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EFFECT OF MERCURIC ACETATE ON SOME SEMEN CHARACTERISTICS IN NEW ZEALAND MALE RABBITS

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SUMMARY

This study deals with the effect of increasing doses of mercuric acetate on semen quantity and quality in male rabbits. The results indicate that there was a significant decrease in semen ejaculate volume, sperm concentration, initial fructose concentration, and osmolality in rabbits treated with mercuric acetate compared with control animals. Meanwhile percentage of dead and abnormal spermatozoa and methylene-blue reduction time (MBRT) were significantly increased in the semen of treated animals.

These deleterious effects of mercury on semen quantity and quality were dose-dependent.

Keywords:Rabbits, mercury intoxication, semen characteristics

INTRODUCTION

Exposure of different organisms to mercury (Hg) is of a great concern because it is highly toxic, persistent and can undergo food chain implication. Because of its neurological disturbances, the majority of mercury researches has been directed towards the CNS (Somjen *et al.*, 1974). However, reproductive performance of several animal groups seems to be greatly impaired by mercury pollution. Increased concentration of mercury in blood was associated with several changes in the reproductive functions of males and females rats and mice (Khera, 1973) and monkeys (Mohamed *et al.*, 1987), including decreased fertility and increased embryonic mortality.

Alexandria represents an important center for Egyptian industry, where it contains about 35-40% of the total industrial units in Egypt (Governorate of Alexandria, 1984). Some of these industries (e.g. fertilizers, papers, chemicals, batteries and electrical machines) use mercury during processing of their products. Therefore, it is expected that a marked amount of mercury could be transferred to the atmosphere and/or deposits on soils, water and plants growing near these units (Shalaby, 1991).

The present study was designed to evaluate the effect of chronic sublethal levels of mercuric acetate on the reproductive functions of male rabbits as measured by semen characteristics.

MATERIALS AND METHODS

The animals used in this study were 12 mature male New Zealand White rabbits at six months of age and three kg mean body weight. They were individually housed in cages where feed and water were provided *ad libitum*. The feed consisted of 48% berseem hay (*Trifolium alexandrinum*), 18% wheat bran, 16% ground corn, 14% soybean meal, 3% molasses and 1% mineral mixture, limestone and vitamins. Chemical analysis indicated that it contained 17% crude protein, 2.75% fat and 12.3% crude fiber. The animals were divided into three equal groups of four rabbits each. Group 1 was used as a general control (representing 100% in all Figures) during the 18 weeks treatment period. Group 2 was left with no treatment for six weeks (preliminary period), then was exposed to low dose of mercuric acetate (20 ppm Hg/L water) for six weeks, followed by a dose of 100 ppm for six weeks. Water intake was 313.7±22.8, 268.3±22.8 and 254.3±22.8 ml/day/animal for the three doses, respectively. Group 3, after the six weeks preliminary period, was treated with high doses of mercuric acetate (100 ppm Hg/L water) for six weeks and with 500 ppm Hg for another six weeks. Water intake was 283.5±22.8, 170.2±22.8 and 62.7±22.8 ml/day/animal for the three doses, respectively. The mercuric acetate was given in drinking water *ad libitum* during the treatment periods.

Semen was collected using artificial vagina once weekly from all animals throughout the 18 weeks experimental period. The ejaculate volume was recorded from the graduated collection tube after the removal of the gel mass. Evaluation of sperm concentration using hemocytometer slide was counted after Smith and Mayer (1955). Assessment of live, dead and abnormal spermatozoa were performed using an eosin-aniline blue staining mixture as described by Shaffer and Almquist (1948). Methylene blue reduction time (MBRT) was measured using methylene blue semen mixture in a capillary tube according to Brochart (1948). Evaluation of seminal initial fructose was determined directly after collection according to Mann (1948). Semen osmolality was determined by measuring the freezing point depression using an osmometer (Osmette A 5002).

Data were analyzed using analysis of variance (Steel and Torrie, 1980) with two factors, namely treatment dose and period. Weeks were nested within periods to avoid confounding.

RESULTS AND DISCUSSION

The marked effect of mercury on reproductive performance of male rabbits was revealed from the decreased libido in treated animals. Additionally, ejaculate volume decreased significantly ($P<0.01$) in the mercury treated animals (Table 1). The magnitude of these changes was dose-dependent. The overall mean ejaculate volume of the control group was 0.76 ml, and animals given 20 and 100 ppm Hg in the 1*st* treatment showed 32.1 and 21.7% decreases than the preliminary values. When the doses were increased to 100 and 500 ppm Hg in the 2*nd* treatment the decreases became 42.7 and 40.6%, respectively (Figure 1 and Table 2).

Table 1. Analysis of variance for the effect of mercury treatment on semen ejaculate volume, sperm concentration, dead sperm, abnormal sperm, methylene-blue reduction time (MBRT), initial fructose and osmolality.

		M.S						
S.O.V.	d.f.	ejaculate volume	sperm concentration	dead sperm	abnormal sperm	MBRT	initial fructose	osmolaity
Treatmen	4	0.41**	41661.73**	149.58**	922.9**	29.62**	114871.71**	16300.1**
Period	2	2.62**	945838.8**	5.536	107.86**	0.07	38058.38**	13701.5**
Week/period	6	0.28**	6647.4**	11.746**	181.64**	51.14**	57172.35**	35.7
Error	194	0.037	2224.61	2.433	19.546	1.5	2902.01	67.9

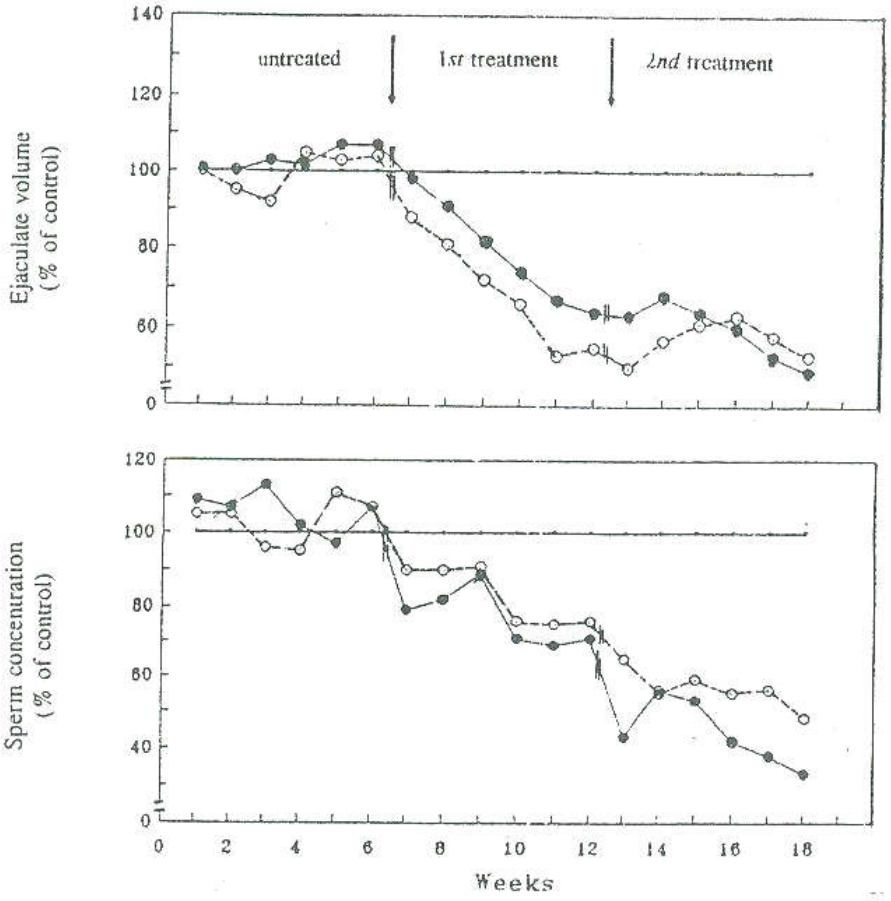

Fig. 1. Changes in semen ejaculate volume and sperm concentration in male rabbits treated with mercuric acetate (o-----o low dose; •_____• high dose).

Table 2. Overall means for the effect of mercuric acetate treatment on semen characteristics

Parameters	cont rol	1st treatment (Hg)			2nd treatment (Hg)		
		0.0 ppm	20 ppm	100 ppm	0.0 ppm	100 ppm	500 ppm
Ejaculative volume (ml)	0.76 ±0.02a	1.06 ± 0.05b	0.72 ±0.05a	0.83 ±0.05a	0.96 ±0.05b	0.55 ±0.05c	0.57 ±0.05c
Sperm concentration (x10^6/ ml)	318.9 ±4.9a	323.2 ±12.3a	279.0 ±12.3b	258.6 ±12.3bd	556.3 ±12.3c	307.4 ±12.3a	244.7 ±12.3d
Dead sperm (%)	2.41 ±0.16a	2.9 ± 0.4a	5.88 ±0.4b	4.88 ±0.4c	4.58 ±0.4a	4.58 ±0.4d	6.9 ±0.4e
Abnormal sperm (%)	11.3 ±0.46a	10.2 ±1.14ac	17.2 ±2.14b	16.7 ±1.14b	9.2 ±1.14c	21.4 ±1.14d	20.6 ±1.14d
Methylene-blue reduction time (min)	5.01 ±0.45a	4.27 ±1.3b	6.15 ±1.3c	6.63 ±1.3cd	3.98 ±1.3b	6.47 ±1.3cd	7.08 ±1.5d
Initial fructose (mg/100 ml)	377.5 ±5.60a	343.5 ±13.9b	308.6 ±13.9c	288.6 ±13.9c	350.0 ±13.9b	294.7 ±13.9c	256.8 ±13.9d
Semen osmolality (mOsml/l)	268.8 ±0.86a	272.8 ±2.12b	246.5 ±2.12c	242.5 ±2.12c	29.7 ±2.12d	228.0 ±2.12e	225.8 ±2.12e

a, b, c, d, e Means within rows, with different superscipt letters differ significantly (P<0.05).

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a, b, c, d, e Means within rows, with different superscipt letters differ significantly (P<0.05).

The overall mean of sperm concentration in the semen of control group was 318.9 x 10^6/ml. Treatment of animals with mercury significantly ($P<0.01$) reduced sperm concentration. The decreases were dose-dependent, and were about 13.7 and 20.0% for animals treated with 20 and 100 ppm Hg in the 1*st* treatment, and were 44.7 and 55.0% for those treated with 100 and 500 ppm Hg in the 2*nd* treatment relative to preliminary values. Values of sperm concentration of the non-treated group were significantly increased ($P < 0.05$) with the advance of age.

The present results concerning the changes in sperm concentration by mercury intoxication agree with those of Popescu (1978) in humans, Mohamed *et al.* (1987) in monkeys, and Chowdhury *et al.* (1989) and Rao (1989a) in rats. Such effect could be due to the cytotoxicity of mercury, since at cellular level mercury is capable of; inhibiting RNA synthesis (Chang *et al.*, 1972), breaking DNA strand (Cantoni and Costa, 1983) as well as altering protein synthesis (Syversen, 1977), mitosis (Miura *et al.*, 1978) and membrane function (Nakada *et al.*, 1978). Also, mercury induces marked disturbances in spermatogenesis (Sakai, 1972).

It is worth noting that the decreases in ejaculate volume and sperm count by Hg treatment were accompanied with parallel increases in abnormal and dead sperm percentages (Figure 2 and Table 2). Analysis of variance (Table 1) showed significant increases ($P < 0.01$) in dead and abnormal sperms in all-mercury-treated animals. The overall means of abnormal and dead spermatozoa in the semen of control rabbits were 2.4% and 11.3%, respectively. Animals treated with 20 and 100 ppm Hg in the 1*st* treatment showed increased dead sperm than control by about 102.8 and 68.3%, and increased abnormal sperm by 68.6 and 63.7%, respectively. When mercury doses were increased to 100 and 500 ppm Hg in the 2*nd* treatment, the increases in dead sperm were 77.5 and 167.4% over the preliminary period, while the increases in abnormal sperm were 132.6 and 123.9%, respectively (Table 2). In the non-treated group percentages of dead sperm were not significantly affected, while those of abnormal sperm declined ($P<0.05$) with the advance of age from 15.4% to 11.8% (Table 3). The increased percentages of dead and abnormal sperms in animals exposed to mercury are in agreement with results obtained by Popescu (1978) in humans, Mohamed *et al.* (1987) in monkeys and Chowdhury *et al.* (1989) in rats.

The hazardous effect of mercury on sperm quality was confirmed by the significant increases ($P<0.01$) in MBRT in the semen of animals treated with mercury. It is worth noting that the response was dose-dependent. The overall mean of MBRT in the semen of control male rabbits was 5.0 minutes. Animals treated with 20 and 100 ppm Hg in the 1*st* treatment showed increased MBRT by 44.0 and 55.3% over the preliminary period; when the doses were increased to 100 and 500 ppm Hg in the 2*nd* treatment the increases became 62.6 and 77.9% relative to the preliminary values (Fig. 3 and Table 2). MBRT of the non-treated group declined significantly ($P<0.05$) with the advance in age from 6.2 minutes at the beginning of experiment to 3.0 minutes at the end of 15 weeks experimental period. The observed changes in MBRT of semen collected from mercury-treated rabbits agree with those of Rao (1989a) in rats and Mohamed *et al.* (1986 a&b) in monkeys. Since the MBRT is a good indicator for viability of spermatozoa and consequently the quality of semen, the increases of this time is a good indicator of a decline in sperm concentration and/or a decrease of sperm viability. Such effect will induce failure of reproductive capability of mercury-intoxicated animals.

Table 3. Means of ejaculate volume (EV) ml, sperm concentration (SC) x 10^6/ml, dead sperm (SD)%, abnormal sperm (AS)%, methylene-blue reduction time (MBRT) min, initial fructose (IF) mg/100 ml and semen osmolality (SO) mOsm/l of male rabbits treated with mercuric acetate

Parameters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Group (1)																		
EV	0.3	0.4	0.65	0.65	0.73	0.75	0.80	0.8	1.3	1.4	1.0	1.05	1.05	0.93	0.95	0.95	0.95	0.95
SC	213.5	221.5	230.0	213.3	250.0	228.7	303.3	285.3	285.0	395.0	393.3	277.5	586.6	555.8	517.5	530.0	275.0	572.8
DS	2.5	2.0	2.6	1.80	2.0	2.3	5.10	3.2	2.5	1.5	1.8	3.5	3.0	3.0	1.0	2.5	3.0	3.0
AS	15.4	14.8	14.6	15.8	11.3	12.0	14.30	13.9	12.0	5.5	7.8	7.2	3.5	9.5	5.5	8.5	11.3	11.8
MBRT	6.2	5.8	6.0	5.5	5.3	5.5	5.00	4.0	4.0	3.6	4.0	5.0	4.3	4.5	4.3	4.3	3.5	3.0
IF	325.6	338.9	316.1	477.8	469.3	434.8	335.1	329.6	387.7	386.0	410.1	212.3	272.5	463.1	392.5	328.6	295.6	340.0
SO	265.0	263.0	266.5	258.8	256.5	256.3	257.8	269.3	270.8	272.3	282.3	284.3	289.3	292.3	295.8	294.5	304.3	303.8
Group (2)																		
EV	0.3	0.38	0.6	0.68	0.75	0.78	0.70	0.65	0.93	0.93	0.53	0.58	0.53	0.53	0.58	0.6	0.55	0.5
SC	255.0	232.5	220.3	202.8	277.5	242.5	273.8	257.5	260.0	300.0	295.0	287.5	378.8	307.5	307.5	290.8	287.5	272.5
DS	2.5	1.9	2.8	1.8	2.0	2.1	7.80	7.3	5.7	5.0	4.0	5.5	3.0	5.5	6.0	4.0	5.0	5.0
AS	11.9	11.9	10.7	13.8	12.0	13.8	21.30	18.0	15.5	11.5	15.3	21.3	22.5	18.3	11.0	17.3	32.5	27.0
MBRT	6.3	5.9	6.0	5.9	5.0	5.8	7.00	5.3	5.5	5.6	6.3	7.2	5.4	6.9	7.0	7.5	6.0	5.0
IF	337.3	352.4	336.6	481.9	464.5	434.9	333.8	328.0	336.0	327.9	345.1	180.2	225.2	415.5	353.7	289.2	254.3	230.0
SO	258.5	256.8	259.5	253.8	254.8	261.5	251.0	251.5	247.3	244.3	244.3	240.3	235.3	234.0	228.0	225.0	224.0	221.8
Group (3)																		
EV	0.3	0.4	0.67	0.66	0.78	0.8	0.78	0.73	1.07	1.03	0.67	0.67	0.66	0.63	0.61	0.53	0.5	0.47
SC	232.0	237.0	260.0	218.0	243.3	243.3	241.7	233.3	253.3	283.3	273.3	266.6	250.0	310.0	273.3	225.0	220.0	190.0
DS	2.4	1.9	2.6	1.8	2.0	2.3	5.70	4.5	4.0	4.0	4.3	7.0	6.3	3.0	4.5	6.0	8.0	8.6
AS	11.3	10.7	11.0	10.7	9.7	11.0	17.00	17.5	15.0	11.0	16.0	23.4	22.3	20.0	10.0	15.0	27.0	29.0
MBRT	5.7	5.4	5.4	5.4	5.2	5.80	6.50	6.7	6.0	5.6	7.0	8.0	7.2	7.3	8.0	8.0	6.0	6.0
IF	346.7	345.4	343.2	459.1	458.2	408.8	331.7	260.0	352.4	341.0	285.8	187.5	196.8	408.7	315.0	223.8	234.2	160.0
SO	253.3	253.3	255.3	257.3	256.7	259.0	247.7	249.3	246.0	240.7	238.3	233.7	230.7	230.7	226.3	224.7	221.3	221.3

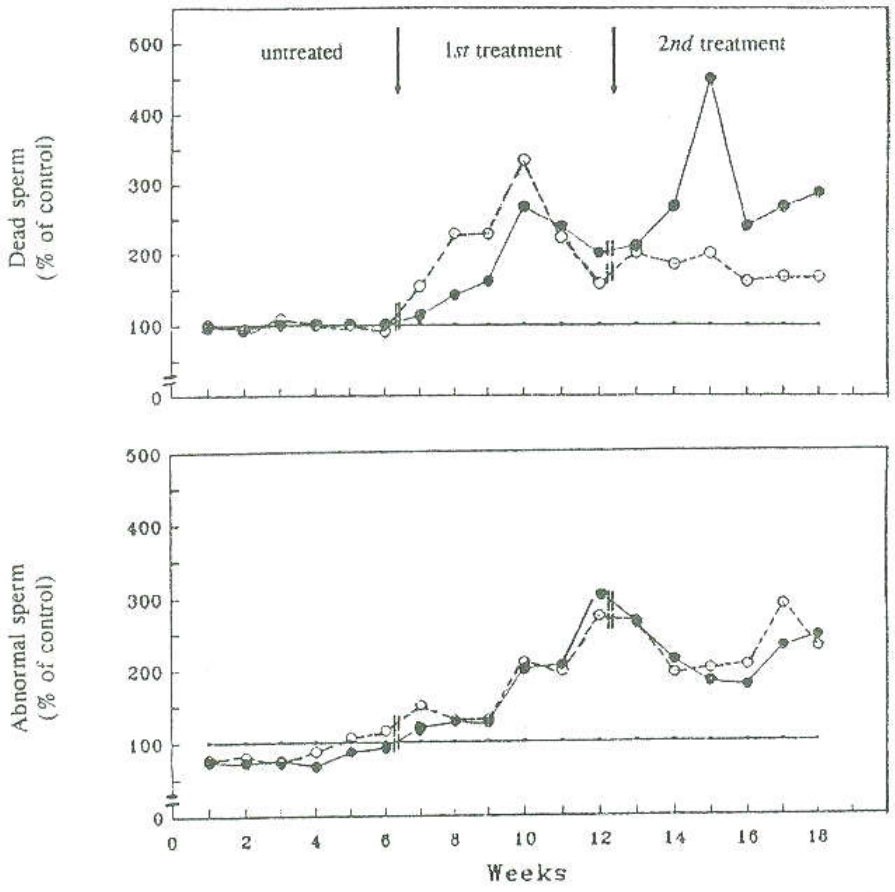

Fig. 2. Changes in percentage of dead and abnormal sperm in semen of male rabbits treated with mercuric acetate (o-----o low dose; •_____• high dose).

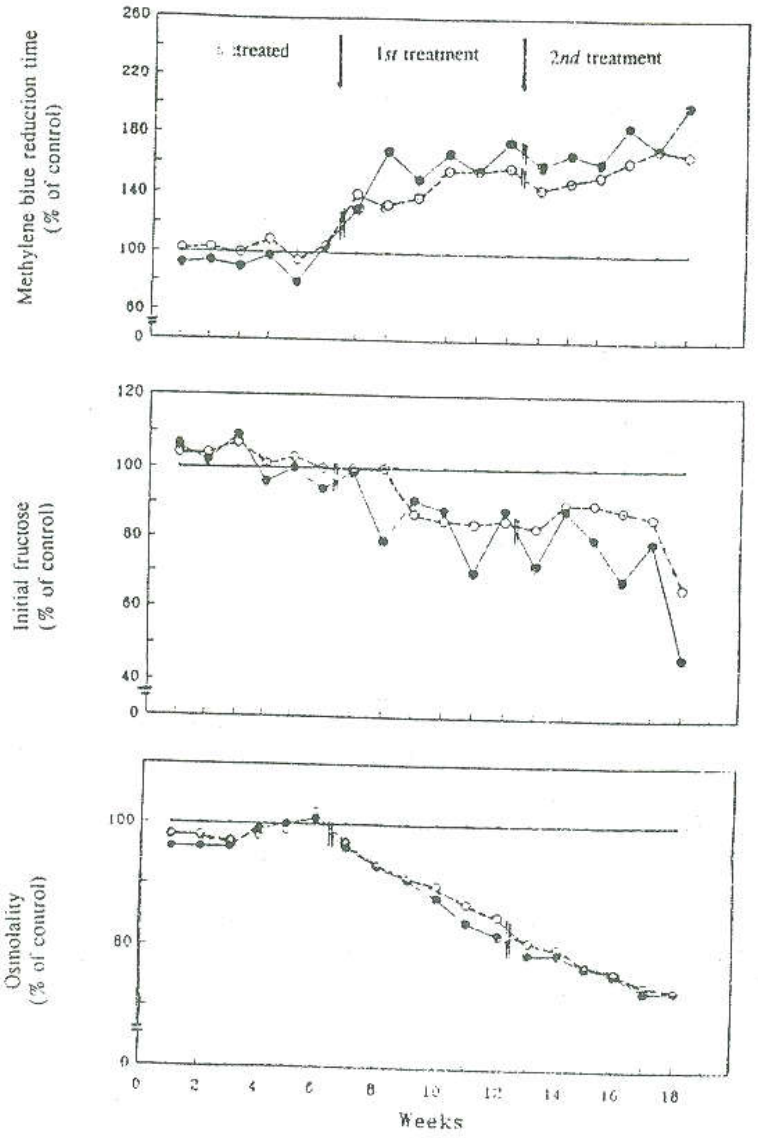

Fig. 3. Changes in semen methylene-blue reduction time, initial fructose and osmolality of male rabbits treated with mercuric acetate (o-----o low dose; •_____• high dose).

Results presented in Table 1 and Fig. 3 indicate that there were significant changes ($P<0.01$) in initial fructose concentration in semen of mercury-treated rabbits compared with control animals, and this effect was dose-dependent. In control animals the overall mean of semen initial fructose was 377.5 mg/100 ml. Animals treated with 20 and 100 ppm Hg in the 1*st* treatment showed a decline in initial fructose in semen by about 10.2 and 16.0% compared with preliminary period, respectively. Increasing the doses to 100 and 500 ppm Hg in the 2*nd* treatment were accompanied with a decrease in initial fructose that amounted to 15.8 and 26.6%, respectively. Initial fructose of the non-treated group declined ($P<0.05$) with the advance of age from 325.6 mg/100 ml at the beginning of experiment to 295.6 mg/100 ml near of the end of experiment (Table 3).

Determination of semen osmolality of control and mercury-treated rabbits (Table 1 and Fig. 3) indicated significant decreases ($P < 0.01$) in semen osmolality by Hg-treatment, and the response was dose-dependent. The overall mean value of semen osmolality in the control group was 268.8 mOsml/L. Decrease of semen osmolality was about 9.6 and 11.1% in the 20 and 100 ppm Hg-treated animals (1*st* treatment); when the dose was increased to 100 and 500 ppm Hg (2*nd* treatment), the semen osmolality decreased than the control by about 23.2 and 23.9%, respectively (Table 2). Semen osmolality of the non-treated group increased ($P < 0.05$) with the advance of age from 265.0 mOsml/L at the beginning of the experiment to 303.8 mOsml/L at the end of the 18 week experimental period (Table 3). Present results indicate that the decreases in semen osmolality in treated rabbits coincides with the decline in semen quality which involved an increase in both percentages of abnormal and dead sperm and a reduction in ejaculate volume. Since the major components controlling the osmolality of colloid solutions are proteins and electrolytes (mainly Na^+ and K^+), it is logic to assume that the reduction of semen osmolality may be due to the decrease in these components (Yousef, 1994).

The present results indicate that semen quantity and quality were markedly declined during mercury intoxication. This effect was accumulative and dose-dependent. These changes in semen characteristics could be due to a direct cytotoxic effect of mercury on testis, and/or to an indirect effect of mercury on the neuroendocrine system. The production of motile spermatozoa in semen is a very complex testicular function that requires the interplay of the hypothalamus, pituitary, testicles and several other glandular secretions. Any adverse effect of mercury on the functions of these organs would be reflected on semen quality and/or testosterone secretion. Thus, a decreased sperm concentration which was revealed in the present study is expected to be due to the known inhibitory effect of methyl mercury on cellular mitosis (Koevker, 1980), and on the induction of disturbances of spermatogenesis as reported in rats by Sakai (1972). Also, a decrease in testosterone secretion in rats treated with mercury has been reported by Burton and Meikle (1980) and Rao (1989b).

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تأثير المعاملة بخلات الزئبق على بعض خصائص السائل المنوى فى ذكور الأرانب النيوزيلاندى

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١- قسم الإنتاج الحيوانى - كلية الزراعة - جامعة الإسكندرية.
٢- قسم الدراسات البيئية - معهد الدراسات العليا والبحوث - جامعة الإسكندرية.

أهتمت هذه الدراسة بتأثير الجرعات المتزايدة من خلات الزئبق على كمية ونوعية السائل المنوى فى ذكور الأرانب النيوزيلاندى. وأظهرت النتائج أن هناك إنخفاضاً معنوياً فى حجم القذفة، تركيز الحيوانات المنوية، تركيز الفركتوز والضغط الأسموزى فى الحيوانات المعاملة بخلات الزئبق مقارنة بالكونترول.

أدت المعاملة بخلات الزئبق إلى زيادة معنوية فى النسبة المئوية للحيوانات المنوية الميتة والمشوهة والوقت اللازم لاختزال صبغة الميثيلين الزرقاء. وهذه التأثيرات الضارة للزئبق على كمية ونوعية السائل المنوى كانت تتناسب طردياً مع مقدار الجرعة المستخدمة.