LEAD ACETATE IMPACT ON FUNDAMENTAL BIOMARKERS OF REPRODUCTIVE FUNCTIONALITY IN FEMALE RATS (*SUCKLING PERIOD EXPOSURE*)

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Summary

Recent researches are emphasizing more and more obvious the perturbance of the health of the reproductive process. the causes including substances with toxic potential (industrial contaminants. pesticides. organic solvents. etc.). However, the information regarding female reproductive toxicology is less than the one regarding males due to the gametogenesis differences and the access ability of the germinal cells and also because of the revolving nature of female breeding function (3, 4).

The aim of the study was the evaluation of lead toxic impact on the femele reproductive system integrity functionality and performances biomarkers.

The objectives of the study were evaluation of the reproductive functionality fundamental biomarkers (duration of sexual cycle and sexual cycle regularity) at sexual maturity of rat female offspring exposed only *during sukling.*

Key words: lead, rats, sexual cycles.

Materials and methods

The study was carried out on 32 adult female rats (age: 90 days) exposed to lead acetate during suckling period as follows: E_1: 50 ppb Pb (the maximum admitted level in drinking water according to the Law 485/2002); E_2: 100 ppb Pb; E_3: 150 ppb Pb.

Control group received tap water not containing lead.

The forages and water have been assured *ad libitum*.

The exposure to lead acetate was stopped from weaning until sexual maturity.

Duration of sexual cycle and of sexual cycle stages regularity were appreciated by examination of vaginal smear cytological characteristics (stained May-Grunwald-Giemsa method examinated by optic microscope. X 20).

The results had been processed by ANOVA method and Student test.

All assays with animals were conduced in accordance with present laws regarding animal welfare and ethics in animal experiments (6, 7, 8, 9, 10, 11).

Results and discussions

The results are presented in table 1, 2 and figures 1, 2.

Table 1

Mean sexual cycle duration (days)

Group	X±Sx	D.S.	C.L. 95%
C	4.96±0.20	0.53	0.32
E1	5.18±0.13	0.34	0.32
E2	5.66±0.10	0.26	0.32
E3	6.02±0.18	0.48	0.32

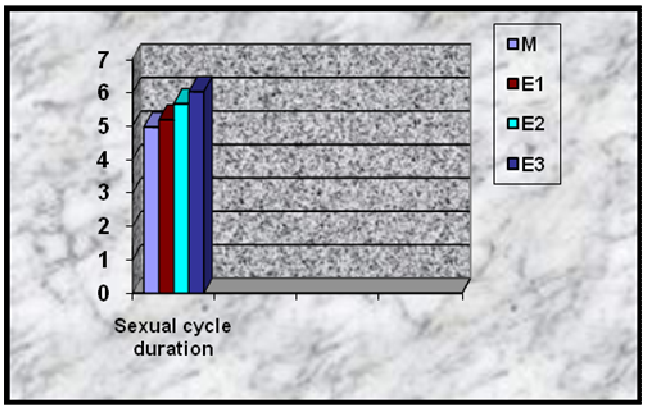

Fig.1. Dynamics of sexual cycle duration (days)

In C group, sexual cycle was in physiological limits – 4-5 days (1) but in exposed groups. the duration was significantly ($p<0.01$) higher than the physiological limits, directly correlated with the exposure level: E_1/C: +4.43% ($p<0.05$); E_2/C:+14.11% ($p<0.01$); E_3/C: +21.37% ($p<0.01$); E_2/E_1: +9.26% ($p<0.01$); E_3/E_2: +6.36 % ($p<0.05$); E_3/E_1:+16.21% ($p<0.01$). In C group all sexual cycle stages were ranged in physiological limits as duration.

Percentage of proestrus in physiological limits was significantly ($p<0.01$) lower comparative to C group: E_1/C: -5%; E_2/C:-6.86%; E_3/C: -12.71% and inversely, significantly ($p<0.01$) correlated with the exposure level (E_2/E_1: -1.95% ($p<0.05$); E_3/E_2: -6.28%; E_3/E_1:-8.11%).

Exposure to lead determined the appearance of sexual cycles with prolonged proestrus, increasing significantly ($p<0.01$) in direct correlation with the exposure level: E_1/C: 5%/0%; E_2/C: 6.86%/0%; E_3/M: 12.71%/0%; E_2/E_1: +37.2%; E_3/E_2: +85.27%; E_3/E_1:+157.2%.

No sexual cycles with absent proestrus were reported.

The percent of sexual cycles with estrus in physiological limits was in E group significantly ($p<0.01$) lower than in C group. Inversely, significantly ($p<0.01$) correlated with the exposure level: E_1/C: -7.43%; E_2/C:-14.29%; E_3/C: -7.57%; E_2/E_1: -7.41%; E_3/E_2: -3.91%. $p<0.05$; E_3/E_1:-10.95%.

No sexual cycles with prolonged estrus were reported.

The percent of sexual cycles with absent estrus was significantly ($p<0.01$) higher in lead exposed groups comparative to C group (E_1/C: 7.43%/0%; E_2/C: 13.29%/0%; E_3/C: 17.57%/0%) inversely, significantly ($p<0.01$) correlated with the exposure level; E_2/E_1: +78.86%; E_3/E_2: +32.20%; E_3/E_1:+136.4%.

The percent of sexual cycles with diestrus I in physiological limits significantly decreased ($p<0.01$) in exposed groups comparative to C group: E_1/C: -5.29%; E_2/C:-9.57%; E_3/C: -15.29% inversely, significantly ($p<0.01$) correlated with the exposure level: E_2/E_1: -4.52%. $p<0.05$; E_3/E_2: -6.32%. $p<0.01$; E_3/E_1:-10.55%, $p<0.01$.

No sexual cycles with absent diestrus I was reported.

The percent of sexual cycles with prolonged diestrus I was significantly ($p<0.01$) higher in lead exposed groups than in C group: E_1/C: 5.29%/0%; E_2/C: 9.57%/0%; E_3/C: 15.29%/0% directly significantly ($p<0.01$) correlated with exposure level: E_2/E_1: +80.9%; E_3/E_2: +59.77%; E_3/E_1:+189.03%

The percent of sexual cycles with diestrus II in physiological limits significantly decreased ($p<0.01$) in exposed groups comparative to C group: E_1/C: 0%; E_2/C:-6%; E_3/C: -14.14% directly significantly ($p<0.01$) correlated with exposure level: E_2/E_1: -6%; E_3/E_2: -8.65%; E_3/E_1:-14.14%.

No sexual cycles with absent diestrus II were reported.

Lead exposure determined the appearance of sexual cycles with prolonged diestrus II in E_2 and E_3 groups (E_1/C: 0%; E_2/C:-6%; E_3/C:-14.14%) correlated with exposure level, significantly to E_3 group: E_3/E_2: +135.66%.

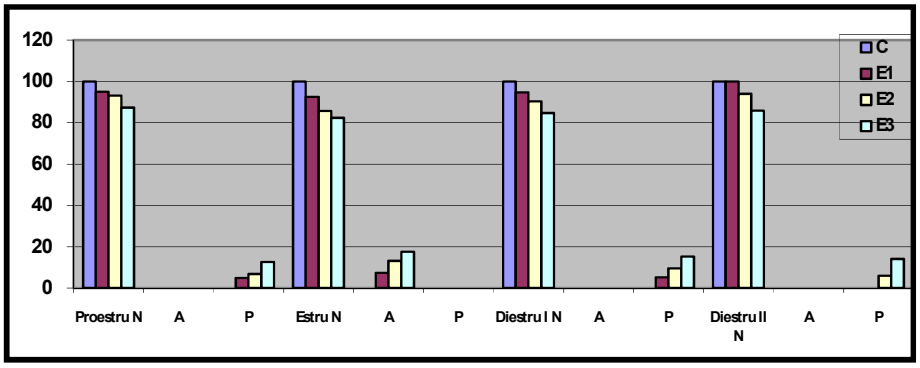

Fig. 2. Sexual cycle stages dynamics after exposure to lead acetate during suckling period

Table 2

Sexual cycle stage						
			C	E_1	E_2	E_3
Proestrus	N	X ± Sx	100± 0.00	95.00±1.48	93.14±1.32	87.29±1.02
		S. D.	0.00	3.92	3.48	2.69
		C.L:	1.33	1.33	1.33	1.33
	A	X ± Sx	0.00± 0.00	0.00±0.00	0.00±0.00	0.00±0.00
		S. D.	0.00	0.00	0.00	0.00
		C.L:	1.33	1.33	1.33	1.33
	P	X ± Sx	0.00± 0.00	5.00±0.49	6.86±0.26	12.71±0.49
		S. D.	0.00	1.29	0.69	0.49
		C.L:	1.33	1.33	1.33	1.33
Estrus	N	X ± Sx	100± 0.00	92.57±1.23	85.71±1.21	82.43±0.75
		S. D.	0.00	3.26	3.20	1.99
		C.L:	1.11	1.11	1.11	1.11
	A	X ± Sx	0.00± 0.00	7.43±0.30	13.29±0.18	17.57±0.20
		S. D.	0.00	0.79	0.49	0.53
		C.L	1.11	1.11	1.11	1.11
	P	X ± Sx	0.00± 0.00	0.00±0.00	0.00±0.00	0.00±0.00
		S.D.	0.00	0.00	0.00	0.00
		C.L	1.11	1.11	1.11	1.11
Diestrus I	N	X ± Sx	100± 0.00	94.71±1.13	90.43±1.07	84.71±0.57
		S.D.	0.00	2.98	2.82	1.50
		C.L:	1.01	1.01	1.01	1.01
	A	X ± Sx	0.00± 0.00	0.00±0.00	0.00±0.00	0.00±0.00
		S.D.	0.00	0.00	0.00	0.00
		C.L:	1.01	1.01	1.01	1.01
	P	X ± Sx	0.00± 0.00	5.29±0.29	9.57±0.30	15.29±0.42
		S.D.	0.00	0.76	0.79	1.11
		C.L:	1.01	1.01	1.01	1.01
Diestrus II	N	X ± Sx	100± 0.00	100±0.00	94.00±1.13	85.86±0.51
		S.D.	0.00	0.00	3.00	1.35
		C.L:	0.79	0.79	0.79	0.79
	A	X ± Sx	0.00± 0.00	0.00±0.00	0.00±0.00	0.00±0.00
		S.D.	0.00	0.00	0.00	0.00
		C.L:	0.79	0.79	0.79	0.79
	P	X ± Sx	0.00± 0.00	0.00±0.00	6.00±0.49	14.14±0.26
		S.D.	0.00	0.00	1.29	0.69
		C.L:	0.79	0.79	0.79	0.79

Sexual cycle stages (% of total sexual cycles)

E_1 – 200 ppb Al
E_2 – 400 ppb Al
E_3 - 1000 ppb Al
N – physiological stage
A – absent stage
P – prolonged stage
NB: 28 supervised sexual cycles /group (7 individuals/group x 4 supervised sexual cycle)

In the studied references was there not found precise information regarding the consequences of lead exposure upon the sexual cycle. In only one bibliographical source Ronis et al., (5) emphasized that the disturbances are appearing (but reversible) only in the case of high level of exposure.

Pillai and Gupta (2) did not observed changes in sexuale cycle after s.c. administration of lead acetate during pregnancy, lactation and before mating.

Conclusions

Exposure to lead acetate *suckling period* determined in female rats at sexual maturity:

- ✓ Significant increase of sexual cycle duration comparative to control group and over the physiological limits. in direct correlation to exposure level;
- ✓ Modification of sexual stages regularity:
  - Significant decrease of sexual cycles percentage with proestrus, estrus, diestrus I and diestrus II in physiological limits as duration comparative to control group and inversely correlated with the exposure level;
  - Appearance of sexual cycles with absent estrus directly correlated with the exposure level;
  - Appearance of sexual cycles with prolonged proestrus, diestrus I and II directly significantly correlated with exposure level.

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