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Dynamics of female sexual hormones in F_1 generation female rats (*Rattus norvegicus*), exposed to potassium dichromate (Cr VI)

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Abstract. The study was carried out on 28 white Wistar adult female rats from F_1 generation (derived from females and males exposed for three months before mating to potassium dichromate, 25, 50 and 75 ppm CrVI). F1 generation was exposed in utero and until sexual maturity to the same Cr VI levels.
The study pointed out: significant increase of FSH, LH and testosterone seric level, directly correlated to exposure level and significant decrease of estradiol and progesterone seric level, inversely correlated with the exposure level.
Key Words: rats, sexual hormones, chromium.

Sažetak. Istraživanje je provedeno na 28 odraslih bijelih ženki Wistar štakora F1 generacije (dobivenih od ženki i mužjaka koji su bili izloženi u roku/vremenu/tijekom (od) tri mjeseca prije parenja na kalij Dichromate, 25, 50 i 75 ppm Cr). F1 generacija je bila izložena već u maternici pa sve do postizanja spolne zrelosti istoj razini Cr VI. Istraživanje je pokazalo: značajan porast/značajno povećanje FSH-a, LH-a, i razine seruma testosterona, u izravnom suodnosu s izloženošću; kao i značajna sniženja razine seruma estradiola i progesterona, u obrnutom suodnosu s razinom izloženosti.
Ključne riječi: štakor, spolni hormoni, krom.

Introduction. Chromium (Cr) is one of the most toxic chemical compounds (Petrovici et al 2010). The high level in the environment is as a result of industrial and agricultural practices. It has become one of the most abundant pollutants in aquatic and terrestrial ecosystems (Coşier & Petrescu-Mag 2008).

Potassium dichromate ($K_2Cr_2O_7$), a hexavalent form of Cr (VI), is widely used in metallurgy, chrome plating, textile manufacture, wood presevation, photography and photoengraving, refractory and stainless steel industry and cooling systems.

Exposure to hexavalent chromium compounds Cr (VI) has several adverse human health effects including important reproductive physical and functional perturbances (Rodriguez et al 2007; Sakhila et al 2008; Soudani et al 2010; Toxicology Profile for Chromium, U.S. EPA 2001).

Material and Method. The study was carried out on 28 white Wistar adult female rats, divided in three experimental (E) groups, exposed from in utero period until sexual maturity to potassium dichromate as follows: 25 ppm Cr – LOAEL (E1) (Toxicology Profile for Chromium, U.S. EPA), 50 ppm Cr – 2 X LOAEL (E2), 75 ppm Cr – 3 X LOAEL (E3) and one control (C) group not exposed to chromium. The F1 generation, represented by mature offspring derived from mothers exposed for three months to the same Cr (VI) levels, mated with similar exposed males.

Potassium dichromate was administered in drinking water.

The female rats were fed with standard diets, corresponding to species and age.

Forages and water were ad libitum.

FSH, LH, estradiol, progesterone and testosterone level were evaluated in proestrus.

The sexual hormones were determined by ELISA technique at Tody's Laboratories (ISO 17025), Bucharest.

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

All assays with animals were conducted in accordance with present laws regarding animal welfare and ethics in animal experiments (Directive 86/609 EEC/1986; Romanian Law 205/26.05.2004; Romanian Law 206/27.05.2004 regarding work in scientific research, technological development and innovation; Romanian Law 471/9.07.2002; Romanian Law 9/11.01.2008; Romanian Law 206/27.05.2004; Romanian Order 143/400/2002).

Results and Discussion. The seric sexual hormones values in F1 generation are summarized in Table 1 and Figs 1-5.

Table 1

Seric hormones level in F1 generation

Seric hormones level (ng/ml)					
		C	E1	E2	E3
FSH	(X±Sx)	10.51±0.22	68.26±0.35	77.92±0.39	98.94±0.17
	S. D.	0.58	0.93	1.02	0.46
	C.L.	0.61	0.61	0.61	0.61
LH	(X±Sx)	30.7±0.47	4619.84±0.25	4700.2±0.04	4800.15±0.05
	S. D.	1.25	0.66	0.12	0.14
	C.L.	0.56	0.56	0.56	0.56
Estradiol	(X±Sx)	6.34±0.01	0.03±0.01	0.02±0.01	0.01±0.01
	S. D.	0.01	0.01	0.01	0.01
	C.L.	0.01	0.01	0.01	0.01
Progesterone	(X±Sx)	43.48±0.14	2.31±0.03	2.06±0.02	1.44±0.05
	S. D.	0.37	0.09	0.05	0.14
	C.L.	0.16	0.16	0.16	0.16
Testosterone	(X±Sx)	0.02±0.01	0.63±0.01	0.73±0.01	1.24±0.01
	S. D.	0.01	0.01	0.01	0.01
	C.L.	0.01	0.01	0.01	0.01

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

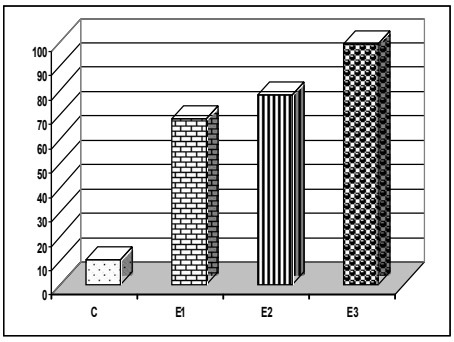

Figure 1. FSH seric level

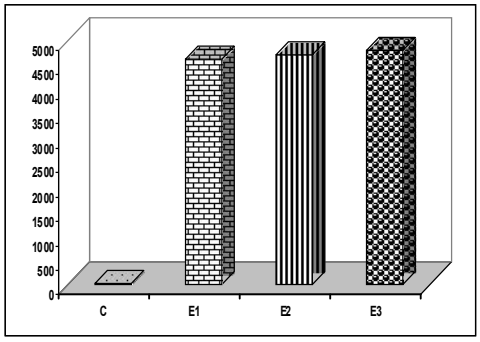

Figure 2. LH seric level

FSH seric level was in physiological limits, up to 500 ng ml^{-1} (Kei-ichiro et al 2000). In both C group as in E groups, FSH level was low, towards the inferior limit.

Exposure to chromium determined significant ($p<0.01$) increase of FSH seric level in C group comparative to E groups: E1/C: +549.47%, E2/C: +641.38%, E3/C: +841.38%, in direct correlation, significantly ($p<0.01$), with the exposure level E2/E1: +14.15%, E3/E2: +26.97%, E3/E1: +44.94%.

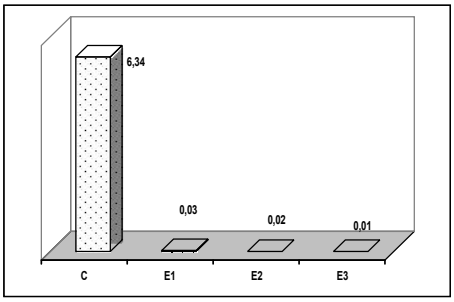

Figure 3. Estradiol seric level

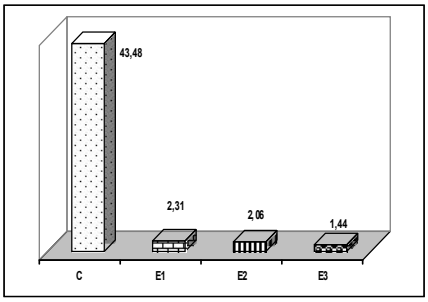

Figure 4. Progesterone seric level

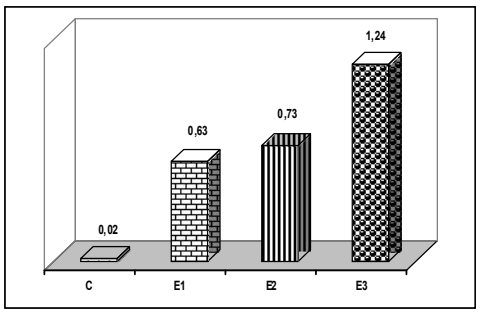

Figure 5. Testosterone seric level

LH seric level exceeded the physiological limits (35 ng ml^{-1}) (Kei-ichiro et al 2000) in all three exposed groups, only in C group, being in physiological limits.

LH seric level was significantly ($p<0.01$) increased in E groups comparative to C group, directly, significantly ($p<0.01$) correlated with the exposure level (E1/C: +14948.33%, E2/C: +15210.09%, E3/C: +15535.66%; E2/E1: +1.73%, E3/E2: +2.12%, E3/E1: +3.9%.

Estradiol level was at the inferior limit of the physiological limits (Kei-ichiro et al 2000), both in C and in E groups.

Exposure to chromium determined significant ($p<0.01$) decrease of estradiol seric level comparative to C group (E1/C: -99.52%, E2/C: -99.68%, E3/C: -99.84%).

Estradiol seric level decreased significantly ($p<0.01$) in E groups, inversely correlated with the exposure level: E2/E1: -33.33%, E3/E2: -50%, E3/E1: -66.66%.

Progesteron seric level was in physiological limits (up to 40 ng ml^{-1}) (Kei-ichiro et al 2000) in C and E groups and decreased significantly ($p<0.01$) in E groups comparative to C group: E1/C: -94.68%, E2/C: -95.26%, E3/C: -96.68%, inversely, significantly ($p<0.01$) correlated with the exposure level: E2/E1: -10.82%, E3/E2: -30.09%, E3/E1: -37.66%.

Testosterone seric level increased significantly ($p<0.01$) in E groups comparative to C group: E1C: +3050%, E2/C: +3550%, E3/C: +6100%.

Testosterone seric level was significantly ($p<0.01$) increased in E groups, directly correlated with the exposure level: E2/E1: +15.89%, E3/E2: +69.86%, E3/E1: +96.82%.

Some researches pointed out that chromium accumulates in the pituitary gland (Nudler et al 2009; Quinteros et al 2007), considerably reduces cell activity, determines apoptosis (Quinteros et al 2008; Quinteros et al 2007), all irreversible effects (Quinteros et al 2007).

Hexavalent chromium has a negative impact on the structure and function of the pituitary gland, its accumulation in the hypothalamic pituitary axis severely affecting normal endocrine function, situation that can explain the encountered hormonal status.

FSH has a very short period of discharge, mostly at the beginning of the proestrus, therefore its secretion peak being very difficult to be surprised (Kei-ichiro et al 2000).

Proestrus is also the moment with the most increased frequency of the LH pulsatile waves (Kei-ichiro et al 2000).

The cause of high LH seric level, over physiological limits, could also be the decrease of the seric progesterone, or the negative impact on estradiol synthesis, which is considered the hormone with the most powerful inhibitor effect on LH (Freeman 1994).

The low level of seric estradiol, in physiological limits (observed even in the C group) is contradictive with the results of Kei-ichiro et al (2000), Newbold & Padilla-Banks (2006), and Sakhila et al (2008), who observed high estradiol levels in proestrous and in accordance with Rodriguez et al (2007) who observed low estradiol level consecutive potassium dichromate exposure.

The decrease of the estradiol level could be the consequence of low FSH seric level, as a result of chromium exposure, that leads to the decrease of the aromatase from the granulose cells and estradiol transformation into androgen hormones (Jones & Thorburn 2001). This affirmation is sustained by the increase of the testosterone level, observed in the performed researches.

The decrease of the seric progesterone in the condition of LH seric level over physiologic level is due to the absence of the optimal response (or delayed) to the secretion of preovulatory ovarian follicles (Kei-ichiro et al 2000).

Other authors have contradictive results regarding Cr (VI) impact on progesterone level: some revealed its decrease (Rodriguez et al 2007), and others observed the opposite (Sakhila et al 2008).

No references regarding the physiological limits of seric testosterone in female rats were found in studied literature. The increase of seric testosterone level was in the present study concomitant with the increased level, over physiological limits, of seric LH. It is known that LH stimulates at maximum the theca androgen production. The theca cells have the enzymes for the cholesterol conversion into androgens (Jones & Thorburn 2001).

Sakhila et al (2008) also observed the increase of testosterone level in the case of female offspring rats derived from mothers exposed during lactation period to potassium dichromate (50, 100, 200, 400 mg l^{-1}).

Conclusions. F1 generation White Wistar female rats exposed to potassium dichromate pointed out:

- Significant increase of FSH, LH and testosterone seric level, directly correlated to exposure level.
- Significant decrease of estradiol and progesterone seric level, inversely correlated with the exposure level.

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