation compared to that of control group, this result may be explained on the bases that implantation depend upon the presence of good amount of estrogen and progesterone [33], these ovarian hormones are crucial for implantation in mice and rats [34], [35], [36] and [37]. Moreover, possible mechanism of action of lead as a cause of infertility might be due to its antiestrogenic activity [38].

Different studies showed that chronic exposure to lead with blood levels at approximately 35 µ/dl resulted in subclinical suppression of circulating LH, and FSH. Furthermore, a reduction in the number of offspring of laboratory animals and in the families of workers occupationally exposed to
lead might be due to the impaired ovarian function as the impairment of normal maturation of this function [26], [42] and [43], and significantly reduced steroid production [44].

At dose 0.1 mg/kg B wt (G1) the significant increase (P< 0.05) in the diameter of the testes at 14 dpc in comparison with control group may be due to receiving the whole dose by embryos at this period of gestation because the defense mechanism was not well developed leading to accumulation of lead in gonads which in turn causing more loose and disrupted structures of the testes, the increase in testes size may be caused by weakening of the surrounding connective tissues and myoid cells due to exposure to lead acetate.

The significant increase in the diameter of testes of the experimental group (G1) at 17 and 20 dpc may be due to rapid growth at the period of this gestation, high dose, and long duration of exposure to lead causes more disruption, necrosis, oedema that results increase of the diameter of the testes these results are in a good agreement with the findings of [39], that cited the presence of oedema due to destruction of lining epithelium of blood vessels which lead to prevalence of plasma and electrolytes and infiltration quantities of plasma to the interstitial tissues of the testes.

The reduction in the diameter of testes recorded in (G2) and (G3) (0.2, 0.4 mg/kg B wt.) might be due to accumulation of lead in the gonads and testicular tissues which affects the physiology of reproduction [40], causing testicular damage and degenerative changes in the testicular tissues [41], [42] leading to dysfunction of the Sertoli cells, which is responsible for the germ cells proliferation and maturation [43]. Also the exposure to lead caused dystrophic changes in the Leydig cells, moreover lead depostis were seen in smooth myocytes, epithelial cells of testes cords [44]. While a dose of 0.4 mg/kg B wt (G3) of lead acetate caused a highly significant decrease (P<0.01) in the diameter of the testes at 14 dpc. This may be attributed to that most of the testicular germ cells might have been destroyed either due to membrane damage or macromolecular degradation leading to a significant decline in testicular size [45].

Furthermore lead caused pathological changes in the testes leading to either arrest of germ cells multiplication and/or differentiation [46], while [47], proved that the testes of developing chick embryos treated with lead exhibited a marked change, this includes decrease of germ cells, and the migration of primordial germ cells are inhibited.

Highly significant decrease (P< 0.01) in (G3) at 17 dpc and 20 dpc in the diameter of testes at the level of this dose may be because embryos at this period of gestation were under organogenesis and rapid growth withdrawing more minerals including lead from their mothers leading to more disrupting, necrosis, and heavy loss of germ cells in the testes which reflected by the significant size loss. Similar results were recorded by [48].

Undescending of the testes to its normal position was observed in experimental group (G1), (G2) and (G3) at 17 and 20 dpc. Transfer of the male gonad from its site of origin at the urogenital ridge, apposed to the kidney, into the scrotum is critical event in male sexual differentiation [49], and since testicular descent is hormonally regulated in which the presence of testosterone induces regression of the cranial suspensory ligaments (CSL), while Insulin Like-III (Insl3) promotes contraction of the gubernacular cord and outgrowth of the gubernacular bulb [50], in fact
gubernaculum contains high levels of androgen receptors [51] and is considered the primary site of androgen action in testicular descent [52] and as it was recorded in this study that lead has a direct insult on the testicular production of the steroid hormone (testosterone), the main male hormone [53] and on the anterior pituitary gland [54] and [55]. Furthermore [27] showed that the Leydig cells are an important target for the harmful action of lead which interferes with several steps in the testosterone biosynthetic pathway, leading to reduction in plasma and intratesticular levels of testosterone. Moreover, in fetal Leydig cells, Insl3 expression is estrogen sensitive [56] a very narrow range of estrogen levels can alter gene expression [37]. Targeted disruption of Insl3 gene, which is specifically expressed in fetal Leydig cells, causes bilateral cryptorchidism [57]. The demonstration of estrogen during fetal and neonatal development has been reported to be associated with a series of male reproductive disturbances, such as cryptorchidism [58]. Estrogen receptors (ERs) and aromatase are found at all stages of testicular development in the rodents [59]. Leydig cells within the rodent fetal testes contain ER alpha until birth [60]. Sertoli cells are also considered as a source of estrogen production by fetal testes [61], several studies have suggested that estrogen exposure may interfere with fetal hypothalamic-pituitary-testes axis, leading to an inhibition of fetal Leydig cells androgen production, thus interfering with testicular descent [62], however, it is well known that in utero exposure of male fetuses to high levels of estrogen can interfere with testicular descent [58], furthermore estrogen deprivation during fetal development is also associated with this process [63]. Lead is well known to cause hormonal disturbance and has antiestrogenic activity [38]. Lead induced hypofertility particularly among the workers of lead factories has been thought to be due to the direct toxic effects of lead on male gonads [64]. The results of this study showed that at higher exposure levels there is a clear association between lead and adverse pregnancy outcomes. At dose 0.2 - 0.4 mg/kg b.wt., there are significant increased cases of abortion and stillbirth, these results are in a good agreement with work of [65], [66], [67] and [68] which stated that the reproductive toxicity of prenatal lead exposure has been associated with reduced fertility, early fetal loss, and stillbirth. Moreover, infertility and stillbirths were common among heavily exposed women lead workers [53], and the actual number of births was lower than the expected number of births for the control group [69]. The study findings of abortion and stillbirth may be attributed to that one way lead may exert its effects on the developing fetus is by precipitating early fetal loss, because inorganic lead has been shown to interfere with embryo implantation in the mouse [70]. Furthermore, the formation of placenta is a critical process in mammalian embryogenesis, without a healthy placenta, the embryo does not survive, its malformation can trigger a spontaneous abortion [71]. Work of [72] showed that placental lead concentration was higher in cases of stillbirth or neonatal death. Also [73] proved that lead crosses the placenta of pregnant women and caused poisoned offspring. The findings of this study suggest that even at 0.1 mg/kg b.wt. of injected lead acetate reveal clear cases of stillbirth, this is similar to that found by [41] who stated that low level exposure to lead may cause many hazards such as developmental toxicity in the offspring.
and stillbirth, and also agree with the findings of [74], which proved that reproductive health hazards by lead cause developmental impairment or death in the embryo, fetus and child.

Reference


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72. Khera, A.K.; Wibberley, D.G. and
التغيرات النمنية الجنينية في مناسل ذكور الفئران المعرضة لاستهلاك الرصاص

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الخليفة:

تتأثر أجهزة حس الإنسان بدرجات متقدمة بالتأثير السني لمادة الرصاص المتشرطة في البيئة. وتعرض النساء في سن الأجداب والحوامل لخطر التلوث بهذه المادة مزودًا إلى التهور أكبر من حالات المعجم كما تتأثر نسج وعاء والفرن بغالب في حالة الحمل مما يؤدي إلى حدوث اختلال في النمو والأداء. ونتيجة لذلك تم إعداد الرصاص المختلفة من قبل الأجهزة للفتيات ومختلفة من خلال بخار الدم وحالة الأداء النسائي للملتزن التكميلي في الحاجة. أجريت هذه الدراسة على (180) فتاة غالية العمر ثنائياً إلى غاية 1987-26-23 أсетاً.

تتأثر السيدات المتشرطة بالمادة الرصاصية (G1) قبل خطط الرصاص في سنا التوليد (G2) و(G3) و(G4) اعتمادًا على لقاح الجرعة المضافة (30 فاراً/جم) و(30 فاراً/جم) ثم قسمت كل مجموعة رئيسية إلى خمس مجموعات ثانية (10 فاراً/جم) على أساس الفترات المختلفة من الحمل التي يتم بها قلق الوليد وهو في اليوم 20-25. أخذت نتائج مهيئة لكل الحيوان يومياً حتى ظهور طفر الشبق في جسم السيدة الأولى للحمل حيث تمت مراقبة هذه الحيوانات مع ذكر بمادة

وحذج حذاء الحمل وحاسب البدء الأول من خلال فحص السيدة المهنية. تحققت مجموعات الأحجام في (G1) و(G2) و(G3) وأ(0.1) و(0.2) و(0.3) ملمع كجم من وزن الجسم على التوالي من منتصف أعداد الرصاص المرتفع (G1) و(G2) و(G3) و(G4).

يرجع الدم المعدن بالكامل (normal saline) في الغالب المعلمي المعدل (P<0.01) ذو نفس الكمية والمصفوف بالكامل المعدل (C1) و(C2) و(C3) و(C4) المصلوف المذهل في كل مجموعة من فحص السيدة المعيشية. أظهرت النتائج وجود خاصية معنوية (P<0.05) في جملة أحديات الحمل تميزها عن غيرها من جملة الحمل حيث تمت تقصين حالات الحمل بعنف الولد

قد تتأثر السيدة في جملة الحمل (P<0.05) في معدات متعلقة بالاستخدام للسيدة النظرية لأشعة الأشعة الذكورية (C1) و(C2) و(C3) و(C4) في موقعين (P<0.05).

الخليفة:

تتأثر أجهزة حس الإنسان بدرجات متقدمة بالتأثير السني لمادة الرصاص المتشرطة في البيئة. وتعرض النساء في سن الأجداب والحوامل لخطر التلوث بهذه المادة مزودًا إلى التهور أكبر من حالات المعجم كما تتأثر نسج وعاء والفرن بغالب في حالة الحمل مما يؤدي إلى حدوث اختلال في النمو والأداء. ونتيجة لذلك تم إعداد الرصاص المختلفة من قبل الأجهزة للفتيات ومختلفة من خلال بخار الدم وحالة الأداء النسائي للملتزن التكميلي في الحاجة. أجريت هذه الدراسة على (180) فتاة غالية العمر ثنائياً إلى غاية 1987-26-23 أсетاً.

تتأثر السيدات المتشرطة بالمادة الرصاصية (G1) قبل خطط الرصاص في سنا التوليد (G2) و(G3) و(G4) اعتمادًا على لقاح الجرعة المضافة (30 فاراً/جم) و(30 فاراً/جم) ثم قسمت كل مجموعة رئيسية إلى خمس مجموعات ثانية (10 فاراً/جم) على أساس الفترات المختلفة من الحمل التي يتم بها قلق الوليد وهو في اليوم 20-25. أخذت نتائج مهيئة لكل الحيوان يومياً حتى ظهور طفر الشبق في جسم السيدة الأولى للحمل حيث تمت مراقبة هذه الحيوانات مع ذكر بمادة

وحذج حذاء الحمل وحاسب البدء الأول من خلال فحص السيدة المهنية. تحققت مجموعات الأحجام في (G1) و(G2) و(G3) وأ(0.1) و(0.2) و(0.3) ملمع كجم من وزن الجسم على التوالي من منتصف أعداد الرصاص المرتفع (G1) و(G2) و(G3) و(G4).

يرجع الدم المعدن بالكامل (normal saline) في الغالب المعلمي المعدل (P<0.01) ذو نفس الكمية والمصفوف بالكامل المعدل (C1) و(C2) و(C3) و(C4) المصلوف المذهل في كل مجموعة من فحص السيدة المعيشية. أظهرت النتائج وجود خاصية معنوية (P<0.05) في معدات متعلقة بالاستخدام للسيدة النظرية لأشعة الأشعة الذكورية (C1) و(C2) و(C3) و(C4) في موقعين (P<0.05).

الخليفة:

تتأثر أجهزة حس الإنسان بدرجات متقدمة بالتأثير السني لمادة الرصاص المتشرطة في البيئة. وتعرض النساء في سن الأجداب والحوامل لخطر التلوث بهذه المادة مزودًا إلى التهور أكبر من حالات المعجم كما تتأثر نسج وعاء والفرن بغالب في حالة الحمل مما يؤدي إلى حدوث اختلال في النمو والأداء. ونتيجة لذلك تم إعداد الرصاص المختلفة من قبل الأجهزة للفتيات ومختلفة من خلال بخار الدم وحالة الأداء النسائي للملتزن التكميلي في الحاجة. أجريت هذه الدراسة على (180) فتاة غالية العمر ثنائياً إلى غاية 1987-26-23 أсетاً.

تتأثر السيدات المتشرطة بالمادة الرصاصية (G1) قبل خطط الرصاص في سنا التوليد (G2) و(G3) و(G4) اعتمادًا على لقاح الجرعة المضافة (30 فاراً/جم) و(30 فاراً/جم) ثم قسمت كل مجموعة رئيسية إلى خمس مجموعات ثانية (10 فاراً/جم) على أساس الفترات المختلفة من الحمل التي يتم بها قلق الوليد وهو في اليوم 20-25. أخذت نتائج مهيئة لكل الحيوان يومياً حتى ظهور طفر الشبق في جسم السيدة الأولى للحمل حيث تمت مراقبة هذه الحيوانات مع ذكر بمادة

وحذج حذاء الحمل وحاسب البدء الأول من خلال فحص السيدة المهنية. تحققت مجموعات الأحجام في (G1) و(G2) و(G3) وأ(0.1) و(0.2) و(0.3) ملمع كجم من وزن الجسم على التوالي من منتصف أعداد الرصاص المرتفع (G1) و(G2) و(G3) و(G4).

يرجع الدم المعدن بالكامل (normal saline) في الغالب المعلمي المعدل (P<0.01) ذو نفس الكمية والمصفوف بالكامل المعدل (C1) و(C2) و(C3) و(C4) المصلوف المذهل في كل مجموعة من فحص السيدة المعيشية. أظهرت النتائج وجود خاصية معنوية (P<0.05) في معدات متعلقة بالاستخدام للسيدة النظرية لأشعة الأشعة الذكورية (C1) و(C2) و(C3) و(C4) في موقعين (P<0.05).