TÜBİTAK

Turkish Journal of Biology

http://journals.tubitak.gov.tr/biology/

Research Article

Turk J Biol
(2014) 38: 581-585
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doi:10.3906/biy-1402-50

Reproductive biology study of dynamics of female sexual hormones: a 12-month exposure to lead acetate rat model

Eugenia DUMITRESCU[1], Romeo Teodor CRISTINA[1,*], Florin MUSELIN[2]

[1]Department of Veterinary Pharmacology and Pharmacy, Faculty of Veterinary Medicine Timişoara, Banat University of Agronomical Sciences and Veterinary Medicine, Timişoara, Romania

[2]Department of Toxicology, Faculty of Veterinary Medicine Timişoara, Banat University of Agronomical Sciences and Veterinary Medicine, Timişoara, Romania

Received: 21.02.2014 • **Accepted:** 08.05.2014 • **Published Online:** 05.09.2014 • **Printed:** 30.09.2014

Abstract: In human and animal organisms, lead can cause reproductive problems beginning with pregnant females. The reproductive axis is particularly sensitive to lead, its influence resulting in a delayed sexual maturity due to biosynthesis suppression of the sexual steroids. An animal model study was carried out on 28 white Wistar adult female rats, divided into 3 experimental (E) groups that were exposed for 12 months to lead acetate in drinking water as follows: 50 ppb Pb (E_1), 100 ppb Pb (E_2), and 150 ppb Pb (E_3), with a control group (M) that received unleaded tap water. Levels of FSH, LH, estradiol, progesterone, and testosterone were evaluated in the proestrus phase by ELISA technique. Data obtained were compared by one-way ANOVA with Bonferroni correction. As a conclusion, compared to the M group, we can ascertain that lead acetate administered over a long-term period to female rats determines (with the exception of estradiol and progesterone), in direct correlation with the exposure levels, the following: significantly decreased FSH, but still within physiological limits of serum levels; significantly higher serum levels of LH; significantly decreased serum levels of estradiol and progesterone; and significantly higher serum levels of testosterone.

Key words: Reproductive biology, females, hormones, rats, lead acetate

1. Introduction

The scientific concerns of the last decade emphasize the importance and timeliness of reproductive health research in animals and humans. Among the numerous causes of reproductive disorders, authors consider, as an important threat, the more and more frequent presence of potential toxic risk disruptors (e.g., industrial contaminants, heavy metals, pesticides, organic solvents, phthalates) in different reproductive pathologies. Among these, lead can act as an important disruptor (EPA, 1997; Altundoğan et al., 1998).

Lead can be found in the soil crust, in mineral form as galena, anglesite, cerussite, mimetite, pyromorphite, linarite, vanadinite, and wulfenite (Humphreys, 1988).

The reproductive axis is particularly sensitive to lead, its influence resulting in a delayed sexual maturity due to biosynthesis suppression of the sexual steroids (Ronis et al., 1998). In this respect, Nicolopoulou-Stamati and Pitsos (2001) confirmed that lead can certainly influence the female endocrine balance, the estrous cycle and fertility being very sensitive to this reproductive disruptor. Unlike other metals, lead has no physiological role in the body and there is no known accepted minimum level that could be considered as nontoxic for humans and animals. In organisms lead can cause reproductive problems beginning with pregnant females (such as premature birth, abortion, or fetal resorption), but debilitated and young individuals are the most affected, lead influencing their viability, normal growth, and development (Wide, 1985; Téllez-Rojo et al., 2004). Peripubertal exposure in females or for long periods determines delay in vulval opening and puberty installation, all associated with low insulin-like growth factor 1 (IGF-1), luteotropic hormone, and estradiol serum levels (Pinon-Lataillade et al., 1995; Dearth et al., 2004).

Nampoothiri and Gupta (2006) demonstrated that lead is also involved in the gonadotrophic metabolism, disrupting the activity of steroidogenic enzymes from ovarian cell granulose. As a result, lead will affect the cellular membrane through free radicals, inducing lipid peroxidation.

Silberstein et al. (2006) showed that accumulation of lead in small amounts over long periods in the ovary will cause irreversible folliculogenesis, with the presence of atretic follicles and heavy diminution of the primary follicles.

* Correspondence: rtcristina@yahoo.com

In Romania, where pollution from the lead industry still exists, information regarding the impact of lead on reproductive function is still needed and the information provided here will be useful for the reproductive biology field, justifying the present study.

2. Materials and methods

The present research was performed in compliance with good laboratory practice; in accordance with the European Convention principles for the protection of vertebrate animals used in experimental and other scientific purposes, adopted in 1986 in Strasbourg (Council of Europe, 1986); in accordance with the 2010/63/EU directive of the European Parliament and of the European Council adopted 22 September 2010 on the protection of animals used for scientific purposes (European Council, 2010); in accordance with Romanian law for animal experimentation (Romanian Government, 2002); and with the approval of the Scientific Ethics Committee of the Faculty of Veterinary Medicine Timisoara.

2.1. Animals

The study was carried out on 28 white Wistar preadult female rats (at 35 days and 220 g average weight). Animals were purchased from the authorized biobase of "Victor Babeş" University of Medicine and Pharmacy, Timişoara, Romania. The animals were acclimatized for 7 days, maintained in standard cages with controlled temperature and humidity. For this purpose, animals were housed in polycarbonate cages with 750 × 720 × 360 mm (L × W × H) dimensions and wood shavings were used as bedding. The environmental temperature was maintained at 20 ± 2 °C and relative humidity was 55 ± 10%. During the acclimatization period, the light cycle was 12 h light and 12 h dark. Nonsterile pelleted diet (Biovetimix, code 140-501, Romania) and water were offered ad libitum.

Rats were divided into 3 experimental groups as follows: E_1 (50 ppb Pb), E2 (100 ppb Pb), and E3 (150 ppb Pb), exposed continuously for a 12-month period to lead acetate in drinking water, with a control group (M) that received unleaded tap water. At 24 h after the last administration, the rats were euthanized and examined according to the standard procedure during necropsy (OECD, 2011). Euthanasia was performed by overdose of anesthetic agents using the following association: ketamine (300 mg kg bw^{-1}) + xylazine (30 mg kg bw^{-1}) (Pierce, 2006).

2.2. Methodology

Levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone, and testosterone were evaluated in the proestrus phase. The sexual hormones were determined by ELISA technique at Tody's Laboratories, Bucharest (ISO 170025 accredited).

2.3. Statistical analysis

Obtained data were analyzed using GraphPad Prism 5.0 (GraphPad Software, USA). The data in different groups were compared by one-way ANOVA with Bonferroni correction. Differences were considered to be significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$.

3. Results

The values of serum hormones after 12 months of exposure are summarized in Figures 1a–1e. In the control group (M) and in the exposed (E) groups, the FSH serum level was within physiological limits (up to 500 ng/mL), toward the inferior limit.

3.1. FSH levels

Exposure to lead caused significant ($P < 0.01$) decrease of FSH level compared to the M group and directly ($P < 0.01$) correlated with the exposure level: E_1 vs. M: –55.64%; E_2 vs. M: –67.72%; E_3 vs. M: –88.05%; E_2 vs. E_1: –27.25%; E_3 vs. E_2: –62.99%; E_3 vs. E_1: –73.08%.

3.2. LH levels

LH level was within physiological limits (35 ng/mL) in the control group, while in experimental groups the LH level was significantly ($P < 0.01$) higher compared to the M group and directly ($P < 0.01$) correlated with the exposure level: E_1 vs. M: +40.31%; E_2 vs. M: + 66.77%; E_3 vs. M: +169.24%; E_2 vs. E_1: +18.86%; E_3 vs. E_2: +61.43%; E_3 vs. E_1: +91.89%.

3.3. Estradiol serum levels

The serum level of estradiol was at the inferior limit of the physiological values (up to 50 ng/mL) both in M and the E groups. Exposure to lead caused significant ($P < 0.01$) decrease of estradiol serum level in comparison to the M group (E_1 vs. C: –32.08%; E_2 vs. C: –67.93%; E_3 vs. C: –88.77%) and inversely significantly ($P < 0.01$) correlated with exposure level (E_2 vs. E_1: –37.89%; E_3 vs. E_2: –73.20%; E_3 vs. E_1: –83.43%).

3.4. Progesterone serum levels

The level of progesterone was within physiological limits (up to 60 ng/mL) in M and the E groups. Exposure to lead caused significant ($P < 0.01$) decrease of serum progesterone level compared to the M group: E_1 vs. M: –12.08%; E_2 vs. M: –33.36%; E_3 vs. M: –44.20%. Progesterone in the E groups was inversely and significantly ($P < 0.01$) correlated with exposure level: E_2 vs. E_1: –24.20%; E_3 vs. E_2: –16.26%; E_3 vs. E_1: –36.52%.

3.5. Testosterone serum levels

No references regarding physiological serum testosterone limits for female rats were found. In our case, serum testosterone level was significantly ($P < 0.01$) higher in the E groups compared to the M group (E_1 vs. M: +72.72%; E_2 vs. M: +163.63%; E_3 vs. M: +200.00%) and in direct correlation ($P < 0.01$) with the exposure level (E_2 vs. E_1: +52.63%; E_3 vs. E_2:+13.79%; E_3 vs. E_1: +73.86%).

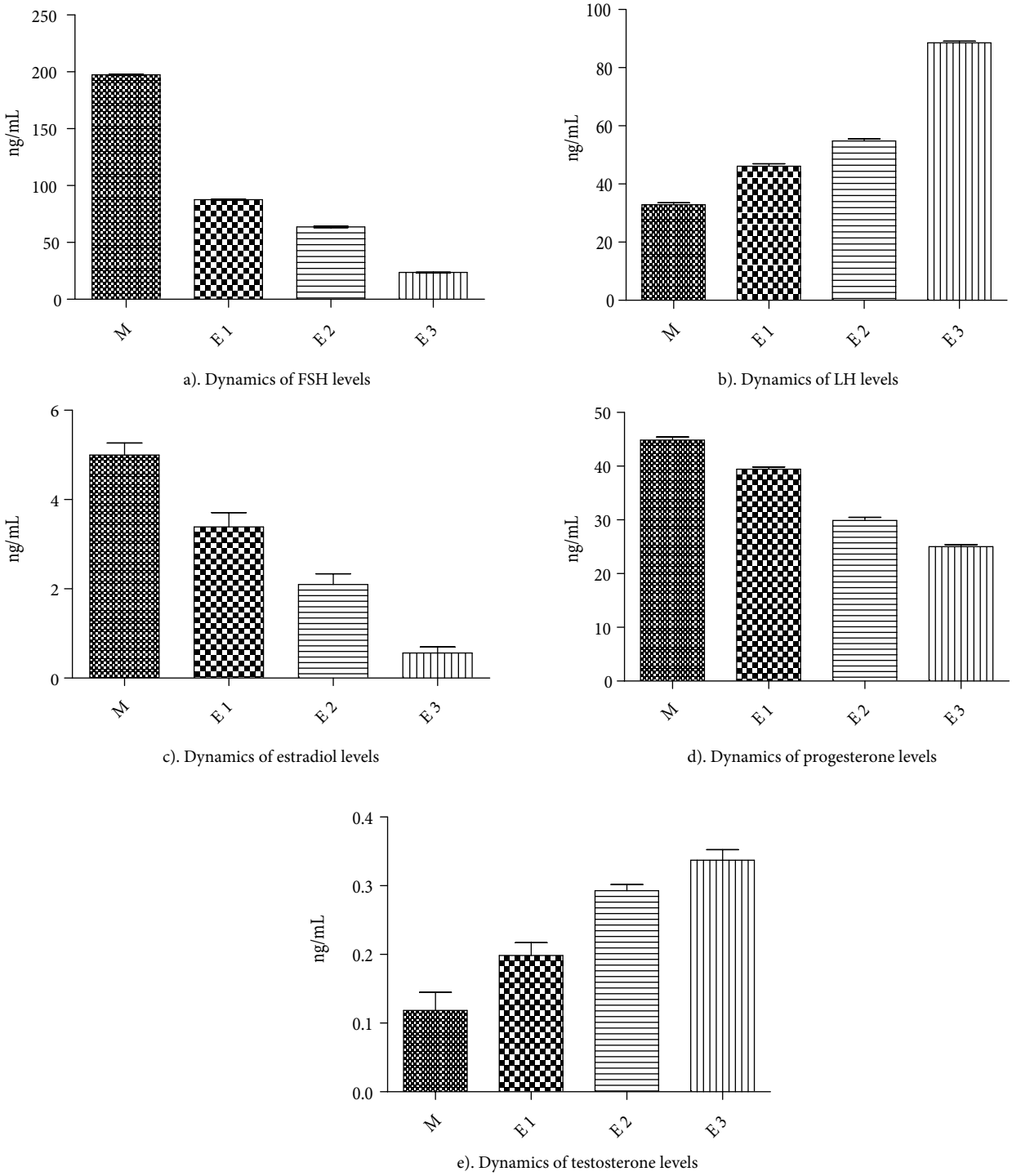

Figure 1. Dynamics of serum FSH (a), LH (b), estradiol (c), progesterone (d), and testosterone (e) levels in the studied group.

4. Discussion

Gonadal activity is under the control of both the hypothalamus and the anterior pituitary gland, the latter being responsible for producing the hormones that are very important for the gonads' control (Cunningham and Klein, 2007). FSH and LH are synergistic hormones in folliculogenesis and ovulation in the ovary. They play a critical role in maintaining the ovarian cycle, governing follicle recruitment and maturation, steroid genesis, completion of ova maturation, ovulation, and luteinization. The low levels of FSH can be explained by the fact that it is very difficult to observe the FSH secretion peak due to its

very short discharge period and because in the proestrus phase the FSH levels are extremely low (Maeda et al., 2000).

Bibliographical information regarding FSH dynamics under lead impact are scarce, though Foster et al. (1996) ascertained that FSH levels significantly decreased after a 10-year lead exposure in monkeys, which confirms the inhibition of ovarian function due to lead. Effects on circulating sex steroids were accompanied by variable effects on levels of circulating LH, pituitary LH, and pituitary LH 'beta'-mRNA, suggesting a dual site of lead action: one at the level of the hypothalamic pituitary unit, and another directly at the level of gonadal steroid biosynthesis (Ronis et al., 1996).

We observed that the LH level increased significantly in comparison to the control group (M) and it was in direct correlation with the exposure level. The increase of LH level over the physiological limits could be explained by the low level of estradiol and progesterone observed by us.

Doumouchtsis et al. (2009) stated that in the subjects exposed in the short term to lead, high levels of FSH and LH are associated with normal testosterone concentrations. The authors also argued that lead will accumulate in the ovarian granulose cells, causing delays in growth and development and infertility in women.

Qureshi and Sharma (2012) showed that lead salts can inhibit the FSH release, leading to atrophy and reduced ovarian secretion of progesterone. Additionally, Dearth et al. (2002) claimed that exposure to lead will result in delayed sexual maturity installation associated with suppression of serum levels of IGF-1, LH, and estradiol.

Estradiol is considered to be the steroid hormone with the biggest inhibitory capacity over LH secretion in rats (Freeman, 1994). Moreover, Maeda et al. (2000) reported the existence of a negative feed-back produced by the estrogens and progesterone secreted by ovaries over LH secretion from late estrus up to the early proestrus phase. In our case, the estradiol levels were at the inferior physiological limit, even in the case of the control group (M), contradicting earlier observations that in proestrus the estradiol level is high. The possible explanation of what we have found could be that the LH high levels beyond the physiological limits as ascertained by us in the experimental lots are linked ($P < 0.01$) with the inhibitory effect exerted by this hormone upon hormonal balance. In our case, we found very low estradiol levels being proportionally inverted ($P < 0.01$) with those of LH, this finding having been also presented by other authors (Freeman, 1994; Taupeau et al., 2001).

Our results obtained on hormonal dynamics are different from those presented by Wiebe et al. (1998), who affirmed that exposure of pregnant females to lead does not have a significant influence on estradiol level.

Progesterone plays an important role influencing the length of sexual cycle in rodents, with progesterone and estrogens working in a synergistic way (Westwood, 2008). The progesterone levels in our case were significantly lower in the experimental groups than in the control group (M). This is in agreement with Freeman's (1994) finding that, in the same period of physiological conditions, the progesterone peak in proestrus is determined by the LH secretion: the progesterone level will decrease and the LH level will increase, probably as the result of lack of or delayed proper/optimal progesterone secretion response by the ovarian preovulatory follicles and as a final consequence of lead's effect on ovary histoarchitectonics. Some authors argued that lead exposure determines decreased serum progesterone (Foster, 1992).

When it comes to the testosterone dynamic during sexual cycles in female rats, no reference values were found by us for this species. Furthermore, no information was found in the bibliographical sources regarding lead's influence on testosterone in female rats. In our case, we have found that the tendency of testosterone dynamics was that of a significant increase ($P < 0.01$) in comparison to the control group (M) and in direct association with the exposure level. In Ryan's (1982) opinion, the increase of testosterone levels in women can be explained by lead's inhibitor activity on aromatase cytochrome P-450, an enzyme necessary to bioconvert androgens into estradiol.

As a conclusion, we can state that lead acetate administered over a long-term period to female rats determines the following, directly and significantly correlated (with the exception of estradiol and progesterone, inversely correlated) with the exposure level: significantly decreased of FSH serum levels, but within physiological limits, as compared to the control group M; significantly higher LH serum levels as compared to the M group; significantly decreased estradiol serum levels as compared to the M group; significantly decreased progesterone serum levels as compared to the M group; and significantly higher testosterone serum levels as compared to the M group.

Extensive studies about the correlation between environmental quality and health and between life quality and health have become a priority for many research teams, such as Karakaş et al. (2013) and Polat et al. (2013), who successfully used rodent models to demonstrate their hypotheses. Here we have confirmed the advantage of the rat model and have shown that this species is well suited for such research on hormonal and reproductive disorders.

Acknowledgment

This work was carried out as part of the project "Postdoctoral School of Agriculture and Veterinary Medicine", POSDRU/89/1.5/S/62371, co-financed by the European Social Fund through the Sectoral Operational Programme for Human Resources Development 2007–2013.

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