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ALUMINIUM SULPHATE IMPACT ON FUNDAMENTAL BIOMARKERS OF REPRODUCTIVE FUNCTIONALITY IN FEMALE RATS (*IN UTERO* EXPOSURE)

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Summary

The study carried out on 32 adult Wistar female rats (90 days) exposed only in utero to aluminium sulphate (E_1: 200 ppb Al, E_2: 400 ppb Al, E_3: 1000 ppb Al) emphasized: significant increase of sexual cycle duration comparative to control group, over the physiological limits and 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 diestrus II, directly correlated with the exposure level; appearance of sexual cycles with prolonged proestrus, estrus, diestrus I and II, directly, significantly correlated with exposure level.

Key words: aluminium rats, sexual cycles

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.) (3, 6, 8, 9).

The studies in the field of reproductive toxicology are of opportunity because in Romania there is primary and secondary aluminium industry, that represents a real risk for the environment, animals and humans health (5).

The aim of the study was the evaluation of aluminium 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 at aluminium sulphate *in utero*.

Materials and methods

The study was carried out on 32 adult Wistar female rats (90 days) exposed to aluminium sulphate during pregnancy period as follows: E_1: 200 ppb Al (the exceptional admitted limit in drinking water according to the Law 485/2002); E_2: 400 ppb Al; E_3: 1000 ppb Al (values representing concentrations found out in water sources destinated for animals and, sometimes, for people, in areas exposed to the risk of aluminium based industry contamination).

Offspring exposure to aluminium sulphate was stopped from birth until sexual maturity. Control group received tap water.

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

Duration of sexual cycle and of sexual cycle stages regularity were appreciated by examination of vaginal smear cytological characteristics (stained May-Grunnwald-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 (10, 11, 12, 13, 14, 15).

Results and discussions

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

Table 1

Mean sexual cycle duration (days)

Group	X±Sx	D.S.	C.L. 95%
C	4.56±0.11	0.29	0.16
E1	4.85±0.05	0.15	0.16
E2	5.43±0.07	0.19	0.16
E3	5.77±0.07	0.18	0.16

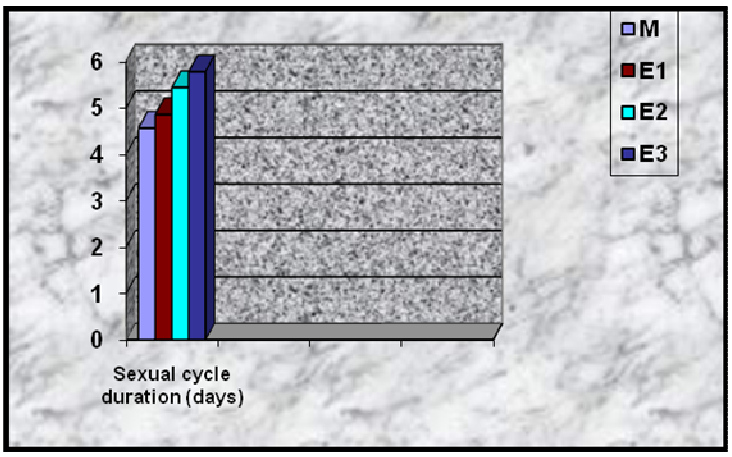

Fig.1. Dynamics of sexual cycle duration (days)

In C group, sexual cycle was in physiological limits – 4-5 days (7), 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: +6.35%,

$p<0.05$; E_2/C:+ 19.07 % $p<0.01$; E_3/C: +26.53% $p<0.01$; E_2/E_1: +11.95% $p<0.01$; E_3/E_2: +6.26% $p<0.01$; E_3/E_1:+ 18.96 % $p<0.01$.

In C group all sexual cycle stages 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: -10.98%; E_2/C: -18.94 %; E_3/C: -27.85% and inversely, significantly ($p<0.01$) correlated with the exposure level (E_2/E_1: -26.41%; E_3/E_2: -18.29%; E_3/E_1:- 35.29%).

No sexual cyles with absent proestrus were reported.

Exposure to aluminium determined the appearance of sexual cycles with prolonged proestrus, increasing significantly ($p<0.01$), in direct correlation with the exposure level: E_1: 15%; E_2:38%; E_3: 45%.

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: -10%; E_2/C:-35%; E_3/C: -40%; E_2/E_1: -27.27%; E_3/E_2: -7.69%; E_3/E_1:-38.46%.

The percent of sexual cycles with prolonged estrus was directly. significantly ($p<0.01$) correlated with the exposure level: E_1: 10%; E_3/E_2: 35%; E_3/E_1:40%; E_2/E_1: +250%; E_3/E_2: +14,28%; E_3/E_1:+300%.

No sexual cycles with absent estrus were reported.

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: 0%; E_2/C:-15%; E_3/C: -25%, inversely, significantly ($p<0.01$) correlated with the exposure level: E_2/E_1: -15%; E_3/E_2: -11,76%; E_3/E_1:-25%.

The precent of sexual cycles with prolonged diestrus I was significantly ($p<0.01$) higher in E group exposed to 400 and 1000 ppb Al than in C group: E_1: 15%; E_3: 25%.

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: -2.5%; E_2/C:-5.5%; E_3/C: -28% inversely, significantly ($p<0.01$) correlated with the exposure level E_2/E_1: -3.07%; E_3/E_2: -23.8%; E_3/E_1:-26.15%.

Aluminium exposure determined the appearance of sexual cycles with absent diestrus II in E_3 groups (E_3:5%).

The percent of sexual cycles with prolonged diestrus II was significantly ($p<0.01$) higher in aluminium exposed groups than in C group: E_1: 2.5%; E_2: 5.4%; E_3: 23% directly, significantly ($p<0.01$) correlated with exposure level: E_2/E_1: +116%; E_3/E_2: +325.92%; E_3/E_1:+820%.

Table 2

Sexual cycle stages (% of total sexual cycles)

			Sexual cycle stage			
			C	E_1	E_2	E_3
Proestrus	N	X ± Sx	100± 0.00	85.00±1.39	62.00±0.57	55.00±1.12
		S. D.	0.00	3.93	1.60	3.16
		C.L:	1.38	1.38	1.38	1.38
	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:	0.18	0.18	0.18	0.18
	P	X ± Sx	0.00± 0.00	15.00±0.42	38.00±0.87	45.00±1.16
		S. D.	0.00	1.20	2.45	3.30
		C.L:	1.38	1.38	1.38	1.38
Estrus	N	X ± Sx	100± 0.00	90.00±1.18	65.00±1.16	60.00±1.68
		S. D.	0.00	3.34	3.30	4.75
		C.L:	1.79	1.79	1.79	1.79
	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	1.79	1.79	1.79	1.79
	P	X ± Sx	0.00± 0.00	10.00±1.27	35.00±1.12	40.00±1.13
		S.D.	0.00	3.59	3.16	3.21
		C.L	1.79	1.79	1.79	1.79
Diestrus I	N	X ± Sx	100± 0.00	100±0.00	85.00±1.12	75.00±2.41
		S.D.	0.00	0,00	3.16	6.80
		C.L:	1.70	1.70	1.70	1.70
	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:	1.70	1.70	1.70	1.70
	P	X ± Sx	0.00± 0.00	0.00±0.00	15.00±0.71	25.00±1.12
		S.D.	0.00	0.00	2.00	3.16
		C.L:	1.70	1.70	1.70	1.70
Diestrus II	N	X ± Sx	100± 0.00	97.50±0.68	94.50±0.65	72.00±0.93
		S.D.	0.00	1.93	1.85	2.62
		C.L:	0.93	0.93	0.93	0.93
	A	X ± Sx	0.00± 0.00	0.00±0.00	0.00±0.00	5.00±0.46
		S.D.	0.00	0.00	0.00	1.31
		C.L:	0.93	0.93	0.93	0.93
	P	X ± Sx	0.00± 0.00	2.50±0.09	5.40±0.10	23.00±0.80
		S.D.	0.00	0.25	0.27	2.27
		C.L:	0.93	0.93	0.93	0.93

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)

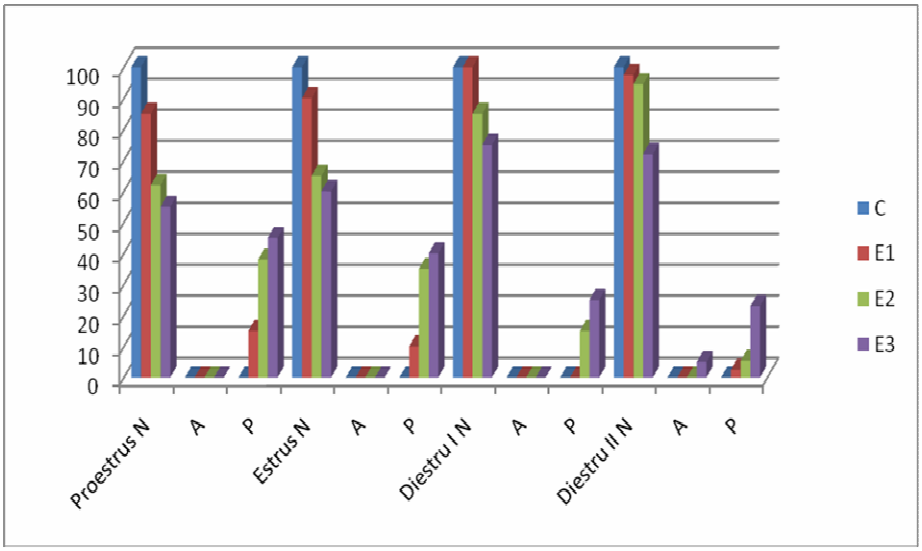

Fig. 2. Sexual cycle stages dynamics

Appearance of anormal sexuale cycles was mentioned by Agrawald et al. (1), Domingo et al. (2, 4) consecutive female exposure from in utero period until sexual maturity.

No data regarding the influence of exposure period, and/ or duration (month, generations) on sexuale cycle characteristics were found.

Conclusions

Exposure to aluminium sulphate *in utero* determined in female rats at sexual maturity:

- ✓ significant increase of sexual cycle duration comparative to control group, over the physiological limits and 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 diestrus II, directly correlated with the exposure level;
  - appearance of sexual cycles with prolonged proestrus, estrus, diestrus I and II, directly, significantly correlated with exposure level.

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