Bulletin UASVM, Veterinary Medicine 65(1)/2008
pISSN 1843-5270; eISSN 1843-5378

THE CONSEQUENCES OF FEMALE RATS CHRONIC EXPOSURE TO LEAD ACETATE ON THE BIOMARKERS EMPHASIZING THE HORMONAL DISRUPTING POTENTIAL OF THE REPRODUCTIVE FUNCTION FOR *IN VIVO* EVALUATION

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Abstract. Lead is a metal which is naturally found in earth crust [4], in mineral from: blue lead, anglesite, cerussite, mimetit, linarit, vanadinite and wulfenite [10, 15]. The products that contain lead are: paints, accumulators, ceramics, shots, gun bullets [5, 10, 14], defense system against X rays and radiations generally [11, 13], pigments, insecticides (lead arsenate), enamels, glass [7, 11], linoleum etc. The are few data referring to lead impact on reproductive system, however, it had been underlined the fact that lead accumulation in ovaries, even less in chronic supply conditions, determines the follicle genesis disturbance, resulting less primordial follicles and more atrezic follicles and also ovary cysts. The aim of the study was to evaluate lead reproductive toxicity for female rats. The concrete objectives were: evaluation of biological markers that emphasize the hormonal disrupting potential of reproductive function for *in vivo* evaluation (sexual hormones: FSH, LH, estradiole, progesterone, and testosterone).

MATERIAL AND METHODS

The study was carried out on 32 white Wistar female, adult rats divided in four groups: three experimental (E) and one control (C). The E groups were exposed to lead acetate in drinking water for six month as follows: E_1: 50 ppb (maximum admitted level in drinking water according to Romanian Law No 485/2002), E_2: 100 ppb, E_3: 150 ppb. Studied sexual hormones were determined by Elisa technique by Tody Laboratoires (ISO 17025). The estimation of sexual hormones dynamics was carried out in proestrus (hormonal surge for majority of studied hormones) at the same hour (around 5 p.m.). The data were statistically analyzed by Anova method and Student test.

RESULTS AND DISCUSIONS

Mean levels of seric FSH are summarized in table 1 and fig.1.

Table 1.

Mean FSH seric level in proestrus stady

Group	FSH (ng/ml)		
	X±Sx	D.S.	Confidence level 95%
M	321.62±49.28	13.39	6.55
E_1	0.58±0.03	0.08	6.55
E_2	69.32±68.67	19,42	6.55
E_3	0.65±0.13	0.38	6.55

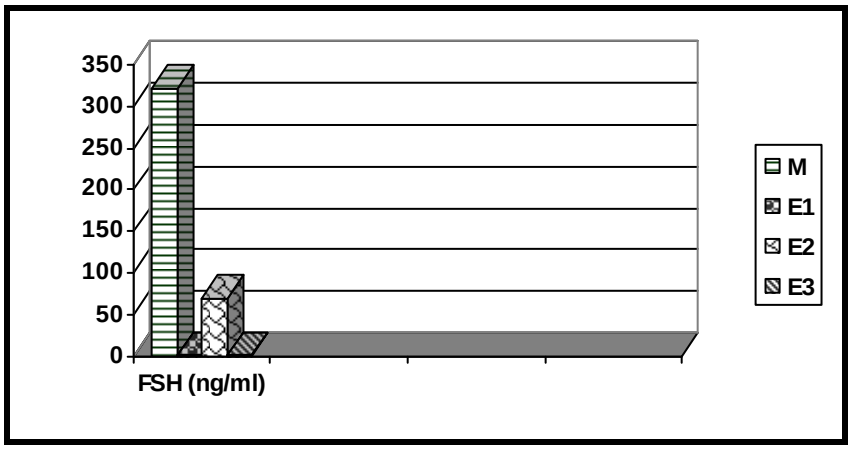

Fig. 1. Dynamics of FSH sric level

The evaluation of FSH seric level pointed out a lot of aspects. At first, the variation of median values in large limits but in physiological limits (until 500 ng/ml -6), in both control and experimental groups. Exposure to lead acetate determined a significant ($p<0.01$) decrease of FSH seric level comparative to C group (E_1/C: -99.82%; E_2/C: -78.45%; E_3/C: -99.79%). The increase of exposure level did not significantly influenced seric FSH level, despite the high differences between groups, maybe because of high individual variation, especially in E_2 group. No references were found regarding the influence of lead on FSH seric level.

The mean values of seric estradiol are presented in table 2 and fig. 2.

Table 2.

Mean estradiole seric level in proestrus stady

Group	Estradiole (ng/ml)		
	X±Sx	D.S.	Confidence level 95%
M	5.55±1.48	4.19	21.17
E_1	2.66±2.62	5.82	21.17
E_2	0.08±0.02	0.06	21.17
E_3	0.21±0.08	0.23	21.17

Exposure to lead determined decrease of estradiol seric level comparative C group, not significantly in E_1 group (E_1/C: -52.07%) and significantly ($p<0.01$) in E_2 and E_3 groups (E_2/C: -98.55%; E_3/C:-96.21%) dznamics.

Exposure level did not significantly influenced estradiol seric level but a decrease tendency was recorded (E_2/E_1: -96.99%; E_3/E_1: -92.10%).

The references about the consequences of lead exposure on seric estradiol are few. The data were refered to prepubertal exposure and pointed out that lead delays puberty appearance the phenomenon being associated to low estradiol and LH level [1, 7].

In utero and during lactation exposure and than until 85 days [8] delayed appearance, of sexual maturity, decrease of steroids hormones level at birth and of plasmatic estradiol concentration during puberty. Wiebe et al [12] pointed out the same as above mentioned authors and also the decrease of steroid hormones.

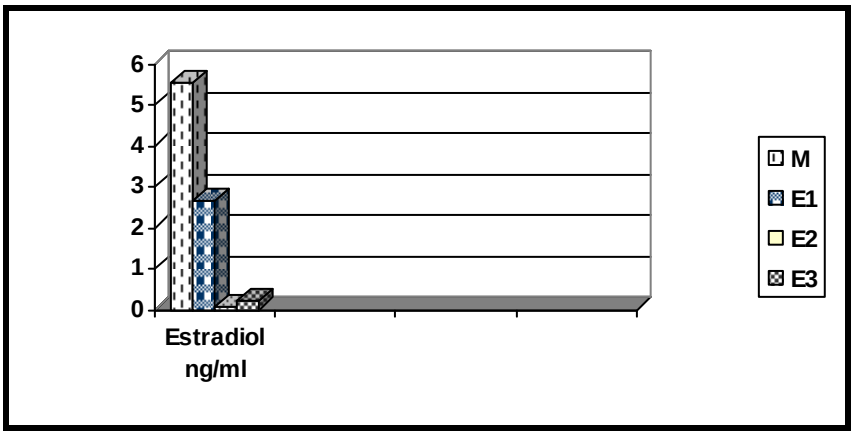

Fig.2.Dynamics of estradiol seric level

The results of present study, at least for proestrus stady, are in agreement with those of the mentioned authors. The decrease of estradiol seric level, correlated with the increase of LH seric level, emphasized the inhibiting effect of pituitary gland exerted through LH.

LH seric level mean values are presented in table 3 and fig.3.

Table 3.

Mean LH seric level in proestrus stady

Group	LH (ng/ml)		
	X±Sx	D.S.	Confidence level 95%
M	33.36±4.92	13.91	106.84
E_1	84.78±32.39	91.62	106.84
E_2	124.65±75.00	212.14	106.84
E_3	138.52±64.71	183.03	106.84

Exposure to lead determined the progressive increase of LH seric level but because of extremely variable individuals values, the differences between E and C groups were not significant (E_1/C: +154.13%; E_2/C:+273.65%;E_3/C; +315.22%). The increase of exposure level was followed by increase of LH seric level but the differences were not significant (E_2/E_1:+47.22%; E_3/E_2: +11.2%; E_3/E_1: +63.38%).

Excepting C group, LH seric level was higher than the physiological limits for the specia and proestrus stady (20-40 ng/ml -6). Ronis et al [8] concluded that *in utero*, during lactation and until 85 days exposure to lead did not influenced LH plasmatic level. Ronis et al [8] emphasized variable effects of lead on circulating LH level suggesting a dual site of lead action at the level of hypothalamic pituitary unit and direct on steroids hormones biosintesis.

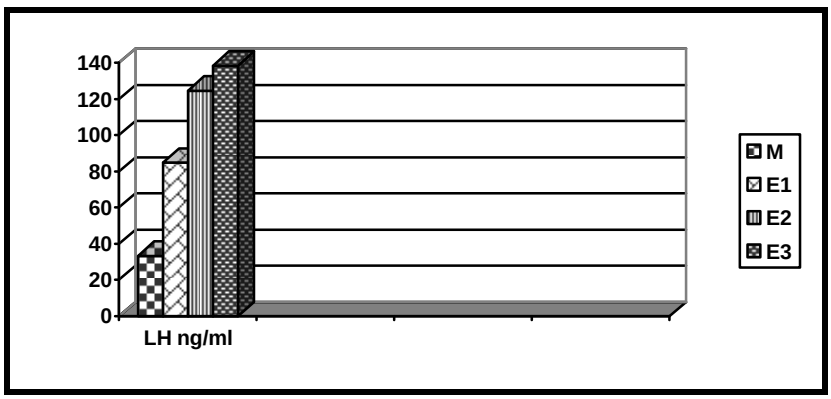

Fig.3. Dynamics of LH seric level

The increase of LH seric level over the physiological limits (PL) (E_1/PL: +182.6%; E_2/PL: +315.5% and E_3/PL: +361.73%) could by explained by the decrease of estradiole and progesterone seric level, both observed after six month exposure to lead acetate. Estradiole is considered a ovarian steroid hormone with the highest inhibiting capacity on LH secretion in rats (Schwartz and Mc Cormach, (1972) quoted by Freeman [3]. Maeda, (1989) quoted by Maeda [6] emphasized a negative feed-back realized by the ovarian estrogens and progesterone on the LH in the period of late oestrus until early proestrus. Possibly, this relation, observed in pathological conditions (exposure to lead), could be transposed in late proestrus too. The mean values of progesterone seric level are summarized in table 4 and fig 4.

Table 4.

Mean progesterone seric level in proestrus stady

Group	Progesterone (ng/ml)		
	X±Sx	D.S.	Confidence level 95%
M	40.55±6.44	18.23	10.28
E_1	32.66±3.98	11.25	10.28
E_2	26.01±4.69	13.28	10.28
E_3	24.41±4.62	13.08	10.28

Progesterone seric level was also in physiological limits (5-60 ng/ml) [6] after exposure to lead too. The progesterone seric level decreased in E groups comparative to C group but the differences were not significant (E_1/C: -19.45%; E_2/C: -35.85%; E_3/C: -39.80%). Progesterone seric level decreased as exposure level increased but the differences were not significant (E_2/E_1:-20.37%; E_3/E_2: -6.16%; E_3/E_1: -25.27%).

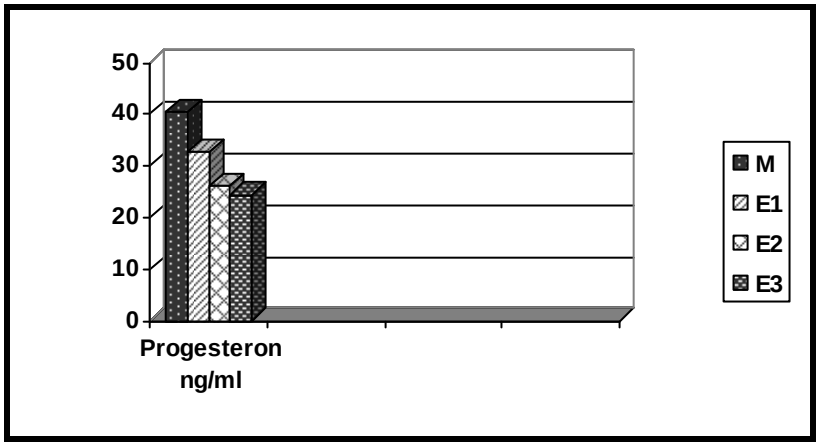

Fig. 4 Dynamics of progesterone seric level

In physiological conditions, the progesterone surge in proestrus is determined by the surge of LH secretion in the same period [3]. In the present experiment, LH level increased but progesterone level decreased comparative to C group, remaining in physiological limits, maybe because of lack or delayed of optimal response of preovulatory ovarian follicles secretion [3], consequence of lead effect on ovary histoarhitecture [2].

Luteal function suppression accompanied by decrease of progesterone level was emphasized too by Franks et al (1989) quoted by Freman [3].

The mean values of testosterone seric level are summarized in table 5 and fig 5.

Exposure to lead increased testosterone seric level, comparative to C group, not significant in E_1 group (E_1/C:+100%) and significant in E_2 (E_2/C:+105.88%, $p<0.01$) and E_3 group (E_3/C:+123.52%, $p<0.05$).

Table 5.

Mean testosterone seric level in proestrus stady

Group	Testosterone (ng/ml)		
	X±Sx	DS	Confidence level 95%
M	0.17±0.04	0.10	0.13
E_1	0.34±0.08	0.23	0.13
E_2	0.35±0.05	0.13	0.13
E_3	0.38±0.07	0.21	0.13

Exposure level did not significantly influenced the testosterone seric level (E_2/E_1:+2.94%; E_3/E_2:+8.57%; E_3/E_1:+11.76%). No data about physiological limits of seric testosterone for sexual cycle stadies were found in rats but in human female, this value range between 0.05 and 0.81 ng/ml (reference values from Tody Laboratoires).

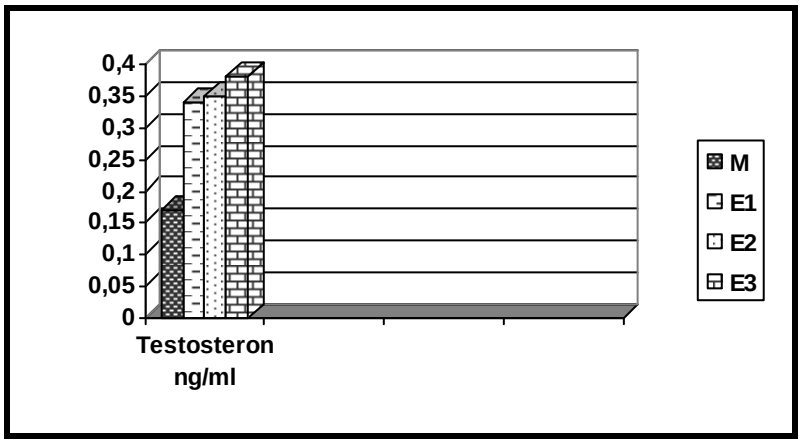

Fig 5. Dynamics of testosterone seric level

Exposure to testosterone determines a rapid masculinization and increase of growth hormone pulse [Painson et al 2000; quoted by.Freeman 3].

CONCLUSIONS

- The consequences of exposure to lead acetate in drinking water (50, 100, 150 ppb Pb) during six month of female rats on sexuale hormones in proestrus stady were:
- significant decrease of FSH seric level comparative to control group, in physiological frame, inverselly correlated, not significant with exposure level;
- increase, over the physiological limits, of seric LH but not significant, comparative to C group and direct correlated, not strictly proportionally to exposure level;
- decrease of seric estradiole, under physiological limits of the specie in proestrus stady, comparative to C group and correlated but not significant to exposure level;
- progressive decrease, not significant, in physiological limits of seric progesterone level comparative to C group and in inversally correlation, not significant, to exposure level;
- increase of testosterone seric level comparative to C group, with different significance degrees and indirect correlation but not significant, to exposure level.

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