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Environmental Concentration of Cadmium in Rabbit Semen and Detection of the Effect on Spermatozoa Motility *In vitro*

[1]*Peter Massanyi,* [1]*Norbert Lukac,* [2]*Robert Stawarz,* [1]*Shubhadeep Roychoudhury,*
[3]*Jaroslav Slamecka,* [3]*Ivan Chlebec,* [4]*Robert Toman,* [1]*Jaroslav Kovacik and* [1]*Jozef Bulla*

[1]Department of Animal Physiology, Faculty of Biotechnology and Food Sciences,
Slovak University of Agriculture, Trieda Andreja Hlinku 2, SK 94976, Nitra, Slovak Republic
[2]Institute of Biology, Faculty of Geography and Biology, Pedagogical University,
Podbrzezie 3, PL 30084, Krakow, Poland
[3]Department for Small Farms, Research Institute of Animal Production,
Slovak Agriculture Research Centre, Trieda Andreja Hlinku 2, SK 94976, Nitra, Slovak Republic
[4]Department of Veterinary Disciplines, Faculty of Agrobiology and Food Resources,
Slovak University of Agriculture, Trieda Andreja Hlinku 2, SK 94976, Nitra, Slovak Republic

Abstract: Cadmium is an environmental risk factor having various toxic effects both in animals and in human. Target of this study was to determine concentration of cadmium in rabbit semen and subsequently to detect *in vitro* effects on spermatozoa motility parameters. Mean cadmium semen concentration in rabbits (n=27) estimated by AAS method was 0.09559 mg/kg. In *in vitro* experiment significant ($p<0.05$) dose-dependent decrease of percentage of motile spermatozoa (> 5 μm/s) estimated by CASA method was detected after 60 and 120 minutes of culture. In groups with the lowest cadmium concentration this decrease was not significantly affected immediately after cadmium addition. Similar tendency of cadmium induced dose-dependent decrease was detected for progressive spermatozoa motility (> 20 μm/s). Comprehensive motility analysis of distance parameters (curve line; average path; straight line) detected significant alteration for all experimental groups after 60 a 120 minutes of culture. At time 0, only the tendency of decrease was detected. Evaluation of curve line distance showed similar results as for average path distance and also significant decrease in the group with maximum cadmium concentration at time 0 of culture was found. For straight line distance the same tendency was recorded. Non-significant ($p>0.05$) differences were detected for straightness, linearity of spermatozoa motility, wobble and amplitude of spermatozoa head displacement. Except for amplitude of head displacement a tendency of increased straightness, linearity and wobble of suffered motile spermatozoa was found. The beat cross frequency was significantly decreased in all experimental groups 60 and 120 minute of co-culture with cadmium. Results of this study clearly confirm negative effects of cadmium on spermatozoa motility and subsequently possible decreased reproductive alterations in environmentally polluted areas.

Key words: Cadmium · Concentration · Semen · Spermatozoa · Toxicology

INTRODUCTION

Cadmium is chemically similar to zinc and occurs naturally with zinc and lead in sulfide ores. Some cadmium has been found in all natural materials that have been analyzed. High concentrations in air, water and soil are, however commonly associated with industrial emission sources, particularly non-ferrous mining and metal refining [1].

In the past, chronic effects due to long-term inhalation of cadmium-containing dust were frequently observed. The type and intensity of symptoms depend on individual disposition as well as on intensity and duration of exposure. Long-term ingestion of large amounts of cadmium has, until now, only been observed in Japan. This has led to kidney dysfunction, as in industrial exposure and to severe bone disease known as Itai-itai disease [2]. Predominant storage in soft tissue

Corresponding Author: Peter Massanyi, Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Trieda Andreja Hlinku 2, SK 94976, Nitra, Slovak Republic

(primary liver and kidney) rather than bone has been reported [3-10].

Cadmium has a diversity of toxic effects including nephrotoxicity, carcinogenity, teratogenicity and endocrine and immune toxicities. Although cadmium is not essential for growth and development in mammals, it generally follows the metabolic pathways of essential elements zinc and copper [11-15].

Cadmium also affects reproductive organs [16,17]. Its action may be either direct, affecting the gonads and accessory organs, or indirect via interference with the hypothalamus-pituitary-gonadal axis [18]. Cadmium chloride administered s.c. induced profound cellular and vascular changes in the ovary of prepubertal rats. The large and medium-size follicles underwent immediate mass atresia and the smaller ones had the same fate after a brief period of resistence [19].

As cadmium is a serious environmental contaminant affecting reproductive ability of animals as well as humans the target of this study was to detect cadmium concentration in rabbit semen and subsequently to determine fine alterations of spermatozoa motility parameters in relation to various cadmium concentrations and compare them with reported concentrations and also to estimate concentrations that might alter the fertilizing ability of spermatozoa.

MATERIALS AND METHODS

Rabbit semen was obtained on a regular collection schedule and sample from 5 adult breeding rabbits (SCPV, Nitra, Slovak Republic) was used.

For cadmium concentration detection semen samples (at least 1 mL) were stored at-20°C and subsequently mineralized in the laboratory. All material of each sample was placed in separate mineralization tubes and mineralized by adding of 2 mL HNO_3-$HClO_4$ (4:1) mixture and heating it at 120°C for 65 minutes in a thermostat-controlled digestion block. The resulting solution was diluted to 10 mL with demineralizated water. Cadmium content was determined by the voltametric method (ASV) by EA9C potentiostat model equipped with working CGMDE electrode and $AgCl_2$ and platinum electrodes. Concentrations are expressed in mg/kg.

For *in vitro* study semen was collected and subsequently diluted (Minitüb, Germany) according to routine methods. After processing the samples were stored in the laboratory at room temperature (20°C). Analysis was carried out using a CASA system-SpermVision (Minitüb, Tiefenbach, Germany) with microscope Olympus BX 51 (Olympus, Japan).

Cadmium ($CdCl_2$; Sigma Chemicals, St. Louis, MO, USA) was added to semen in various concentrations starting with 0.1 g per 10 mL of medium (basic medium; 10 g/1000 mL)-group MAX. Subsequently the concentrations were diluted as described in Table 1.

Each sample was placed into Makler Counting Chamber (depth 10 μm, Sefi-Medical Instruments, Germany) and in each sample the following parameters were evaluated-percentage of motile spermatozoa (motility > 5 μm/s), percentage of progressive motile spermatozoa (motility > 20 μm/s), DCL (distance curved line; μm), DAP (distance average path, μm), DSL (distance straight line, μm), VCL (velocity curved line, μm/s), VAP (velocity average path, μm/s), VSL (velocity straight line, μm/s), LIN (linearity-VSL:VCL), STR (straightness-VSL:VAP), WOB (wobble-VAP:VCL), ALH (amplitude of lateral head displacement, μm) and SCF (beat cross frequency, H_2) [20].

Obtained data were statistically analyzed with the help of PC program Excel and SAS using t-test and Scheffe's test.

Table 1: Cadmium concentrations used in the study

Group	Semen volume	$CdCl_2$ (0.1 g/10 mL)|	Physiological solution (μL)	Dilution rate
Control	20 μL	0	100	0:1
MAX	20 μL	100	0	1:0
G	20 μL	100	100	1:1
F	20 μL	100	200	1:2
E	20 μL	100	300	1:3
D	20 μL	100	400	1:4
C	20 μL	100	500	1:5
B	20 μL	100	600	1:6
A	20 μL	100	700	1:7

Maximum used: 10000 mg/3000 L; final concentration: 3333 mg/L; Group A: 0.1 g/730 mL; 0.137 mg/L; Normal: 0.10 mg/kg [L]

RESULTS

Mean cadmium semen concentration in rabbits (n=27) estimated by AAS method was 0.09559 mg/kg.

In three time periods (Time 0, 30, 60) *in vitro* effects of co-culture of rabbit spermatozoa with cadmium were analyzed.

Evaluation of the percentage of motile spermatozoa showed significantly ($p<0.05$) decreased values in all experimental groups cultured for 60 and 120 minutes (Table 2). In two groups (F, G) with the lowest cadmium concentration the difference was not significant, suggesting the onset of the cadmium spermatozoa motility alteration from the concentration used for group E. The highest decrease of spermatozoa motility was detected after 120 minutes of culture in group with the highest cadmium concentration decreasing from 59.75% in control to 9.77%.

Identical spermatozoa motility inhibition was detected also for the percentage of progressive motile spermatozoa with significant decrease in all experimental groups after 60 and 120 minutes of culture (Table 3).

Detail distance parameter analysis detected for DAP significant decrease in all cadmium addition groups after 60 and 120 minutes of culture (Table 4). The decrease tendency at Time 0 was more than 5 μm, but the differences were not significant. Evaluation of DCL-distance curve line, showed similar results as for DAP, with except for Time 0 where also a significant decrease was detected in the group with the highest cadmium concentration (Table 5). Straight line distance has copied other distance parameters with significant values after 60 and 120 minutes of culture with cadmium (Table 6).

Measurement of spermatozoa path velocity detected significant ($p<0.05$) decrease of velocity average path (VAP), velocity curved line (VCL) as well as velocity

Table 2: Spermatozoa motility (%) in groups and time periods

Group	Control	G	F	E	D	C	B	A	MAX
Time 0									
x	60.95	46.93	47.63	41.77*	39.80*	39.05*	35.25*	31.68*	26.64*
minimum	50.00	23.80	27.36	25.64	15.78	13.51	10.34	0.00	5.94
maximum	76.19	70.21	74.36	60.27	63.78	73.03	61.58	59.70	50.00
S.D.	7.52	14.38	15.67	10.27	15.39	19.74	15.99	15.01	12.06
C.V.	12.34	30.65	32.89	24.58	38.67	50.56	45.35	47.36	45.26
Time 60									
x	58.66	18.11*	17.93*	17.22*	15.05*	14.75*	12.33*	12.29*	11.95*
minimum	33.33	4.00	3.22	4.34	6.00	0.00	6.25	0.00	0.00
maximum	81.50	40.27	35.62	26.92	34.83	25.00	28.57	24.77	28.43
S.D.	13.03	11.62	9.92	6.55	6.57	5.42	5.34	6.01	5.86
C.V.	22.21	64.15	55.34	38.00	43.64	36.78	43.31	48.87	49.06
Time 120									
x	59.75	14.99*	16.70*	15.32*	14.52*	15.79*	13.28*	13.60*	9.77*
minimum	26.31	0.00	5.71	7.93	2.43	5.35	4.95	0.00	6.08
maximum	82.06	40.00	34.02	26.98	25.00	24.35	22.22	25.25	14.95
S.D.	15.37	8.69	7.88	4.91	5.82	4.57	4.45	7.69	2.92
C.V.	25.72	57.97	47.19	32.02	40.12	28.96	33.50	56.59	29.89

$p<0.05$

Table 3: Progressive spermatozoa motility (%) in groups and time periods

Group	Control	G	F	E	D	C	B	A	MAX
Time 0									
x	36.62	24.01	26.17	20.12*	14.40*	16.93*	14.13*	12.58*	7.68*
minimum	18.86	4.76	8.88	6.12	0.00	0.00	0.00	0.00	0.00
maximum	52.00	57.85	54.77	41.09	39.63	50.00	46.89	42.53	21.73
S.D.	10.76	19.49	15.50	9.34	14.03	18.99	14.68	11.35	6.55
C.V.	29.39	81.17	59.25	46.44	97.41	112.18	103.87	90.19	85.31
Time 60									
x	43.64	5.17*	3.03*	2.42*	2.08*	1.55*	1.41*	1.01*	2.09*
minimum	26.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	66.27	18.75	12.44	11.61	7.36	6.51	5.42	5.06	6.86
S.D.	12.97	6.72	4.33	3.19	2.20	1.96	1.68	1.37	1.96
C.V.	29.72	130.09	142.82	131.70	105.85	126.64	119.50	136.23	93.99
Time 120									
x	41.26	1.34*	1.45*	2.32*	2.01*	1.80*	1.54*	1.09*	1.99*
minimum	3.84	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	71.72	6.25	6.04	15.87	10.00	9.73	6.97	7.22	6.53
S.D.	20.75	1.66	1.72	3.37	2.61	2.43	1.58	1.76	1.79
C.V.	50.29	124.21	118.85	145.44	129.80	135.13	102.64	160.67	90.02

*$p<0.05$

Table 4: Distance Average Path (DAP, in µm) in groups and time periods

Group	Control	G	F	E	D	C	B	A	MAX
Time 0									
x	15.29	13.81	13.55	13.16	9.92	15.83	11.47	11.28	9.20
minimum	6.92	7.04	8.26	8.60	0.00	0.00	0.00	0.00	0.00
maximum	24.67	22.32	19.26	18.49	27.24	44.76	28.14	34.87	14.24
S.D.	3.79	3.74	2.66	2.53	6.66	11.79	8.26	7.38	4.23
C.V.	24.80	27.06	19.65	19.22	67.11	74.46	71.99	65.47	46.02
Time 60									
x	18.11	6.24*	5.42*	5.89*	5.92*	3.75*	5.12*	4.51*	6.03*
minimum	13.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	26.03	11.36	11.04	13.39	11.17	9.58	12.74	10.44	17.01
S.D.	3.06	4.77	4.72	5.30	4.78	4.06	4.63	4.42	4.83
C.V.	16.90	76.44	87.00	89.97	80.75	108.27	90.54	97.91	80.04
Time 120									
x	19.32	4.50*	5.15*	5.93*	4.72*	4.88*	6.59*	4.07*	7.37*
minimum	11.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	32.50	10.06	16.70	14.53	10.21	11.60	14.12	13.79	16.14
S.D.	5.22	4.31	4.88	4.98	4.17	5.10	4.75	4.68	3.66
C.V.	27.01	95.72	94.84	83.90	88.38	104.34	72.09	114.91	49.65

*p<0.05

Table 5: Distance Curved Line (DCL, in µm) in groups and time periods

Group	Control	G	F	E	D	C	B	A	MAX
Time 0									
x	34.10	33.16	31.70	33.62	22.55	35.84	26.46	23.43	17.01*
minimum	17.27	9.10	14.06	18.36	0.00	0.00	0.00	0.00	0.00
maximum	44.48	46.46	50.63	44.98	50.20	104.98	66.01	45.49	34.17
S.D.	6.10	10.75	9.45	6.60	14.70	26.93	19.41	13.33	9.44
C.V.	17.90	32.42	29.82	19.63	65.19	75.13	73.37	56.86	55.49
Time 60									
X	39.68	10.26*	8.24*	9.42*	8.81*	5.45*	7.02*	5.77*	7.76*
Minimum	30.82	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	69.84	25.76	21.98	30.89	20.42	16.87	25.53	15.68	18.17
S.D.	9.13	8.98	8.17	9.64	7.63	6.11	7.14	5.77	6.49
C.V.	23.01	87.54	99.19	102.24	86.58	112.09	101.73	100.07	83.71
Time 120									
X	44.71	8.06*	8.29*	9.57*	7.17*	7.41*	10.83*	6.23*	10.77*
Minimum	18.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Maximum	61.04	26.62	29.57	38.68	31.80	25.12	37.64	21.94	46.97
S.D.	9.78	8.58	8.52	10.34	7.76	8.23	9.15	7.35	9.60
C.V.	21.88	106.48	102.82	107.98	108.19	110.99	84.49	117.89	89.08

*p<0.05

Table 6: Distance Straight Line (DSL, in µm) in Groups and Time Periods

Group	Control	G	F	E	D	C	B	A	MAX
Time 0									
x	11.51	9.84	9.42	9.13	7.16	12.01	7.82	8.91	7.33
minimum	5.03	5.48	6.00	5.69	0.00	0.00	0.00	0.00	0.00
maximum	20.93	17.00	12.71	14.27	23.57	41.76	22.62	32.59	12.19
S.D.	3.74	2.64	1.78	2.03	5.33	10.50	5.67	6.47	3.28
C.V.	32.47	26.88	18.95	22.23	74.40	87.42	72.50	72.63	44.69
Time 60									
x	13.86	5.42*	4.60*	4.93*	4.80*	3.39*	4.40*	4.05*	5.44*
minimum	9.60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	20.82	10.96	9.35	10.57	10.25	8.14	9.86	9.60	16.97
S.D.	2.83	4.24	4.00	4.33	3.97	3.66	3.94	3.97	4.52
C.V.	20.41	78.07	86.93	87.79	82.55	108.06	89.62	97.96	83.05
Time 120									
x	14.62	3.62*	4.05*	4.91*	4.07*	4.28*	5.65*	3.74*	6.43*
minimum	7.79	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	28.47	9.58	10.40	12.59	8.88	11.01	11.31	12.89	9.19
S.D.	4.62	3.57	3.67	4.10	3.63	4.51	3.96	4.30	2.79
C.V.	31.62	98.58	90.53	83.55	89.11	105.50	70.12	115.21	43.33

*p<0.05

straight line (VSL) in all experimental cadmium group after 60 and 120 minutes of culture (Table 7-9). On the other hand, we have to state that any of the velocity parameters were not significantly altered immediately at the beginning of culture (Time 0), even an evident decrease tendency of path velocity was detected.

Non-significant differences were found for straightness of spermatozoa motility (STR), linearity of curved path motility (LIN), wobble (WOB) as well as for amplitude of lateral head displacement (ALH) (Table 10-13). Except amplitude of lateral head displacement tendency of increased straightness of

Table 7: Velocity Average Path (VAP, in µm/s) in groups and time periods

Group	Control	G	F	E	D	C	B	A	MAX
Time 0									
x	35.08	29.85	31.78	29.51	22.47	34.71	25.54	26.29	23.27
minimum	17.58	17.42	21.65	18.08	0.00	0.00	0.00	0.00	0.00
maximum	53.36	46.18	45.70	40.50	56.47	92.60	58.53	72.14	35.38
S.D.	7.78	7.91	5.81	5.96	14.67	24.39	17.68	16.10	10.44
C.V.	22.18	26.49	18.28	20.18	65.32	70.25	69.24	61.23	44.89
Time 60									
x	40.18	15.16*	13.71*	14.88*	15.25*	9.74*	13.61*	12.53*	16.84*
minimum	31.89	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	53.87	28.23	28.31	31.32	30.65	26.15	32.14	35.86	48.49
S.D.	5.75	11.52	12.03	13.05	12.45	10.60	12.28	12.87	13.64
C.V.	14.32	76.01	87.74	87.73	81.61	108.78	90.24	102.73	81.03
Time 120									
x	42.42	11.19*	13.49*	15.94*	12.35*	13.16*	17.70*	9.93*	18.47*
minimum	22.87	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	69.40	26.92	44.65	36.54	25.70	36.71	44.59	36.83	37.26
S.D.	12.64	10.89	13.41	13.45	10.83	14.08	12.91	11.62	8.83
C.V.	29.81	97.28	99.37	84.38	87.69	106.96	72.94	116.99	47.79

*p<0.05

Table 8: Velocity Curved Line (VCL, in µm/s) in groups and time periods

Group	Control	G	F	E	D	C	B	A	MAX
Time 0									
x	77.29	71.26	72.74	74.10	50.10	77.53	58.14	54.21	42.31
minimum	41.85	20.22	35.49	40.35	0.00	0.00	0.00	0.00	0.00
maximum	98.75	101.87	117.59	102.46	104.19	217.20	139.77	94.13	81.08
S.D.	12.85	23.11	20.20	14.49	32.06	55.65	41.32	30.44	22.43
C.V.	16.63	32.43	27.77	19.56	63.99	71.79	71.07	56.14	53.00
Time 60									
x	87.27	24.05*	20.22*	22.59*	21.87*	13.73*	18.41*	15.96*	21.14*
minimum	66.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	144.50	56.74	51.98	63.92	48.07	40.45	60.55	48.47	51.71
S.D.	17.78	20.32	19.81	21.33	18.44	15.28	18.40	16.52	17.27
C.V.	20.37	84.48	97.95	94.45	84.32	111.33	99.95	103.54	81.67
Time 120									
x	97.62	19.16*	20.85*	25.54*	18.64*	19.73*	28.20*	14.92*	26.28*
minimum	37.87	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	132.78	63.90	76.17	116.75	79.57	67.18	90.35	54.70	108.40
S.D.	23.03	19.77	21.36	28.38	19.79	21.95	23.04	17.60	21.79
C.V.	23.59	103.20	102.44	111.10	106.16	111.25	81.70	117.97	82.89

*p<0.05

Table 9: Velocity Straight Line (VCL, in µm/s) in groups and time periods

Group	Control	G	F	E	D	C	B	A	MAX
Time 0									
x	26.50	21.32	22.35	20.50	16.29	26.43	17.66	20.77	18.58
minimum	12.97	13.17	13.84	11.79	0.00	0.00	0.00	0.00	0.00
maximum	45.16	35.18	31.55	31.28	48.86	86.40	48.16	67.44	30.66
S.D.	7.77	5.63	4.36	4.82	11.57	21.75	12.58	13.86	8.18
C.V.	29.33	26.39	19.49	23.51	71.02	82.28	71.25	66.72	44.02
Time 60									
x	30.85	13.26*	11.66*	12.76*	12.60*	8.86*	11.76*	11.25*	15.30*
minimum	22.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	43.08	27.71	27.57	25.97	30.00	22.53	28.54	34.92	48.34
S.D.	5.48	10.37	10.25	11.28	10.72	9.60	10.62	11.60	12.92
C.V.	17.78	78.22	87.87	88.41	85.08	108.35	90.31	103.11	84.42
Time 120									
x	32.14	9.19*	10.71*	13.19*	10.69*	11.69*	15.28*	9.16*	16.26*
minimum	16.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	60.86	26.90	32.74	26.66	21.63	34.44	31.97	34.70	25.55
S.D.	10.91	9.52	10.46	10.94	9.46	12.73	10.71	10.77	7.12
C.V.	33.95	103.52	97.63	82.96	88.43	108.88	70.11	117.58	43.78

*p<0.05

Table 10: Straigtness (STR) in groups and time periods

Group	Control	G	F	E	D	C	B	A	MAX
Time 0									
x	0.74	0.72	0.70	0.69	0.55	0.61	0.53	0.65	0.71
minimum	0.65	0.52	0.52	0.58	0.00	0.00	0.00	0.00	0.00
maximum	0.86	0.94	0.81	0.84	0.86	0.93	0.97	0.96	0.97
S.D.	0.07	0.10	0.07	0.07	0.32	0.32	0.35	0.31	0.29
C.V.	9.20	14.03	10.05	9.83	57.39	52.19	65.27	46.84	40.93
Time 60									
x	0.76	0.57	0.50	0.50	0.51	0.43	0.50	0.49	0.60
minimum	0.68	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	0.88	0.99	0.98	0.98	0.99	0.97	0.99	0.99	0.99
S.D.	0.06	0.43	0.43	0.44	0.42	0.46	0.44	0.46	0.44
C.V.	7.89	76.21	86.17	87.72	81.90	107.02	88.00	94.95	72.97
Time 120									
x	0.75	0.43	0.47	0.52	0.50	0.44	0.62	0.42	0.77
minimum	0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	0.91	0.99	0.98	0.98	0.96	0.98	0.99	0.99	1.00
S.D.	0.07	0.42	0.42	0.42	0.44	0.45	0.41	0.47	0.34
C.V.	9.87	97.20	88.65	80.13	87.50	103.22	66.24	111.59	44.32

p>0.05

Table 11: Linearity (LIN) in groups and time periods

Group	Control	G	F	E	D	C	B	A	MAX
Time 0									
x	0.33	0.32	0.32	0.28	0.25	0.29	0.25	0.33	0.43
minimum	0.23	0.20	0.20	0.17	0.00	0.00	0.00	0.00	0.00
maximum	0.47	0.93	0.60	0.40	0.49	0.72	0.76	0.88	1.00
S.D.	0.06	0.14	0.10	0.06	0.16	0.18	0.20	0.20	0.26
C.V.	18.35	43.70	29.85	20.90	62.57	63.57	78.71	61.32	60.67
Time 60									
x	0.35	0.40	0.38	0.36	0.38	0.32	0.40	0.40	0.50
minimum	0.23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	0.45	0.93	1.00	0.89	1.00	0.85	1.00	1.00	1.00
S.D.	0.05	0.35	0.38	0.36	0.36	0.35	0.38	0.40	0.39
C.V.	14.26	88.06	98.09	98.67	93.51	109.59	94.54	101.78	78.81
Time 120									
x	0.33	0.29	0.32	0.39	0.38	0.31	0.42	0.29	0.62
minimum	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	0.67	1.00	0.99	1.00	0.94	1.00	0.84	0.87	1.00
S.D.	0.10	0.33	0.32	0.36	0.37	0.35	0.30	0.33	0.33
C.V.	31.52	115.88	98.04	91.13	96.47	110.89	73.11	115.40	52.56

p>0.05

Table 12: Wobble (WOB) in groups and time periods

Group	Control	G	F	E	D	C	B	A	MAX
Time 0									
x	0.45	0.44	0.45	0.40	0.35	0.38	0.34	0.41	0.52
minimum	0.35	0.33	0.35	0.28	0.00	0.00	0.00	0.00	0.00
maximum	0.56	0.99	0.74	0.51	0.58	0.77	0.78	1.00	1.04
S.D.	0.05	0.13	0.10	0.05	0.20	0.20	0.22	0.23	0.27
C.V.	10.31	29.83	21.49	12.39	58.25	54.24	64.89	55.94	51.55
Time 60									
x	0.46	0.44	0.44	0.41	0.45	0.35	0.46	0.43	0.55
minimum	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	0.56	1.03	1.08	0.90	1.03	0.90	1.00	1.02	1.05
S.D.	0.06	0.37	0.41	0.38	0.39	0.38	0.41	0.43	0.42
C.V.	12.20	82.75	93.38	92.81	85.23	109.34	89.76	98.61	76.30
Time 120									
x	0.43	0.34	0.39	0.46	0.44	0.35	0.47	0.31	0.68
minimum	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	0.74	1.04	1.07	1.01	0.99	1.01	0.86	0.91	1.05
S.D.	0.09	0.36	0.36	0.40	0.41	0.38	0.34	0.35	0.33
C.V.	21.78	104.93	91.60	87.89	93.89	107.50	72.15	113.92	48.48

p>0.05

Table 13: Amplitude of Lateral Head Displacement (ALH, in μm) in groups and time periods

Group	Control	G	F	E	D	C	B	A	MAX
Time 0									
x	2.90	2.94	3.39	2.93	2.51	3.15	2.34	2.82	2.98
minimum	1.27	1.00	2.22	1.74	0.00	0.00	0.00	0.00	0.00
maximum	3.70	4.85	4.24	4.06	4.94	8.20	3.99	6.08	4.67
S.D.	0.58	0.88	0.48	0.53	1.65	1.97	1.46	1.55	1.29
C.V.	20.15	30.08	14.12	18.14	65.69	62.29	62.61	54.98	43.38
Time 60									
x	3.02	2.22	1.87	1.84	1.87	1.36	1.83	1.69	2.08
minimum	1.72	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	4.41	4.94	3.90	4.57	3.79	3.36	5.05	4.20	6.48
S.D.	0.75	1.79	1.63	1.67	1.54	1.47	1.72	1.70	1.73
C.V.	24.91	80.47	86.80	90.55	82.18	107.88	93.84	100.55	83.51
Time 120									
x	3.25	1.52	1.74	1.78	1.66	1.69	2.44	1.57	2.75
minimum	1.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	4.77	4.24	5.72	4.07	4.39	4.96	6.51	5.14	4.03
S.D.	0.97	1.53	1.69	1.47	1.49	1.81	1.93	1.86	1.20
C.V.	29.95	100.57	97.56	82.78	89.67	107.32	78.85	118.59	43.73

$p>0.05$

Table 14: Beat Cross Frequency (BCF) in groups and time periods

Group	Control	G	F	E	D	C	B	A	MAX
Time 0									
x	24.90	23.21	20.53	23.75	15.53	20.81	17.30	18.35	14.44
minimum	17.12	10.56	5.36	16.11	0.00	0.00	0.00	0.00	0.00
maximum	33.85	29.94	32.30	31.81	30.46	42.45	36.68	38.62	40.00
S.D.	4.74	5.32	5.46	4.03	10.84	13.35	12.44	11.35	11.12
C.V.	19.04	22.92	26.58	16.97	69.81	64.14	71.90	61.83	77.01
Time 60									
x	29.64	9.04*	5.02*	6.59*	6.26*	4.99*	4.40*	3.42*	5.18*
minimum	24.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	39.18	23.56	19.99	20.55	22.37	25.00	27.69	26.04	28.57
S.D.	4.61	9.29	7.70	7.96	8.15	7.43	7.57	6.56	6.68
C.V.	15.56	102.75	153.33	120.76	130.11	148.98	172.03	191.65	128.97
Time 120									
X	29.41	7.10*	5.73*	8.44*	3.76*	10.75*	11.24*	8.75*	10.89*
minimum	19.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
maximum	44.40	30.00	29.99	26.66	17.34	56.33	51.04	42.35	45.00
S.D.	5.98	9.02	7.72	10.23	5.43	15.49	13.53	12.95	13.81
C.V.	20.33	127.07	134.77	121.19	144.57	144.12	120.31	147.91	126.81

*$p<0.05$

motility, linearity of curved path motility and wobble was detected in those spermatozoa which stayed motile and alive.

Beat cross frequency (BFC) was significantly ($p<0.05$) decreased in all experimental groups at Time 60 and 120 of cadmium co-culture (Table 14). Immediately on the on set of culture (Time 0) the decrease of beat cross frequency was not detected ($p>0.05$).

Results clearly demonstrate that the effect of cadmium on spermatozoa motility in rabbits is very significant mainly in relation to the time (Time 60 and 120) and that in practice the semen control has to be realized according to cadmium concentration in semen as well as to the spermatozoa ability to move in relation to time.

DISCUSSION

Mean cadmium semen concentration in rabbits estimated by AAS method was 0.09559 mg/kg. Cadmium accumulates mainly in the kidney and liver [12] but it has various effects on male as well as female reproductive organs [21, 22]. In a human study significant correlations were observed between blood cadmium levels and volume of semen, midpiece defects and immature forms of

spermatozoa [23] The excessive cadmium intake can decrease testes weight, testosterone and spermatozoa production and cause histological alterations in mammals [24, 25]. In comparison with other animals the level of cadmium in all studied animals does not show any significant differences [26-28].

Also decreased testosterone production has been observed in cultured Leydig cells exposed to cadmium [29, 30]. Cadmium (present) in cigarettes could be a possible causative agent for the low sperm density among smokers, especially in heavy smokers [31]. Heavy smoking was associated with low spermatozoa count, motility and morphology and increased seminal cadmium levels [32, 33]. Cigarette smoking enhances the levels of Cd and Pb in seminal plasma and blood and the extent of oxidative damage associated with a decrease in components of the antioxidant defenses in the spermatozoa of infertile males [34]. Smoking affects semen quality and oxidative lipid and protein damage in human spermatozoa.

There are also very significant correlation between cadmium and other trace elements affecting semen quality. In the study of 107 fertile and 103 subfertile male blood and semen specimens the concentrations of calcium, magnesium, zinc and copper in blood and seminal plasma were not different between the subfertile and fertile group. Weak correlations were demonstrated between blood plasma zinc concentrations and spermatozoa count, spermatozoa motility and abnormal spermatozoa morphology. Zinc concentrations in seminal plasma correlated weakly with spermatozoa count and copper concentrations in blood plasma with motility [35]. In relation to zinc the main alteration cause the status of hypozincaemia. The zinc deficiency cause degenerative changes in spermatogenic cells after meiosis, their depletion and cumulation in the lumen of seminiferous tubuli. The increased occurrence of malformed spermatids indicates to an impaired course of spermatogenesis. It has been stated that zinc is an indispensable element for a normal course of spermatogenesis [36, 37]. As it is well known that zinc can prevent toxic effect of many toxic elements we can suggest that the high zinc concentration in boar semen protect mainly their spermatozoa. The toxic effects of copper on seminal plasma are manifested in the decrease in the percentage of motile spermatozoa and in the decrease of malformed sperm cells [38]. In the study of male reproductive toxicity of inorganic lead at current European exposure levels have been found an adverse effect of lead on sperm concentration and susceptibility to acid induced denaturation of sperm chromatin [39]. The data of the time-course study indicate that the effect of nickel on testosterone production is both time and concentration dependent and not due to cytotoxicity [40, 41].

In relation to cadmium previous studies report that a low cadmium dose (40 µg/ml natrium citrate) does not significantly decreases spermatozoa motility over a lenght of time, progressively decreases motility and decreases the percentage of spermatozoa with the highest motility [42]. When comparing the four different cadmium doses (0.02; 0.1; 0.2 and 2.0 mg $CdCl_2$/ml), it was found that the progressive motility, path velocity and straightness were most affected in the group with the highest cadmium concentration [43].

Compared to classic spermatozoa analysis using visual criteria of motility, a new system of computer assisted spermatozoa analysis (CASA) enables more objective and exact evaluation of spermatozoa quality including determination types of motility. Despite this fact the correlation between multiple characteristics of semen quality measured by CASA and actual fertility in rabbits is 0.53 [44]. This correlation likely will be increased with refinement in instrumentation.

Spermatozoa motility is one of major important factors of ejaculate characteristics. Evaluation of motility based on visual feelings of operator is rather subjective and needs some improvements. CASA is a high specific measuring system which allows defining different forms of spermatozoa motility, which is not possible to determine using classic method (for example using Burker Turk slide). Motility parameters, determined by this system, in combination with spermatozoa morphology analyses can provide additional information about the fertilizing capacity of spermatozoa [45]. Analysis of the motile spermatozoa revealed several types of trajectories (irregular, small circular, large circular and arcs, jagged and straight-line). Accuracy of classification varied from 70% to 96%, depending on the type of track [46].

CONCLUSIONS

This study demonstrates that cadmium is a serious toxic element that occurs in animal tissues and fluids in variable concentration. Analysis of cadmium concentration in rabbit semen detected in this study might solve as a control value. On the other hand our study detected serious alterations in spermatozoa motility parameters even in low concentration related to time of

in vitro culture that suggest that even a weak enhance of this toxic element might cause reproductive disorders in animals and subsequently probably also in human.

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