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Selenium protects reproductive system and foetus development in a rat model of gestational lead exposure

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Abstract. – OBJECTIVE: Lead is a common environmental contaminant. Lead accumulation in the body is especially dangerous for pregnant women and newborns. Selenium is a trace element which may rectify the damaging effects of lead. Here we tested potential protective effects of selenium against gestational lead exposure.

MATERIALS AND METHODS: Pregnant SD rats were exposed to 200 mg/L of lead acetate (given with water), with or without sodium selenite supplementation (2-8 mg/kg/day via intragastric administration). Pregnant rats not exposed to lead or selenium served as control animals. The outcomes in pregnant rats were serum lead and selenium levels, reproductive hormone (follicle-stimulating hormone, luteinizing hormone, prolactin, oestradiol, progesterone) levels, and uterine and ovarian morphological changes. The outcomes in the offspring were sex differentiation, survival rates (day 21 after birth), weight (days 0-35 after birth), weight of reproductive organs, and puberty onset (foreskin separation or vaginal opening).

RESULTS: Selenium supplementation dose-dependently decreased serum lead levels, rectified reproductive hormone levels, and attenuated reproductive morphological changes caused by lead exposure. Lead exposure did not affect sex differentiation, but significantly ($p < 0.05$ vs. control animals) decreased the offspring weight on days 0-28 and the weight of their reproductive organs. Furthermore, lead exposure delayed the onset of puberty. These pathological changes were dose-dependently rectified or attenuated by selenium supplementation.

CONCLUSIONS: Gestational lead exposure causes damages to the reproductive system of pregnant rats, and negatively modulates growth and reproductive system development of the offspring. These adverse effects are rectified or attenuated by selenium supplementation.

Key words:
SD rats, Lead, Selenium, Reproductive hormones, Reproductive system, Puberty onset.

Introduction

Lead is a common environmental contaminant toxic to humans. Lead accumulation in the body causes systemic damage, especially in pregnant women and their newborns[1-3]. Excessive serum lead levels are associated with increased risk of premature membrane rupture, prematurity, gestational hypertension, spontaneous abortion, and disturbances of foetal growth and development[3-5]. Therefore, it is important to prevent the damaging effects of lead on reproductive system of pregnant women and on foetal health.

Selenium is a trace element essential for human and animal health. Selenium is present ubiquitously in the body and is involved in metabolic processes[6-8]. During pregnancy, selenium levels gradually decrease, reaching the minimum just before the delivery[9]. It is plausible to hypothesize that adverse effects of lead during pregnancy may be related to selenium deficiency. Conversely, selenium supplementation may reverse the damaging effects of lead. In this study, we exposed pregnant SD rats to lead with or without selenium supplementation. The outcomes were changes of reproductive system of pregnant female rats, and body growth and reproductive system development of their offspring. We demonstrate below beneficial effects of selenium supplementation on these parameters.

Materials and Methods

Reagents and assays

Sodium selenite (Na_2SeO_3) was purchased from Beijing United Chemical Co. (Beijing, China). Lead acetate ($[Pb(C_2H_3O_2)]_2$) was obtained from Guangzhou Chemical Reagent Factory

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(Guangzhou, China). As downstream assays, we utilized five magnetic microparticle radioimmunoassay assays (iodine [^{125}I]-human follicle stimulating hormone, ^{125}I-human luteinizing hormone, prolactin, ^{125}I-oestradiol, and ^{125}I-human serum progesterone), purchased from Beijing Beimian-Dongya Institute of Biological Technology (Beijing, China).

Instruments

H-7500 transmission electron microscope was obtained from Hitachi (Tokyo, Japan), and WFX-120 atom spectrophotometer and AF-610A atomic fluorescence spectrometer were obtained from Beijing Ruili Analytical Instrument Company (Beijing, China). HMIAS-2000 high-resolution color medical image analysis system was obtained from Wuhan Qianping Imaging Technology Company (Wuhan, China), whereas SN-684 type γ RIA counter was from the Shanghai Institute of Nuclear Research (Shanghai, China).

Animals

Animal experiments conducted in this study were approved by the Animal Research Committee of Hebei Medical University and were conducted in accordance with the guidelines of the Animal Experimental Center of Hebei Medical University.

One hundred and eighty adult SD rats (60 males and 120 females, weight 240-270 g) were used in this study. The animals were purchased from the Experimental Animal Center of Hebei Medical University (Certificate of Conformity: DK0503-0031). The animals were incubated at ambient temperature of 20 ± 2º C, humidity of 50 ± 20%, and a circadian cycle of 12 dark/light hours. Male and female SD rats were placed in the same cage everyday at 20:00. At 8:00 each morning, vaginal plugs of female rats were assessed, and vaginal smears were procured. Smears positive for sperm were used as an indication of pregnancy.

Exposure of pregnant rats to lead and selenium

One hundred pregnant SD rats were randomly divided into five groups (20 rats/group). The groups were control group, lead-exposed group, lead + low dose of selenium group, lead + intermediate dose of selenium group, and lead + high dose of selenium group. Beginning from the first day after conception, the rats in the lead-exposed group were provided with normal diet and double-distilled water supplemented 200 mg/L of lead acetate[10]. The rats in three selenium groups were similarly exposed to lead acetate and received a daily intragastric administration of, respectively, 2, 4, or 8 mg/kg Na_2SeO_3[11]. The rats in control group consumed normal diet and double-distilled H_2O. Twenty days after conception, 10 rats from each group were randomly selected for pathology studies. The remaining rats were provided with normal diet and double-distilled water, with or without selenium gavage, after delivery of their offspring.

Measurements of serum lead and selenium levels, and reproductive hormones, and observations of genital tissue under electron microscope

Twenty days after conception, blood was obtained from hearts of pregnant rats after intraperitoneal anesthesia with 2% pentobarbital sodium (30 mg/kg). Serum lead levels were measured with the WFX-120 graphite furnace spectrophotometer, and serum selenium levels were quantified with the AF-610A atomic fluorescence spectrometer. The follicle stimulating hormone, luteinizing hormone, prolactin, estradiol, and progesterone were measured with respective radioimmunoassays.

In addition, uterine and ovarian tissues were extracted from the rats and fixed in 2.5% glutaraldehyde. Then, the tissues were rinsed with phosphate buffer (pH 7.2) and fixed with 1% osmium tetroxide for 1.5 hours. After ethanol dehydration, embedding, and ultrathin sectioning, ultrastructures of uterine and ovarian ultrastructures were observed under the Hitachi H-7500 transmission electron microscope.

Growth and physiological development of the offspring

The number of the offspring was recorded at birth. The offspring gender was distinguished via anogenital distance measurements using a Vernier caliper. Anogenital distance is defined as the distance between the frontmost edge of the anus and the backmost edge of the genitals. Any genital malformation was noted. Weights of the offspring were measured every 7 days (i.e., on days 0, 7, 14, 21, 28, and 35) after birth. Survival rates at days 4 and 21 were recorded. On day 21 after birth, the litter was separated by gender. Twenty rats (10 males and 10 females) were randomly selected from each group for pathology studies. Specifically, their reproductive organs were removed and weighed.

Then, 20 other rats (10 males and 10 females) were randomly selected from each group. Male rats were monitored for foreskin separation starting from day 35 after birth. Observations were continued until all of male rats exhibited foreskin separation. Vaginal openings of female rats were checked every morning at 9:00 starting from 27 days after birth until all female rats exhibited vaginal openings.

Statistical Analysis

Data are presented as mean ± SD. The groups were compared using the One-Way ANOVA or chi-square tests. The SPSS 12.0 (SPSS Inc., Chicago, IL, USA) statistical package was used for statistical analysis. Differences at $p < 0.05$ were considered statistically significant.

Results

General condition, and serum levels of lead and selenium

During the experiment, we did not observe abnormal behaviours or abnormal physical appearance of pregnant female rats exposed to lead. Specifically, we did not witness indifferent reactions, or extreme levels of excitement, changes in dynamicity, or presence of dry, yellow, or shedding fur. In addition, no delivery or nursing abnormalities (labour dystocia, stillbirth, or malformation) were observed.

Nevertheless, when we obtained serum specimens and determined the levels of lead, these were 100% higher in the lead-exposed animals compared with control animals (respectively, 122.162 ± 9.378 *vs.* 60.317 ± 4.721 µg/L, $p < 0.05$; Figure 1A). Supplementation with selenium dose-dependently decreased serum lead levels. Thus, low, intermediate, and high dose supplementation respectively reduced serum lead levels by 3.862%, 29.529%, and 51.853% (Figure 1A). The decrease in serum lead levels in the groups supplemented with intermediate and high doses of selenium was significant ($p < 0.05$ *vs.* lead group; Figure 1A). As expected, selenium supplementation dose-dependently increased selenium levels in serum (Figure 1B). Thereby, there was a reverse association between selenium and lead serum levels.

Reproductive hormone levels

Exposure to lead caused a significant ($p < 0.05$) drop in serum levels of follicle-stimulating hormone and estradiol (respectively, Figures 2A and 2D), and significant increases in serum levels of luteinizing hormone (Figure 2B), prolactin (Figure 2C), and progesterone (Figure 2E; all $p < 0.05$). Selenium supplementation normalized the levels of all tested reproductive hormones. Thus, follicle-stimulating hormone and oestradiol levels increased, whereas the levels of luteinizing hormone, prolactin, and progesterone decreased (Figures 2A-2E). These results demonstrated that protective effects of selenium supplementation occurred at the level of reproductive hormones.

Uterine and ovarian morphologies

We further observed uterine and ovarian morphologies to detect the damaging effects of lead exposure to the reproductive system. We documented the presence of moderate oedema and fusion disappearance in the mitochondrial arrangements in specimens of rats exposed to lead (Figure 3). Furthermore, rough endoplasmic reticulum expansion and degranulation were also evident (Figure 3). These changes

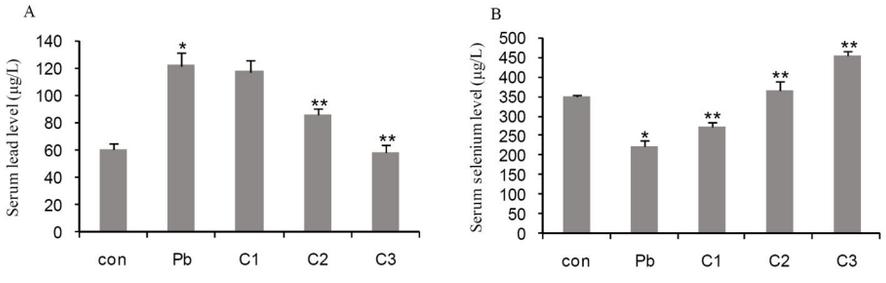

Figure 1. Serum lead and selenium levels in pregnant rats. **A**, Serum lead levels. Con: control group; Pb: lead-exposed group; C1: lead + low dose selenium group; C2: lead + intermediate dose selenium group; C3: lead + high dose selenium group. Values are mean ± SD of 10 rats. *$p < 0.05$ *vs.* Con. **$p < 0.05$ *vs*. Pb. **B,** Serum selenium levels. Abbreviations and significances as above.

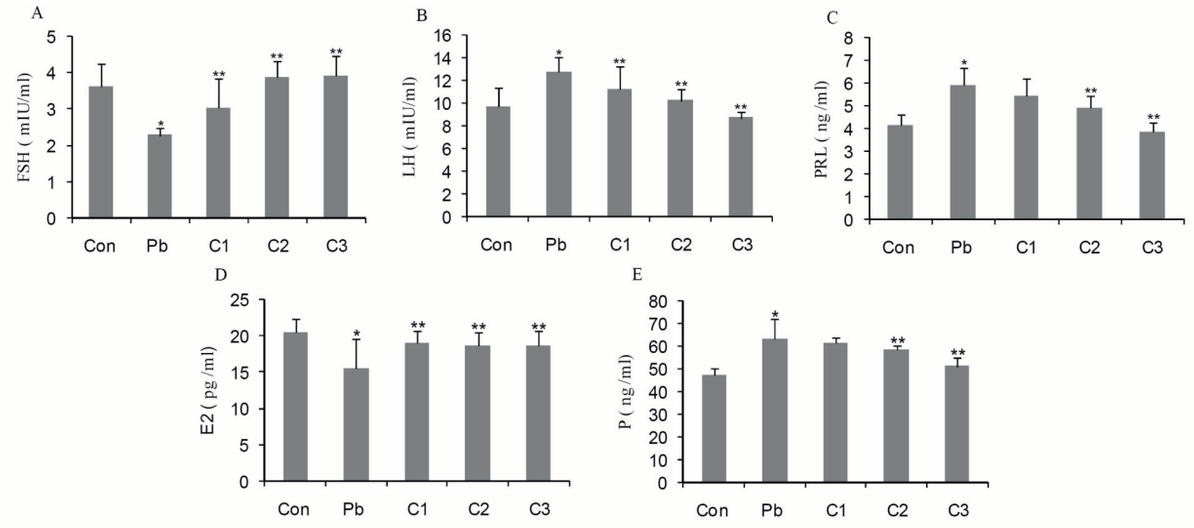

Figure 2. Reproductive hormone levels in pregnant rats. **A**, Human follicle stimulating hormone (FSH). Con: control group; Pb: lead-exposed group; C1: lead + low dose selenium group; C2: lead + intermediate dose selenium group; C3: lead + high dose selenium group. Values are mean ± SD of 10 rats. *$p < 0.05$ *vs.* Con. **$p < 0.05$ *vs.* Pb. **B**, Luteinizing hormone (LH). Animal groups, abbreviations and significances as above. **C**, Prolactin (PRL). Animal groups, abbreviations and significances as above. **D**, Oestradiol (E2). Animal groups, abbreviations and significances as above. **E**, Progesterone (P). Animal groups, abbreviations and significances as above.

were also present in rats exposed to lead and supplemented with low dose of selenium (Figure 3). However, starting from the intermediate dose of selenium, the observed morphological changes were attenuated (Figure 3). Thus, the extent of oedema became less pronounced, and there was only partial fusion disappearance in the mitochondrial arrangements, as well as mild rough endoplasmic reticulum expansion and degranulation (Figure 3). Rats supplemented with high dose of selenium demonstrate no morphological changes (Figure 3). All of the above observations were applicable to uterine and ovarian tissues (respectively, A and B panels of Figure 3).

Sex differentiation in the offspring

Sex differentiation is influenced by genetics, hormonal regulation, and other factors. The mean anogenital distance was calculated in the next ex-

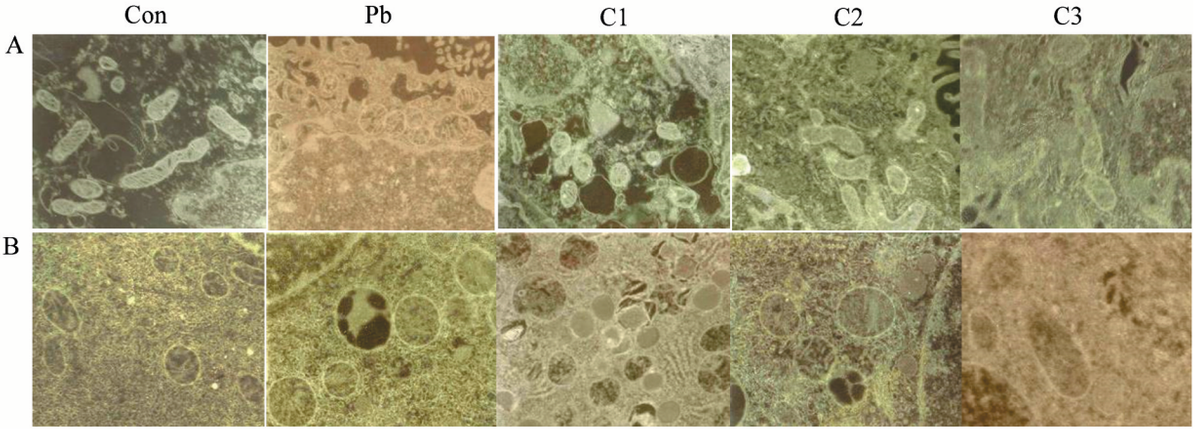

Figure 3. Uterine and ovarian cell morphologies. **A**, Electron microscopy of uterine tissue (magnification ×20,000). Con: control group; Pb: lead-exposed group; C1: lead + low dose selenium group; C2: lead + intermediate dose selenium group; C3: lead + high dose selenium group. **B**, Electron microscopy of ovarian tissue (magnification ×20,000). Animal groups as above.

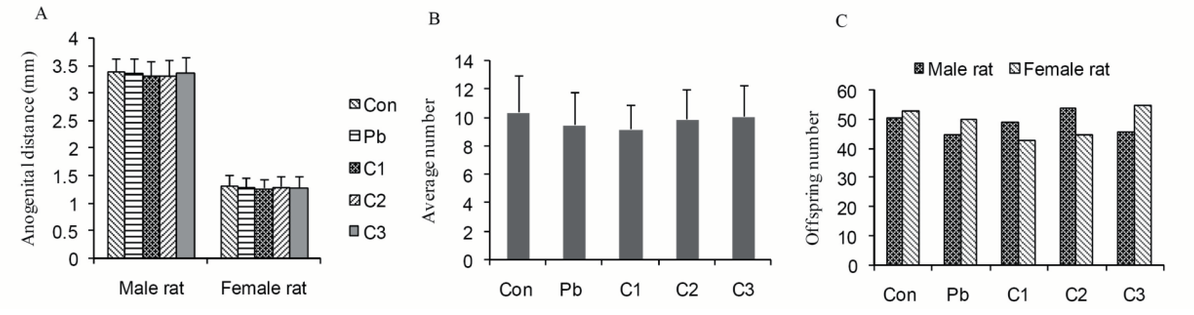

Figure 4. Sex differentiation of the offspring. **A**, Average female and male anogenital distance. Con: control group; Pb: lead-exposed group; C1: lead + low dose selenium group; C2: lead + intermediate dose selenium group; C3: lead + high dose selenium group. Values are mean ± SD of 10 rats. **B**, Average number of offspring. Animal groups as above. **C**, Number of male and female offspring. Animal groups as above.

periment as a measure of sex differentiation. We observed no statistically significant differences in the mean anogenital distances among animal groups (Figure 4 A). Furthermore, there were no differences in the pups' number among groups (Figure 4 B). Finally, we did not observe differences in the sex ratios (i.e., number of male *vs.* female offspring) (Figure 4 C). These observations excluded effects of lead exposure or selenium supplementation on the tested parameters of sex differentiation.

Growth and development of the offspring

Next, we evaluated offspring survival rates on days 4 and 21. Furthermore, since weight is one of the key indicators of the growth and development of the offspring, we measured the weights of the offspring at days 0, 7, 14, 21, 28, and 35.

While survival rates of the lead-exposed group tended to be lower on days 4 and 21, these differences did not reach statistical significance (Figure 5A). However, average weights of this group were

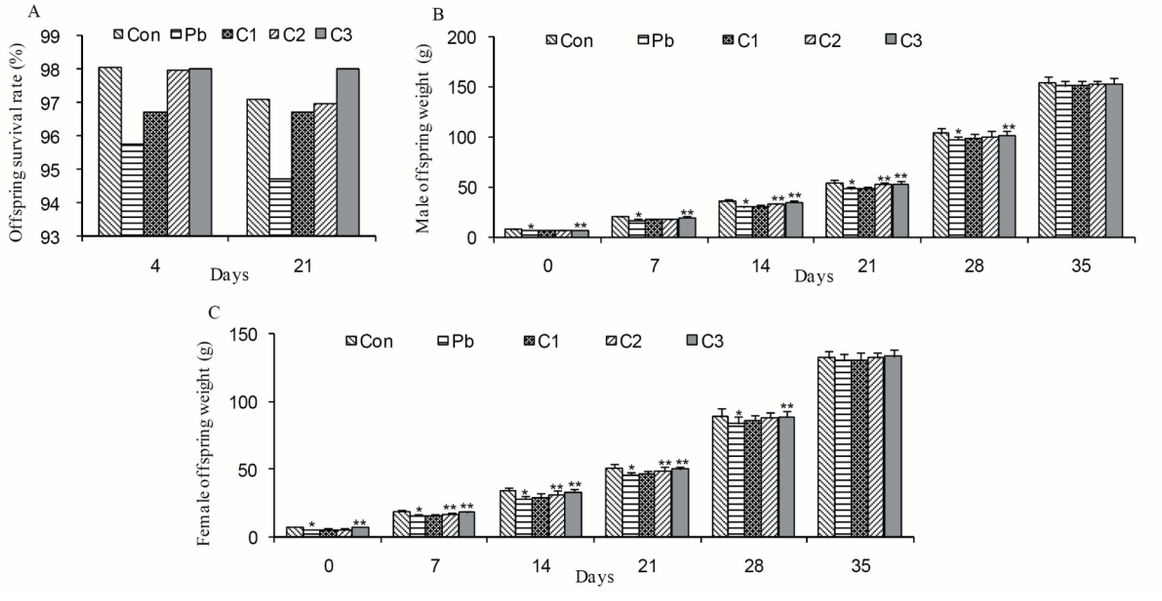

Figure 5. Survival rates and increase of weights of the offspring. **A**, Offspring survival rate recorded on days 4 and 21 after birth. Con: control group; Pb: lead-exposed group; C1: lead + low dose selenium group; C2: lead + intermediate dose selenium group; C3: lead + high dose selenium group. Values are mean ± SD of 10 rats. **B**, Mean weight of male offspring. Body weights were measured on days 0-35 after birth. Animal groups as above. Values are mean ± SD of 10 male rats. *$p < 0.05$ *vs.* Con. **$p < 0.05$ *vs.* Pb. **C**, Mean weight of female offspring. Animal groups and significances as above. Values are mean ± SD of 10 female rats.

significantly lower on days 0-28 after birth (Figures 5 B and 5 C). After 35 days, no significant differences in weights existed among the groups. Rat weights, attenuated on days 0-28, were significantly enhanced by supplementation with high dose selenium (Figures 5 B and 5 C).

These observations indicate that exposure to lead during pregnancy markedly decreases the growth of the offspring, which is attenuated by selenium supplementation.

Reproductive organ development of the offspring

Development of reproductive organs affects sexual maturation and reproductive functioning of the offspring. We next studied how lead exposure, with or without selenium supplementation, affects reproductive organ development of the offspring. Specifically, we measured the weights of the testis and epididymides, or ovaries and uterus.

Lead exposure significantly ($p < 0.05$ for all observations; Figure 6) decreased the reproductive organ weights. Selenium supplementation dose-dependently attenuated these pathological changes, and significant differences were achieved by either intermediate or high dose supplementation (Figure 6), further highlighting protective effects of selenium.

Puberty onset in the offspring

Puberty onset is indicated by foreskin separation or vaginal opening. To test for this outcome, we recorded the number of days required for foreskin separation or vaginal opening.

Both foreskin separation and vaginal opening were significantly delayed in lead-exposed animals (respectively, 3.664 and 3.968 days; both $p < 0.05$; Figure 7). Selenium supplementation, especially with intermediate and high dose, decreased the time required for foreskin separation (respectively, by 1.887 and 3.013 days, both $p < 0.05$; Figure 7). In a similar manner, high dose selenium significantly decreased the number of days required for vaginal opening (decrease by 2.994 days; $p < 0.05$; Figure 7). These results indicated that selenium supplementation attenuated deleterious effects of lead exposure on the onset of puberty in the offspring.

Discussion

Supplementation with selenium has been reported to benefit patients with the prostate, lung, colorectal, and bladder cancers[12-14]. Selenium supplementation can also promote resistance to viruses and maintain reproductive function[15,16]. Furthermore, it attenuates the damage to the kidneys, the liver, and other organs caused by lead exposure, and improves cognitive ability[17-21].

Lead damages blood cells and affects the nervous, urinary, and immune systems[22,23]. Due to accumulation in the body, long-term exposure to low doses of lead can affect reproductive function[24,25]. Here we exposed SD rats to lead during pregnancy and tested potential protective effects of supplementation with different doses of selenium. Our observations indicated that gestational lead exposure damaged reproductive systems of pregnant rats and caused uterine and ovarian cell morphology changes, leading to changes in reproductive hormones. These toxic effects of lead were reduced by selenium supplementation.

Lead causes the damage to both pregnant rats and, by crossing the placental barrier and passing through breast milk, the offspring. Thus, it affects hormone secretion and organ development in the offspring[26-29]. Exposure to lead can also cause abnormal morphological changes and dysfunction of the reproductive system, including the oestrogen-like activity during sex differentiation in the offspring. Under normal circumstances, the anogenital distance of offspring is strictly regulated by endogenous sex hormones. Androgens ex-

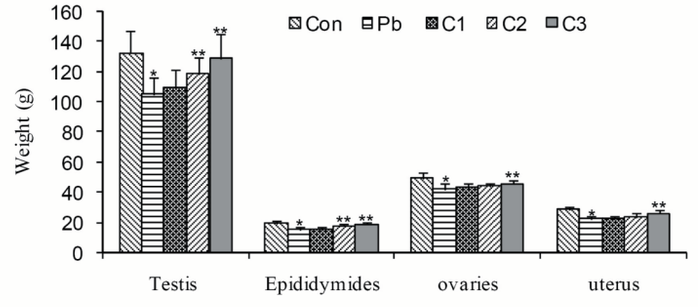

Figure 6. Weights of reproductive organs of the offspring. Ten male and ten female rats were randomly selected on day 21 after birth. In these animals, the weights of testis and epididymides, or ovaries and uterus, were measured. Con: control group; Pb: lead-exposed group; C1: lead + low dose selenium group; C2: lead + intermediate dose selenium group; C3: lead + high dose selenium group. Values are mean ± SD of either 10 male or 10 female rats. *$p < 0.05$ *vs.* Con. **$p < 0.05$ *vs.* Pb.

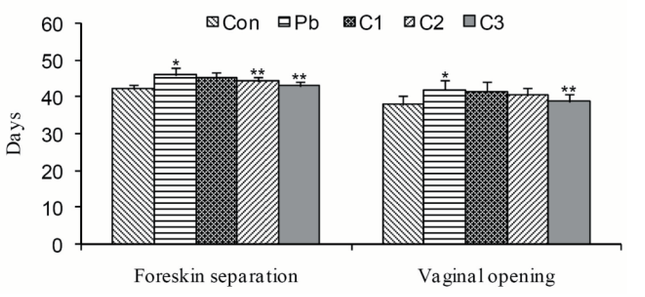

Figure 7. Time required for foreskin separation and vaginal opening in the offspring. Ten male and ten female rats were randomly selected, and the number of days required for foreskin separation or vaginal opening were recorded. Con: control group; Pb: lead-exposed group; C1: lead + low dose selenium group; C2: lead + intermediate dose selenium group; C3: lead + high dose selenium group. Values are mean ± SD of either 10 male or 10 female rats. *$p < 0.05$ *vs.* Con. **$p < 0.05$ *vs.* Pb.

tend the anogenital distance, whereas oestrogens reduce it[30,31]. In our study, anogenital distances and gender ratios were not affected by lead exposure, and morphological abnormalities of the reproductive tract were observed. Therefore, lead exposure does not seem to disrupt normal differentiation or development. Similarly, we did not observe any adverse effects of selenium exposure to these parameters.

Thyroid hormone secretion may affect the growth and development of animals. Since selenium regulates thyronine deiodinase of thyroid hormone, long-term selenium deficiency may affect growth and development[32-35]. In our study, body weights and reproductive organ weights were lower in lead-exposed animals on days 0-28. However, no significant differences in the body weights were noted after 35 days, indicating that the effects of lead exposure on the offspring could be limited to specific development time.

Foreskin separation and vaginal opening are considered to be external indicators of the onset of puberty, which is a subject to complex regulation by oestrogens and androgens[36,37]. We observed that gestational lead exposure delayed the onset of puberty in the offspring, and that this was attenuated by selenium supplementation. Thus, the delayed onset of puberty in the offspring associated with lead exposure can be treated with selenium supplementation, possibly via regulation on hormonal level.

Conclusions

In a rat model of gestational lead exposure, we demonstrate damages to the reproductive system during pregnancy, and negative modulation of growth and reproductive system development of the offspring. These adverse effects are reverted or attenuated by selenium supplementation. Therefore, selenium supplementation may be used to treat lead poisoning during pregnancy.

Conflicts of interest

The Authors declare that there are no conflicts of interest.

Reference

1. Koller K, Brown T, Spurgeon A, Levy L. Recent developments in low-level lead exposure and intellectual impairment in children. Environ Health Perspect 2004; 112: 987-994.
2. Fadrowski JJ, Abraham AG, Navas-Acien A, Guallar E, Weaver VM, Furth SL. Blood lead level and measured glomerular filtration rate in children with chronic kidney disease. Environ Health Perspect 2013; 121: 965-970.
3. Taylor CM, Golding J, Hibbeln J, Emond AM. Environmental factors predicting blood lead levels in pregnant women in the UK: the ALSPAC study. PloS one 2013; 8: e72371.
4. Caserta D, Graziano A, Lo Monte G, Bordi G, Moscarini M. Heavy metals and placental fetal-maternal barrier: a mini-review on the major concerns. Eur Rev Med Pharmacol Sci 2013; 17: 2198-2206.
5. Sowers M, Jannausch M, Scholl T, Li W, Kemp FW, Bogden JD. Blood lead concentrations and pregnancy outcomes. Arch Environ Health 2002; 57: 489-495.
6. Roman M, Jitaru P, Barbante C. Selenium biochemistry and its role for human health. Metallomics 2014; 6: 25-54.
7. Molnar J, MacPherson A, Dixon J. Effect of supplementation with selenium on whole blood glutathione peroxidase activities and on plasma and tissue selenium concentrations in lambs. Biol Trace Elem Res 1996; 55: 253-262.
8. Sordillo LM. Selenium-dependent regulation of oxidative stress and immunity in periparturient dairy cattle. Vet Med Int 2013; 2013: 154045.
9. Smith AM, Picciano MF. Evidence for increased selenium requirement for the rat during pregnancy and lactation. J Nutr 1986; 116: 1068-1079.
10. Geng HO, Zhang JC, Zhou L, Cai HY, Wang JB. [The effect of lead exposure in utero on the teeth eruption and enamel development of rat offspring.]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi 2005; 23: 27-30.
11. Othman AI, El Missiry MA. Role of selenium against lead toxicity in male rats. J Biochem Mol Toxicol 1998; 12: 345-349.

12. Jablonska E, Gromadzinska J, Sobala W, Reszka E, Wasowicz W. Lung cancer risk associated with selenium status is modified in smoking individuals by Sep15 polymorphism. Eur J Nutr 2008; 47: 47-54.
13. Richman EL, Chan JM. Selenium and prostate cancer: the puzzle isn't finished yet. Am J Clin Nutr 2012; 96: 1-2.
14. Li Z, Meng J, Xu TJ, Qin XY, Zhou XD. Sodium selenite induces apoptosis in colon cancer cells via Bax-dependent mitochondrial pathway. Eur Rev Med Pharmacol Sci 2013; 17: 2166-2171.
15. Brown KM, Arthur JR. Selenium, selenoproteins and human health: a review. Public Health Nutr 2001; 4: 593-599.
16. Yu L, Sun L, Nan Y, Zhu LY. Protection from H1N1 influenza virus infections in mice by supplementation with selenium: a comparison with selenium-deficient mice. Biol Trace Elem Res 2011; 141: 254-261.
17. Akbaraly TN, Hininger-Favier I, Carriere I, Arnaud J, Gourlet V, Roussel AM, Berr C. Plasma selenium over time and cognitive decline in the elderly. Epidemiology 2007; 18: 52-58.
18. Atroshi F, Rizzo A, Biese I, Veijalainen P, Saloniemi H, Sankari S, Andersson K. Fumonisin B1-induced DNA damage in rat liver and spleen: effects of pretreatment with coenzyme Q10, L-carnitine, alpha-tocopherol and selenium. Pharmacol Res 1999; 40: 459-467.
19. Flora SJ, Singh S, Tandon SK. Role of selenium in protection against lead intoxication. Acta Pharmacol Toxicol (Copenh) 1983; 53: 28-32.
20. Nehru B, Dua R, Iyer A. Effect of selenium on lead-induced alterations in rat brain. Biol Trace Elem Res 1997; 57: 251-258.
21. Nehru B, Iyer A. Effect of selenium on lead-induced neurotoxicity in different brain regions of adult rats. J Environ Pathol Toxicol Oncol 1994; 13: 265-268.
22. Chan YH, Gao M, Wu W. Are newborn rat-derived neural stem cells more sensitive to lead neurotoxicity? Neural Regen Res 2013; 8: 581-592.
23. Doumouchtsis KK, Doumouchtsis SK, Doumouchtsis EK, Perrea DN. The effect of lead intoxication on endocrine functions. J Endocrinol Invest 2009; 32: 175-183.
24. Hertz-Picciotto I. The evidence that lead increases the risk for spontaneous abortion. Am J Ind Med 2000; 38: 300-309.
25. Miranda ML, Edwards SE, Swamy GK, Paul CJ, Neelon B. Blood lead levels among pregnant women: historical versus contemporaneous exposures. Int J Environ Res Public Health 2010; 7: 1508-1519.
26. Anjum MR, Sainath SB, Suneetha Y, Reddy PS. Lead acetate induced reproductive and paternal mediated developmental toxicity in rats. Ecotoxicol Environ Saf 2011; 74: 793-799.
27. Antonio MT, Leret ML. Study of the neurochemical alterations produced in discrete brain areas by perinatal low-level lead exposure. Life Sci 2000; 67: 635-642.
28. Gulson BL, Mahaffey KR, Jameson CW, Mizon KJ, Korsch MJ, Cameron MA, Eisman JA. Mobilization of lead from the skeleton during the postnatal period is larger than during pregnancy. J Lab Clin Med 1998; 131: 324-329.
29. Hu H, Hashimoto D, Besser M. Levels of lead in blood and bone of women giving birth in a Boston hospital. Arch Environ Health 1996; 51: 52-58.
30. Levy JR, Faber KA, Ayyash L, Hughes CL Jr. The effect of prenatal exposure to the phytoestrogen genistein on sexual differentiation in rats. Proc Soc Exp Biol Med 1995; 208: 60-66.
31. Nielsen M, Bjornsdottir S, Hoyer PE, Byskov AG. Ontogeny of oestrogen receptor alpha in gonads and sex ducts of fetal and newborn mice. J Reprod Fertil 2000; 118: 195-204.
32. Carlson DB, Reed JJ, Borowicz PP, Taylor JB, Reynolds LP, Neville TL, Redmer DA, Vonnahme KA, Caton JS. Effects of dietary selenium supply and timing of nutrient restriction during gestation on maternal growth and body composition of pregnant adolescent ewes. J Anim Sci 2009; 87: 669-680.
33. Kohrle J, Jakob F, Contempre B, Dumont JE. Selenium, the thyroid, and the endocrine system. Endocr Rev 2005; 26: 944-984.
34. Meyer AM, Reed JJ, Neville TL, Taylor JB, Hammer CJ, Reynolds LP, Redmer DA, Vonnahme KA, Caton JS. Effects of plane of nutrition and selenium supply during gestation on ewe and neonatal offspring performance, body composition, and serum selenium. J Anim Sci 2010; 88: 1786-1800.
35. Zwolak I, Zaporowska H. Selenium interactions and toxicity: a review. Selenium interactions and toxicity. Cell Biol Toxicol 2012; 28: 31-46.
36. Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M. Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiol Rev 2012; 92: 1235-1316.
37. Roth C, Leonhardt S, Theiling K, Lakomek M, Jarry H, Wuttke W. Ontogeny of the GNRH-, glutaminase- and glutamate decarboxylase-gene expression in the hypothalamus of female rats. Brain Res Dev Brain Res 1998; 110: 105-114.