Paternal exposure to low-dose lead acetate: effect on implantation rate, pregnancy outcome, and sex ratio in mice

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Background/aim: It has been reported from in vivo exposed experimental animals that paternal lead exposure reduces birth rate; however, there is limited evidence to suggest a decrease in the proportion of male births. This study investigated the role of paternal exposure to lower lead acetate doses on early embryonic development (implantation) and the sex ratio of their offspring.

Materials and methods: In total 180 Swiss Webster mice were used (60 male and 120 female). The males were divided into 3 groups: G1 (untreated group), G2 (treated daily with 50 µg/kg BW lead acetate), and G3 (treated daily with 100 µg/kg BW lead acetate). The implantation success rate, pregnancy outcome, and sex ratio were measured.

Results: The results showed a highly significant reduction in both the percentage of implantation rate and the number of offspring in the G3 mice, but there was no significant difference for the G2 mice. There was a slight insignificant reduction in the number of newborn males compared with females for both G2 and G3 mice.

Conclusion: The findings of this study suggest lead exposure in experimental animals reduces implantation rate with paternal BLL of 28 µg/dL and the sex ratio for offspring showed a slight insignificant reduction with both paternal BLLs of 23.5 µg/dL and 28 µg/dL.

Key words: Lead acetate, mice, implantation rate, sex ratio

1. Introduction

An increase in the human population, rapid industrialization, and motorized vehicular traffic are thought to be responsible for the increased release of toxic metals into the environment. Among these metals is lead, increased exposure to which can cause adverse effects on both male and female reproductive systems (1).

Lead is a highly toxic metal for humans and other mammals. It is abundant in the environment and accumulates in the human body over time, including the prenatal period (2). Toxicity is manifested in the male reproductive system with lead deposition in testes, epididymis, vas deferens, seminal vesicle, and seminal ejaculate (3).

The adverse effects of lead on reproductive functions are not controversial; however, it has been difficult to establish a clear threshold. This is due to difficulty in selecting an exposure indicator and a reproductive endpoint, which continues to be an area for investigation. Nevertheless, many studies suggest there is no adverse effect at lead concentrations of 35–50 µg/dL in blood (4–8). There are conflicting results concerning the effect of low lead exposures on semen quality. Hernandez-Ochoa et al. (9) found that low lead concentrations in seminal fluid (0.2 µg/dL) were associated with impaired semen quality, motility, morphology, and sperm concentration. In contrast, Mendiola et al. (10) found a relationship between levels of lead in seminal fluid and low motility, but found that measured lead concentrations of 9.75 µg/dL in blood and 2.78 µg/dL in blood plasma had no significant effect on morphology, motility, or sperm concentration. Meeker et al. (11) measured a lead concentration of 1.5 µg/dL in blood and reported no effect on sperm concentration or motility.

Environmental lead exposure has dropped in recent decades due to the dominant use of unleaded petrol and the ban on lead-based paint and lead solder in food cans. Nevertheless, specific population groups are disproportionately at high risk of lead exposure; one example is Iraq, where the past two decades have seen an
increased dependency on electrical generators that require leaded gasoline.

Our study aimed to determine the effect of relatively long-term paternal exposure to low doses of lead acetate on early embryonic development (implantation) and the outcomes of normal fertilization of intact oocytes with polluted sperms, and finally to find the relationship between paternal exposure to lead acetate and the sex ratio of their offspring.

2. Materials and methods

This study was carried out at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq, from May 2010 to November 2011.

One hundred and eighty mature and healthy Swiss Webster mice were used, composed of 60 males aged 6–8 weeks and 120 females aged 8–10 weeks with average body weights of 20–24 g (average = 22 g), obtained from an inbred colony at the animal house of the Institute. They were housed in plastic cages (North-Kent Plastic Ltd, UK) and kept in a room with a 12:12 h light:dark cycle and controlled temperature. Food (as standard diet pellets) and water were available ad libitum.

The experiments were approved by the Animal Research Committee at the Institute and the treatment of rats in this study adhered to the guidelines of the United States Environmental Protection Agency (12).

The males mice studied were divided into three groups: G1, G2, and G3, with 20 mice/group:

1. G1 (untreated group) was the control group and received distilled water only.
2. G2 (low-dose group) was treated with 50 µg/(kg body weight) daily dose of lead acetate (23.5 µg/dL) dissolved in drinking water for 16 weeks.
3. G3 (high-dose group) was treated with 100 µg/(kg body weight) daily dose of lead acetate (28 µg/dL) dissolved in drinking water for 16 weeks.

The female mice were used for mating and in vivo fertilization of their oocytes with the polluted sperms of the treated males. They were divided into three equal groups (with 40 mice/group); the first group was used for mating with the G1 control males, the second for mating with the G2 treated males, and the third for mating with the G3 treated males.

Two weeks prior to the beginning of the experiment, for each group, only 4 mice were housed per cage, which contained a glass bottle filled with 250 mL of distilled water. The amount of consumed water was recorded daily. By the end of the 2 weeks, a mean of 22.5 mL/cage (mean of 5.6 mL for each mouse) was consumed. This was used to calculate the weight of lead acetate required to dissolve in the water to obtain the exact dosage of lead.

Standard lead solutions containing 1 mg of lead acetate (Merck, Germany) were prepared. Two stock solutions of lead acetate were prepared according to the determined doses (each mouse received 1.1 µg for the low dose group and 2.2 µg for the higher dose group). Sixteen weeks later, the mice were anesthetized using diethyl ether (Fluka, Germany) and quickly the heart was punctured by a fine disposable needle and the blood drawn into tubes containing EDTA. After the tubes were shaken for a few minutes, they were left in the refrigerator at 4 °C to be used later for measurement of lead concentration in each mouse's blood.

The blood samples were thoroughly mixed for at least 1 h prior to the determination using a mixer (Kahen-Shacker, Italy). They were diluted with an equal volume of 10% trichloroacetic acid for lysis of RBCs and to release their lead contents. The diluted samples were centrifuged at 10,000 rpm for 5 min. The supernatant fluid was then transferred to another tube and centrifuged again at 7000 rpm for another 5 min. The new supernatant fluid was tested for lead using an atomic absorption spectrophotometer (Shimadzu, Japan) at a wavelength of 217 nm.

The animals were weighed monthly and their average body weight at the beginning of the experiment was 22 g.

2.1. Effect of paternal exposure to lead acetate on embryonic implantation and pregnancy outcome

A stained vaginal smear with 1% aqueous methylene blue for 3–5 min was examined under a light microscope to check the estrus cycles of the females (13). At the end of treatment with lead acetate, two females in the metestrus phase were housed with one male (the control and lead-treated). The morning following housing, the presence of a vaginal plug indicated successful mating, and vaginal smears were taken the day after supposed mating to detect the presence of sperm in the vagina. This was repeated daily until the appearance of sperm in the vagina, and that day was considered the first day of pregnancy.

Seventy-four pregnant mice were moved into a separate cage (36 belong to G1, 26 to G2, and 22 to G3; half of which (from all three groups) were sacrificed at day 5 of pregnancy to detect the number of implanted embryos. The rest were left to complete parturition.

2.2. Determining the effect of lead on implantation rate

The implantation sites in female mice were detected late at night on day 4 and onward. This was achieved by intravenous injection of macromolecular blue dye via the tail vein (13). The animals were sacrificed 3–5 min after the dye injection to identify blue bands in the uterus. The blue bands represent the implantation sites. The whole uterus was then transferred into a small petri dish, washed with normal saline, and checked under the dissecting microscope to record the number of dark bands, which represents the number of implanted embryos.
2.3. Detecting the effect of lead on pregnancy outcome
The remaining half of the pregnant mice were left until the time of parturition. The number and the body weight of newborn mice were recorded for each group and sex ratio was calculated.

2.4. Statistical analysis
All statistical analysis was done using the Statistical Package for Social Sciences (SPSS) version 19. Categorical data were presented as count and percentage. The chi-square test of significance was used. Quantitative data were presented as mean and standard error of mean and the paired sample t-test was used for comparison between the two groups. The probability level was less than or equal to 0.05 (P ≤ 0.05).

3. Results
3.1. Blood lead level (BLL)
The BLL in G2 was 23.5 ± 0.7 µg/dL and in G3 was 28 ± 0.071 µg/dL. These values were significantly higher than 19.9 ± 0.38 µg/dL in the control group (P < 0.005; P < 0.001, respectively).

3.2. Effect of lead on body weight
Figure 1 showed the mice in the control group G1 exhibited a gradual increase in their body weight over the duration of the experiment as compared to the start of the experiment (P < 0.05). The mice in G2 demonstrated a slight decrease in their body weight during the first month of treatment and a gradual increase in their weight was noticed during the following 3 months. The body weight of the mice in G3 similarly showed a significant increase. At the end of the experiments, G2 and G3 mice showed a slight insignificant decrease in mean body weight in comparison to the control group.

3.3. Effect of lead acetate on implantation rate
Figure 2 shows the two horns of pregnant uteri at the early embryonic implantation period (day 5 of gestation) stained with Chicago blue. In the control group there is a dark blue band indicating multiple implantation sites. In G1 the number of dark blue band approximates that of the control group and in G2 there was a reduction in uterine size and the number of implantation sites.

The females that mated with the G2 males had a slight insignificant decrease (P = 0.078) in the percentage of implantation rate, compared with that of the control group (6.8 ± 0.29% and 7.6 ± 0.31%, respectively). In contrast, the females fertilized by males of G3 showed a highly significant reduction (P < 0.01) in the percentage of implantation rate (3.0 ± 0.56%), as shown in Figure 3.

3.4. Effect of lead acetate on pregnancy outcome
3.4.1. Body weight of offspring
All offspring belonging to G2 and G3 showed decreased body weight but to a nonsignificant level as compared to that of the control group. The body weights of the male offspring from females fertilized by the control group, G2, and G3 males were then compared. The reduction in body weight of male offspring from polluted sperm was found to be insignificant compared with the control sperm. Similarly, female offspring born to males of G3 showed an insignificant decrease in their body weight, while those born from males corresponding to G2 demonstrated a significant increase (P < 0.05) in body weight compared to those of the control group (Figure 4).

3.4.2. Number and sex ratio of offspring
Next, the effect of paternal exposure to lead acetate on the number of offspring was measured. The number of male
and female offspring born to the G1 mice was 74 and 64, respectively. Although the number of offspring born to the G2 mice was decreased to 62 females and 28 males, it was not significant ($P = 0.244$). Meanwhile, the number of offspring to the G3 mice was 22 females and 12 males, which was a significant reduction ($P = 0.022$).

Finally, the ratio of male to female offspring born to the control group, G2 and G3 was calculated and compared. The study revealed a slight insignificant reduction in the ratio of newborn males to females; in the control group, the sex ratio was 0.865, whereas G2 and G3 offspring had a sex ratio of 0.45 and 0.55, respectively (Figure 5; Table).

### 4. Discussion

Although the adverse effects of lead on human pregnancy outcomes have been reported to be associated with high-level occupational exposures (14), the present study revealed no significant differences between G2 mice and the control group, a finding previously reported by Speyer et al. (15).

The implantation rate in the present study was significantly reduced in G3 mice, but not in G2 mice. This contradicts the results produced by Lindbolim et al. (16), who found no significant relationship between paternal lead exposure to BLL greater than 30 µg/dL and spontaneous abortion. Moreover, a striking dose-response relation between BLL and risk of spontaneous abortion was found without confirmation to be associated with maternal or paternal exposures (17); however, similar studies reported no such association (18).

An expected consequence of a reduced implantation rate is a reduction in the total number of the offspring (pregnancy outcomes) in G3 mice. These results concur with the reduction in live birth and paternal exposure to lead (19,20); however, they contradict the findings from Bonde and Kolstad (21), who noted no association between birth rate and paternal exposure to BLL of 35.9 µg/dL.

Previously published data demonstrated a higher percentage of DNA fragmentation in high-dose treated mice, which reflected a significant reduction in the number of newborn males and females.
of implanted embryos in females mated with males with BLL of 28 µg/dL (22). This was also reported by Brahem et al. (23), who found increased recurrent pregnancy loss to be significantly associated with the number of sperm with fragmented DNA. These genetic changes within the spermatozoa of treated male mice could be the cause behind the reduced implantation ratio.

For a long time the ratio of male to female offspring at birth has been a simple method for monitoring the reproductive health of a population (24). Despite this, the extensive literature on the adverse effects of lead on reproductive health and birth outcomes scarcely contains studies investigating the effect of exposure on the sex ratio (25). In humans, there is increasing evidence that the birth sex ratio is altered in areas close to industry where there is increased exposure to environmental and industrial chemicals (26).

In the present study, a clear reduction in the offspring sex ratio of both treated groups was demonstrated. Surprisingly, the control group also showed a slight decrease from the normal range of 1.05–1.07. The results showed that even a low dose of paternal BLL could affect the ratio of males to females in the offspring. A sex ratio of 0.69 was noted by Ansari-Lari et al. (27) in exposed offspring, compared to 1.07 of unexposed offspring from men in the general population. Furthermore, Simonsen et al. (28) demonstrated a sex ratio of 0.86 to BLL equal to 60 µg/dL, reaching a value of 1.24 to BLL of only 0–20 mg/dL. Contrary to our results, some studies on humans show no effect on the sex ratio (29,30). The alteration in the sex ratio due to fathers exposed to toxicant agents could be explained by endocrine disruption, and epigenetic and gene–environment interaction (25,27,31).

The results of the present study show that rats in vivo exposed to lead with blood levels of 23.5 µg/dL or 28 µg/dL had no significant adverse effects on the body weight of offspring. However, a significant reduction in implantation rate was recorded when paternal BLL was 28 µg/dL, after mating with healthy females, and the sex ratio for offspring showed a significant reduction from the normal value with both paternal BLLs of 23.5 µg/dL and 28 µg/dL.

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References


