

Effect of lead acetate injection into pregnant mice on the testis function of their male offspring at puberty

تأثير حقن اناث الفئران الحوامل بمادة خلات الرصاص على وظيفة الخصى لموالدها الذكور عند البلوغ

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Abstract

The study aimed to follow up the effects of injection lead acetate into pregnant females on the testicular development and semen quality of their male births at puberty time. Forty mature female Swiss Webster mice (8-10) weeks old were divided into two equal groups (experimental and control). After mating with fertile males, the pregnant females injected intraperitoneally with 0.1 mg/kg of body weight of lead acetate from the first day of gestation and continued throughout the gestation period 20 days. While the control groups were injected by the same dose and rout of injection with normal saline. At parturition, numbers of the neonate were recorded. After six weeks the male births only isolated and weighted then mated with normal fertile females to evaluate their fertility through assessing their birth rate, then sacrificed to get their testes; each testis was weighted and the left one was fixed, then histological sections with a thickness of 5 microns were prepared. The epididymis was minced with warm normal saline to get a spermatozoal suspension to evaluate the sperm concentration, sperm motility, and abnormal sperm morphology and sperm viability. Results showed a highly significant decrease in the body and testicular weights of experimental male animals. A highly significant reduction in their birth rate after mating with normal partners was recorded together with a significant inverse changes in all semen parameters.

المستخلص

تهدف هذه الدراسة الى متابعة تأثيرات التعرض لمادة خلات الرصاص السامة من قبل الاناث الحوامل على التطورات النمائية الجنينية للخصى ونوعية السائل المنوي لموالدها الذكور عند البلوغ . اجريت هذه الدراسة على 40 انثى فأر بالغة بعمر ثمانية الى عشرة اسابيع حيث قسمت الى مجموعتين (اختبار وسيطرة) . بعد مزوجة هذه الحيوانات مع ذكور بالغة حقنت الاناث الحوامل بـ 0.1 ملغم/كغم من وزن الجسم من محلول خلات الرصاص في الخلب حيث تم الحقن منذ اليوم الاول واستمر خلال فترة الحمل 20 يوم فيما حقنت مجموعة السيطرة بنفس الكمية ولنفس الفترة بالمحلول الملحي الفزيولوجي فقط . تم تسجيل اعداد المواليد عند الولادة . بعد ستة اسابيع تم عزل ووزن المواليد الذكور فقط . ثم شُرحت لاستئصال الخصى . وزنت كل خصية ووضعت اليسرى فقط في محلول مثبت لاغراض الدراسة النسيجية . ليتم تحضير مقاطع بسمك (5) مايكرون اما البربخ فتم تقطيعه ووضعها بمحلول ملحي دافئ للحصول على النطف لتقييم تركيز وحركة النطف كذلك اشكالها غير الطبيعية وحيويتها . اظهرت النتائج وجود انخفاض معنوي جدا في اوزان اجسام الامهات والمواليد . وكذلك اوزان الخصى لمجاميع الاختبار وانخفاضا معنويا جدا في معدلات موالدها بعد مزوجتها بحيوانات طبيعية كما سجلت تغيرات سلبية معنوية في كل معايير السائل المنوي للحيوانات المعاملة بخلات الرصاص .

Introduction

Lead has become widely distributed and mobilized in the environment. Human exposure and uptake of this element has consequently increased [1]. Lead stays in the body for much longer than most metals, with a half-life of 11 years in the bone [2]. It is used widely in industry for its waterproofing and electrical and radiation-shielding properties [3]. At higher level of human exposure, there is damage to almost all organs and systems, most importantly the central nervous system (CNS), kidneys, blood, skeletal, gastro-intestinal and respiratory systems [4]. Lead poisoning impairs both male and female reproductive function in adult and developmental stages lead to death in the embryo, fetus or child [5]. Reported effects on men include; reduction in libido, sperm quality (motility, numbers, and morphology) chromosomal damage, infertility, abnormal prostatic function and changes in serum testosterone level [6]. Increased lead concentration may contribute to production of unexplained male infertility [7]. In order to follow up the effect of lead on the function of testes and semen quality of young male offspring born from mothers exposed to lead acetate during gestation, this study was conducted.

Materials and methods

Forty mature female Swiss Webster mice (8-10)weeks old were divided into two equal groups: experimental (G) and control (C). Vaginal smear were performed to all adult female mice to detect heat stage for mating. Females in the metestrus phase were left with mature healthy males (1male/ 1female), the occurrence of vaginal plug considered as the first day of pregnancy [8]. The pregnant female was removed into separate cage. Intraperitoneally injection of lead acetate (0.1 mg/ kg body weight/day) [9], was started at the first day of gestation and continued through gestation period 20 day for the experimental group, while the parallel control group was injected normal saline by the same rout and dose as that used in the experimental group. At parturition, numbers of the offspring for all experimental and control mothers were recorded. After six weeks (around the puberty age) the newborn animals (males only) were weighted, mated with fertile females (to detect their ability for fertilization and then recording their birth rate), and then sacrificed to get their testes. Each testis was weighted, then the left one was fixed with 10% normal saline, and histological sections with a thickness of 5 microns were prepared using the routine histological technique [10]. Thickness and diameter of seminiferous tubules in each testis were recorded. The epididymis was put in a small Petri dish containing worm normal saline, organ were minced into tiny pieces with micro surgical scissors until getting a homogenized solution, which contain the spermatozoal suspension then incubated at 37C°befor subjected to sperm function test, including microscopic examination to record the concentration of spermatozoa (sperm / ml), sperm motility (Grade A, B, C, D), abnormal sperm morphology and sperm viability by using eosin stain. Statistical analysis were performed using version 7.5 SPSS (Statistical analysis Package for Social Scientists) to determine the mean and standard error of mean. P- Value < 0.05 was considered significant in this study [12].

Results

Effect of lead on fertility of young males

The young males borne from mothers treated with lead acetate during gestation period showed a reduction in their fertility when mated with fertile normal females, revealed by a highly significant decrease ($P<0.01$) in their litter size as compared to the parallel control group Table (1).

Effect of lead on body and testes weights of young male mice

The result showed that there was a highly significant decrease ($P<0.01$) in the mean of the body weights and a significant decrease ($P<0.05$) in testes weight of experimental groups as compared to the control group Table (1).

Effect of lead on semen quality (sperm function test) of young males:

Results showed significant decrease ($P<0.05$) in the percentage of epididymal viable sperms of experimental group with a highly significant reduction in sperm concentration ($P<0.01$) associated with a highly significant decrease in percentage of grade activity (A, B, C) and a highly significant increase in percentage of grade activity (D). Also a significant increase ($P<0.05$) in the number of sperms with abnormal morphology was recorded in the experimental animals in comparison with that of control group Table (2).

Histological observations:

The histological observations of the testes of male offspring showed a significant decline ($P<0.05$) in the thickness and diameter of seminiferous tubules with a degenerative features of germ cells, disrupting of basal lamina, sloughing cells and absence of sperms from some of seminiferous tubules was also recorded Figure (1,2).

Table (1): Effect of exposure to lead during the intrauterine life on the fertility and weights of body and testes of young males offspring.

	Experimental group(G)	Control group(C)
Birth rate	5.6** ± 0.063	7.7 ± 0.04
Body weight of offspring at puberty (g)	16.3** ± 0.038	19.3 ± 0.028
Weight of testis (g)	0.13* ± 0.0007	0.14 ± 0.0008
Thickness of seminiferous tubules (µm)	1.25* ± 0.0003	1.77 ± 0.0005
Diameter of seminiferous tubules (µm)	12.5* ± 0.03	13.6 ± 0.002

Values are mean ± standard error (SEM).

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

* : significant decrease ($P<0.05$).

Table (2): Effect of exposure to lead during the intrauterine life on the semen quality of young males offspring.

Seminal fluid analysis	Experimental group(G)	Control group(C)
Sperm concentration (million /ml)	45** ± 0.07	51.5 ± 0.08
Sperm motility	10.6**	21
Grade A	± 0.1	± 0.07
Grade B	37.5** ± 0.03	48 ± 0.1
Grade C	13** ± 0.07	19.2 ± 0.2
Grade D	39** ± 0.07	11.5 ± 0.1
Abnormal Morphology	78.5* ± 0.21	72.5 ± 0.17
Viability	18.5* ± 0.06	20 ± 0.05

Values are mean ± standard error (SEM).

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

*: significant decrease ($P < 0.05$).

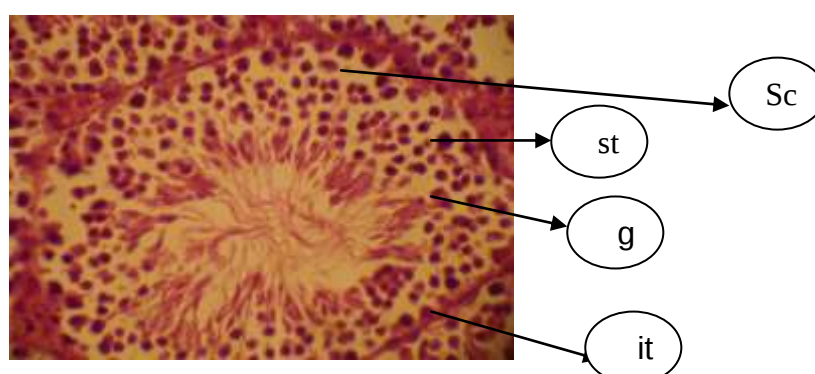


Fig (1): Testis section of male mouse offspring (control group). Note the well organized seminiferous tubules (st) filled with germ cells (g) and Sertoli cells (Sc), with extensive interstitial tissue (it) (H&E, 40X).

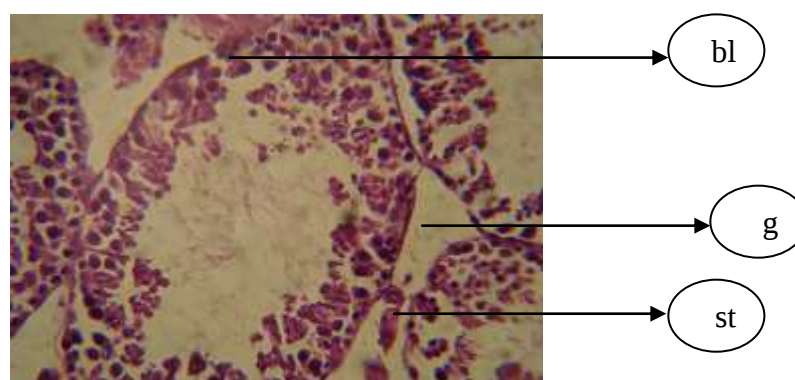


Fig (2): Testis section of male mouse offspring belong to mother treated with (0.1mg /kg b.w.) of lead acetate. Note a significant decline in the thickness and diameter of seminiferous tubules (st) with degenerative features germ cells (g), disrupting of basal lamina (bl), sloughing cells and absence of sperms from some of seminiferous tubules (st) (H&E, 40X).

Discussion

Several studies confirm that lead is able to pass through the placenta of pregnant female mice, presumably by passive diffusion and accumulate in embryo tissues interfering with embryo implantation [13]. This passage of lead with what is known about its persistence in body for longer time than other metals [14], may explain all the adverse results recorded in this study including the reduction their ability to fertilize normal females which expressed by the significant decrease in their birth rate compared to that of control group. This reduction in fertility goes with other findings including impaired semen parameters and reduction in body and testes weight. The decrease in body weights of experimental males may be related to the decrease in feeding habit, which may lead to growth retardation indicating that lead has an inhibitory action on metabolism [15], which agreed with finding recorded that human with elevated level of lead have depressed secretion of several hormones including metabolic hormones [16]. On the other hand the significant reduction in sperm concentration recorded, may be due to reduction in sperm production in testes since lead could disturb mitosis *in vivo* and alter Sertoli cells proliferation at perinatal period which could produce a significant decrease of sperm count in the testes of adult offspring [17]. The sperm motility and grade of activity are one of the most important criteria of semen quality and a determinant in the success of fertilization [11], the reduction of motility may be due to the direct effect of lead on the sperm cells [17], or it may affect the epididymis since Prased and Raja [18] showed that sperm motility is dependent on the phospholipids that found in caput epididymis as a source of energy. In the present study there was a significant increase in abnormal sperm morphology within epididymis, similar results were also reported by [19] in mice semen collected after an acute exposure to lead acetate. [20] reported that the lead was accumulate in all the male reproductive organs with especially high concentration of lead in the epididymis where it have caused alteration in the functions of epididymis which lead to the abnormal morphology of sperm. Results of present study showed a significant decrease in the percentages of viability in experimental group as compared with control group. [21] showed that the presence of lead in the seminal plasma exerts toxic effects on sperm parameters such as morphology, motility and viability. Therefore, adverse changes in epididymal and testicular sperm structure lead to non-viable sperm production. Degenerative features in the testes of male offspring of experimental group revealed by a significant decline in the thickness of seminiferous tubules and reduction in the diameter of testes may be due to accumulation of lead in the gonads and testicular tissues which affects the physiology of reproduction [22], necrosis of testicular cords, degenerative primordial germ cells, disrupting of basal lamina, sloughing cells and absence of sperm from some seminiferous tubules. These results agree with those obtained by [23] in which the weight of testes was dropped significantly in 22 day old male rats exposed to lead prenately. It was proved [24] that lead exposure during a period of pregnancy when structures related to the Hypothalamus Pituitary Gonadal (HPG) axis are undergoing rapid proliferation, the

exposed animals at significant risk for reduced reproductive capacity in adulthood, and elicit toxic pathological changes in the testes leading to atrophy of the organ.

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