HVM BIOFLUX
Human & Veterinary Medicine
International Journal of the Bioflux Society

The consequences of aluminium sulphate intake on exposure and integrity biomarkers in female rats at sexual maturity (two generation study)

Alexandra Trif, Eugenia Dumitrescu, Diana Brezovan, and Snejana Petrovici

Banat's University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Timisoara, Romania, EU.
Corresponding author: A. Trif, e-mail: al_trif@yahoo.com

Abstract. The aim of the study was the assesment of alumnium toxic impact on female reproductive system integrity and performances biomarkers. The objectives were: the evaluation of aluminium levels in ovary, Fallopian tubes and uterus (exposure biomarker) and the structural changes in ovary, Fallopian tubes and uterus (integrity biomarker) at sexual maturity consecutive exposure to aluminium sulphate (200, 400, 1000 ppb Al) in drinking water along two generations (F_0, F_1). The study pointed out significant increase of aluminium level in ovaries, Fallopian tubes and uterus comparative to the control group and direct corelation, with different degrees of significance, with the exposure level; severe congestive and degenerative changes in ovary and uterus. The studies regarding aluminium reproductive toxicity are few and sometimes controversially (Andrews 1993).
Key words: aluminium, ovary, uterus, level, histoarhitecture.

Material and Methods. The evaluation of aluminium toxic effect on reproductive system exposure and integrity biomarkers was carried out on 28 withe Wistar female rats, from F_0 and on 28 withe Wistar female rats from F_1 generation divided lack in four groups: three experimental (E) and one control (C).

Females and males from F_0 generation were exposed before mating for three month to aluminium sulphate in drinking water as follows: E_1: 200 ppb Al (the exceptional admitted limit in drinking water); 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 industrial contamination).

Females from F_0 generation were mated with other males corresponding as exposure level as mentioned above in ratio of 1♂:2♀ to obtain F_1 generation. The F_1 offspring were exposed to the same levels of aluminium sulphate until sexual maturity.

Control group received tap water. The forages and water have been assured *ad libitum.*

The females from F_0 generation, after weaning of the F_1 generation offspring and the F_1 female offspring at sexual maturity were sacrificed following protocols and ethical procedures and ovary, Fallopian tubes and uterus were taken of for aluminium level determination and histological exam.

The aluminium level was determined in genital organs (ovary, Fallopian tubes and uterus) by atomic absorbtion spectrometry in the Laboratory of Nutrition and Toxicology from Facutly of Veterinary Medicine Timişoara, with the spectrometer-AAS AA-6650 Shimadzu, with graphite oven, provided by the company Viola Bucharest and the structural changes on histological section trichromic Mallory stained (after fixation in alcohol 80^c, sectioned at 5µ).

All assays on animals were performed in accordance with present laws regarding animal welfare and ethics in animal experiments (Directive 86/609 EEC/1986; Romanian

Law 205/2004; Romanian Law 206/2004; Romanian Law 471/2002; Romanian Law 9/2008; Romanian Order 143/400).

The results were statistically processed by the software Anova and the Student test.

Results and Discussions. The results regarding aluminium level in genital organs (ovary, Fallopian tubes and uterus) are summarized in Table 1 and Figure 1.

The study emphasized: higer, significant (F_0: $p<0.05$; F_1: $p<0.01$) accumulation in ovary in E groups comparative to C group in both generations and not correlated with exposure level in F_0 and direct, not significantly ($p>0.05$) correlated with exposure level in F_1 generation (F_0: *ovary*- E_1/C: +150.23%; E_2/C: +165.45%; E_3/C: +102.10%; E_2/E_1: +6.08%; E_3/E_2: -23.87%; E_3/E_1: -19.24%; F_1: *ovary* -E_1/C: +148.76%; E_2/C: +155.78%; E_3/C: +162%; E_1/E_2: -2.82%; E_3/E_2: -2.42%; E_3/E_1: +5.35%).

In Fallopian tubes and uterus, aluminium level was significantly ($p<0.01$) higher than in C group in both F_0 and F_1 generations, not correlated with exposure level in F_0 generation and direct correlated with exposure level in F_1 generation (F_0: *uterus* -E_1/C: +28.08%; E_2/C:+16.9%: E_3/C: +24.78%; E_2/E_1: -8.73%; E_3/E_2: +6.74%; E_3/E_1: -2.58%; F_1:*uterus* -E_1/C: +17.36%; E_2/C: +19.92%; E_3/C: +30.52%).

The increase of exposure level determined the progresive increase of aluminium level in Fallopian tubes and uterus, but the differences were significant ($p<0,01$) only when exposure level increased from 400 to 1000 ppb Al (E_3/E_2: +8,83%) ($p<0.01$) and from 200 to 1000 ppb Al (E_3/E_1: +11.2%) ($p<0.01$).

Exposure along two generations (F_0, F_1) has had as consequences in female offsprings from F_1 at sexuale maturity higher aluminium level in ovary, Fallopian tubes and uterus than in F_0 generation (*ovary*: X_EF_1/X_EF_0: +11.57%, $p<0.01$).

Table 1

Aluminium sulphate average concentration (µg/g) in ovary, Fallopian and tubes uterus

Group		X±Sx F_0	D.S.	X±Sx F_1	D.S.	C.L. 95%
Ovary	C	38.04±7.11	10.05	39.74±0.47	1.04	0.90
	E1	95.19±7.35	10.39	98.86±0.32	0.72	0.90
	E2	100.98±11.38	16.09	101.65±0.46	1.03	0.90
	E3	76.88±1.30	1.84	104.12±0.43	0.95	0.90
	X_E	91.01		101.54	-	-
Uterus and Fallopian tubes	C	6.98±1.90	2.68	7.83±0.16	0.35	0.31
	E1	8.94±1.51	2.14	9.19±0.15	0.34	0.31
	E2	8.16±0.81	1.15	9.39±0.16	0.35	0.31
	E3	8.71±0.31	0.44	10.22±0.25	0.25	0.31
	X_E	8.60		9.60	-	

SD=standard deviation, CL=limits of confidence, X=mean, Sx=the sample standard deviation of the variable "x", X_E= mean for experimental groups.

In studied references there were not found data about the presence and level of aluminium in female sexual organs even the aluminium accumulation in soft tissue was mentioned by some authors.

The presence of aluminium was pointed out only in male sexual organs, their level as hierarchy beening after bones, liver and kidney.

Exposure to aluminium sulphate determined severe structural changes in genital organs - *ovary*: vacuolar epithelial cells, follicles with large oocytes, very evident edema of the parenchymatosa zone, follicles destruction, destruction of parenchyma; *uterus*: necrosis of uterine glands, partial destruction of the lining uterine epithelium, passive

vascular congestion, necrosis of the connective tissue, rarefaction of connective tissue, almost complete detachment of the uterine epithleium. The histological images are presented in Figs 2-13.

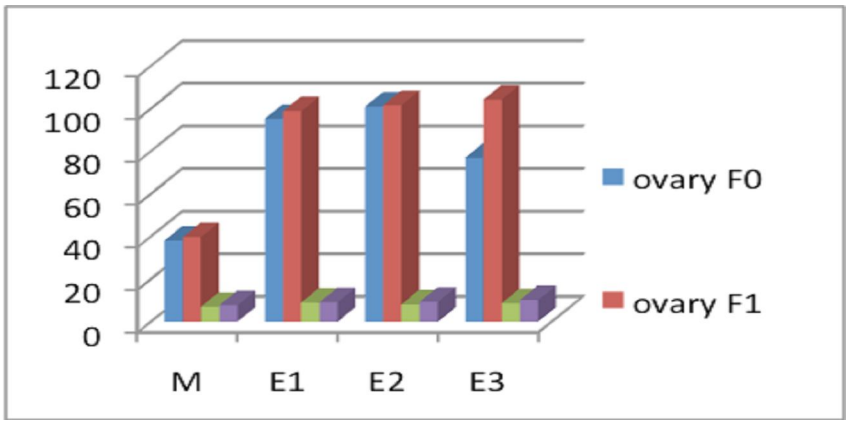

Figure 1. Dynamics of aluminium sulphate levels in ovary, Fallopian tubes and uterus

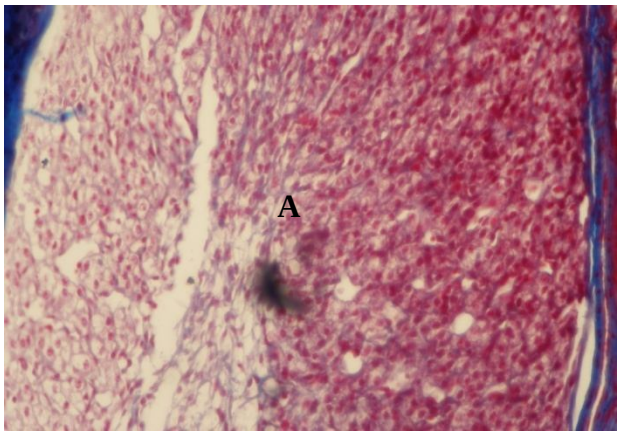

Figure 2. Histological section in rats' ovary after exposure to 200 ppb Al (F_0)
Trichromic Mallory stain, X 200; vacuolar epithelial cells (A).

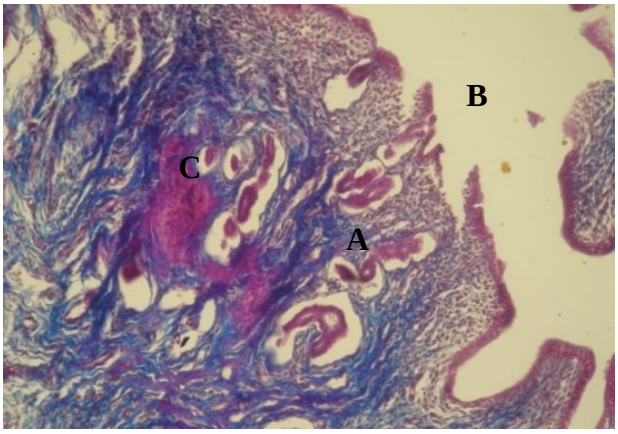

Figure 3. Histological section in rats' uterus after exposure to 200ppb Al (F_0)
Trichromic Mallory stain, X 100; necrosis of uterine glands (A), partial destruction of the lining uterine epithelium (B) pasive vascular congestion (C).

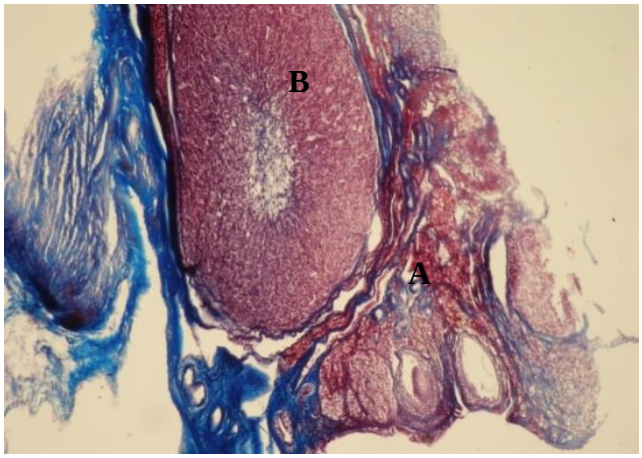

Figure 4. Histological section in rats' ovary after exposure to 400ppb Al (F_0)
Trichromic Mallory stain, X 100, follicles with large oocyte (A); vacuolar epithelial cells (B).

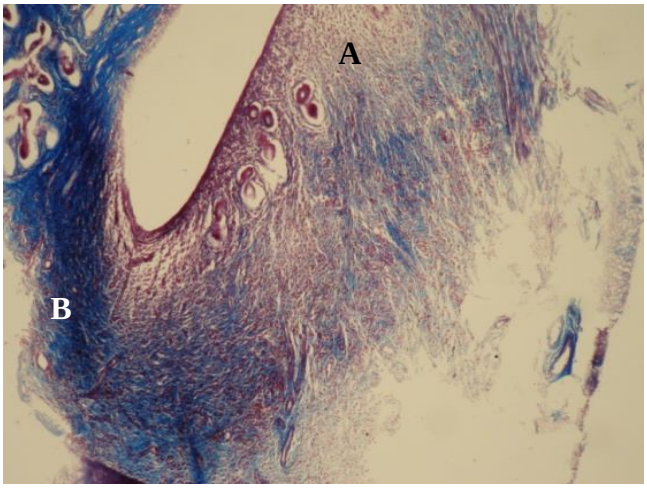

Figure 5. Histological section in rats' uterus after exposure to 400ppb Al (F_0)
Trichromic Mallory stain, X 100; necrosis of uterine glands (A),
partial destruction of the lining uterine epithelium (B).

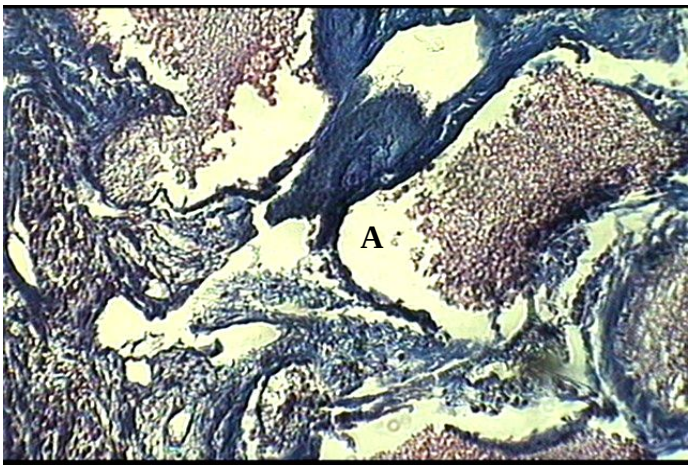

Figure 6. Histological section in rats' ovary after exposure to 1000 ppb Al (F_0)
Trichromic Mallory stain, X 150; very evident edema of the zona parenchymatosa (A).

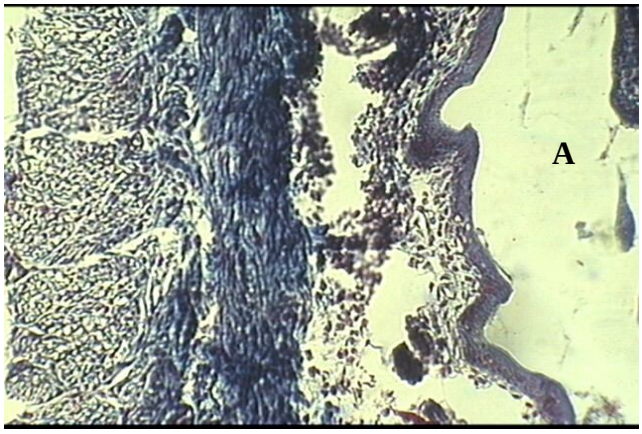

Figure 7. Histological section in rats' uterus after exposure to 1000 ppb Al (F_0)
Trichromic Mallory stain, X 300;
almost complete detachment of the uterine epithleium (A).

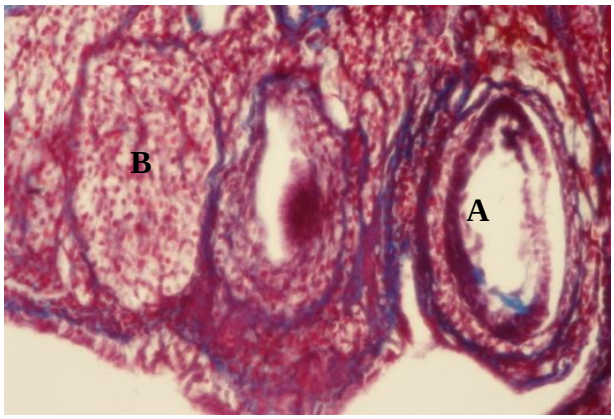

Figure 8. Histological section in rats' ovary after exposure to 200ppb Al (F_1)
Trichromic Mallory stain, X 400; follicles destruction (A),vacuolar epithelial cells (B).

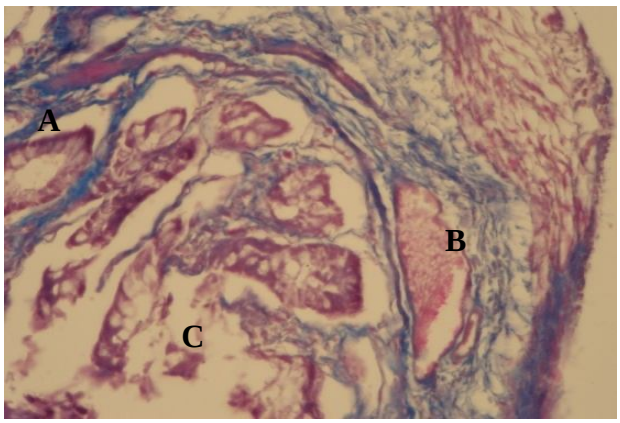

Figure 9. Histological section in rats' uterus after exposure to 200ppb Al (F_1)
Trichromic Mallory stain, X 100; necrosis of uterine glands (A), passive congestion (B), rarefaction of connective tissue (C).

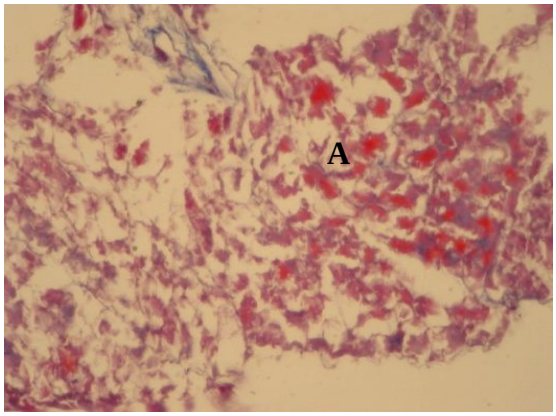

Figure 10. Histological section in rats' ovary after exposure to 400ppb Al (F_1)
Trichromic Mallory stain, X 100; destruction of parenchyma (A).

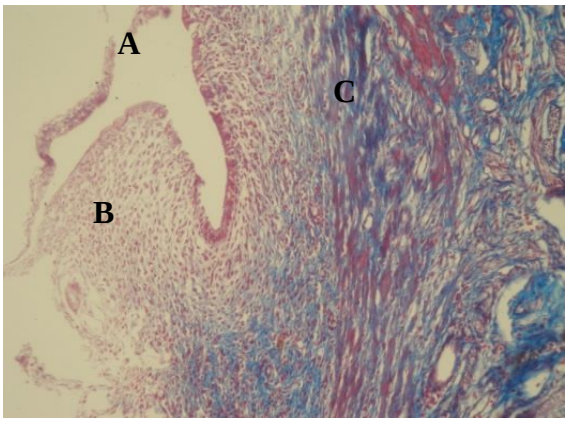

Figure 11. Histological section in rats' uterus after exposure to 400ppb Al (F_1)
Trichromic Mallory stain, X 100; partial destruction of uterine lining cells (A), cells rarefaction from underlying epithelium (B).

The morphological changes pointed out by this study were different from those of Schauffer et al (2002) quoted by ICHEM. Other authors as Chinoy et al (2001), Domingo et al (1994), Trif et al (2008), described: ovaries with numerous ovarian follicles, decrease of follicles size.

Conclusions. Exposure to aluminium sulphate of female rats along two generation determined in adult period at sexual maturity:

- Significant increase of aluminium level in ovaries, Fallopian tubes and uterus comparative to the control group not correlated with exposure level in F_1 generation and in direct correlation, with different degrees of significance, with the exposure level in F_1 generation;
- Higher, significant aluminium level in ovaries and Fallopian tubes and uterus in F_1 generation comparative to F_0 generation;
- Severe congestive and degenerative changes in ovary and uterus.

References

Andrews J. S., 1993 Biologic Monitoring and Biomarkers, ATSDR-Hazardous Waste Conference; http://www.atsdr.cdc.gov/cx6a.html

Chinoy N. J., Patel T. N., 2001 Effects of sodium fluoride and aluminium chloride on ovary and uterus of mice and their reversal by some antidotes. Fluoride **34**:1: 9-20.

Domingo J. L., 1994 Reproductive and developmental toxicity of aluminium: A Review**,** Neurotoxicology and Teratology **17**(4):515-521.

Trif A., Dumitrescu E., Muselin F., 2008 The consequences of chronic aluminum sulphate intake on exposure and morphological integrity biomarkers (aluminum level and weight of sexual organs) in female rats. Lucr Şt Med Vet Timişoara **XLI**:977-981.
*** INCHEM, 2002 Aluminium WHO Food Additives Series; Available online at: http://www.inchem.org./documents/jecfa /jecmono/v024je07.
*** Directive 86/609 EEC from 24.11.1986, for protection of animals used in scientific purposes and other scientific means, http://ec.europa.eu/food/fs/aw/aw_legislation/scientific/86-609-eec_en.pdf
*** Romanian Law 205/26.05.2004 regarding animal protection.
*** Romanian Law 206/27.05.2004 regarding work in scientific research, technological development and inovation.
*** Romanian Law 471/9.07.2002 for O.G. nr. 37/2002 approval for animal protection when used in scientific purpuses and other experimental means.
*** Romanian Law 9/11.01.2008 for modification and addendum of 205/2004 Law regarding animal protection.
*** Romanian Order 143/400 for approval of instruction for housing and attendance of animals used in scientific purposes and other scientific means.

Received: 22 May 2010. Accepted: 14 June 2010. Published online: 14 June 2010.
Authors:
Alexandra Trif, Faculty of Veterinary Medicine, Timişoara, Romania, Calea Aradului no 119, 300645, al_trif@yahoo.com
Eugenia Dumitrescu, Faculty of Veterinary Medicine, Timişoara, Romania, Calea Aradului no 119, 300645, cris_tinab@yahoo.com
Diana Brezovan, Faculty of Veterinary Medicine, Timişoara, Romania, Calea Aradului no 119, 300645, dargherie@yahoo.com
Snejana Petrovici, Faculty of Veterinary Medicine, Timişoara, Romania, Calea Aradului no 119, 300645, petrovicisnejana@yahoo.com
How to cite this article:
Trif A., Dumitrescu E., Brezovan D., Petrovici S., 2010 The consequences of aluminium sulphate intake on exposure and integrity biomarkers in female rats at sexual maturity (two generation study). HVM Bioflux **2**(1):11-17.