REPRODUCTIVE PERFORMANCE, SEMEN QUALITY AND TESTICULAR BLOOD FLOW IN RABBITS FOLLOWING SUBCHRONIC TOXICITY OF FORMALDEHYDE WITH POSSIBLE PROTECTIVE EFFECT OF L- CARNITINE

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ABSTRACT

This study aimed to evaluate testicular function of rabbit exposing to subchronic toxicity of formaldehyde and possible protective role of L-carnitine. A total twenty Newzealand adult male rabbits divided randomly into four equal groups were used. Received at: 22/9/2012 For Three months, the first control group (CG) received saline, second group (FA) received formaldehyde in milk, third group (FA+LC) received the L-carnitine in Accepted: 18/11/12 combination with formaldehyde in milk and the fourth group (LC) received 250 mg/kg L-Carnitine orally. Animal weight, sexual desire, reaction time, semen analysis and testicular blood flow estimated each 15 days. There were no significant differences in the sexual desire, reaction time between groups. The body weight, ejaculate volume, sperm motility, alive sperm percent and sperm cell count were dramatically decreased (P<0.001), while sperm abnormality percent and pH increased in FA group. On the other hand, the motility, viability percent and sperm cell count increased with marked decrease (P < 0.001) in sperm abnormality percent in L-carnitine group. FA group showed an increase in the intratesticular blood perfusion. Formaldehyde subchronic toxicity has dramatic adverse effects on the rabbit semen and could be overcomed using L-carnitine.

Key words: Semen evaluation, Testicular blood, L-canitine, Formaldehyde, Male rabbit

INTRODUCTION

Formaldehyde produced naturally in our bodies by normal metabolism and it is found in the air, food, some skin-care products as well as preservatives in processed food, especially dried food and frozen food (Weng et al., 2009). Formaldehyde is a common environmental contaminant. Although preventive measures aimed at reducing formaldehyde levels have been implemented, exposure to formaldehyde remains one of the most prominent occupational and environmental health problems (Sari et al., 2004; Gurel et al., 2005 and Wong et al., 2006). Homes containing large amounts of formaldehvde in pressed wood products and fiber beard. Outdoor also contains large amounts of formaldehyde which may exceeds the NIOSH (The National Institute of Safety and Health) which is 16 ppb. In Egypt (Cairo) this level reached 33 ppb (Zhang et al., 2009). Formaldehyde used as bacteriostatic in cheese and milk to prevent Closridium sp. from forming gas holes during the manufacture of cheese. If the amount of formaldehyde is small, it does not harm health. However, it can cause minor to serious problems. It has great toxic effects on liver and testicles (Majumder and Kumar, 1995; Odeigah, 1997 and Tang et al., 2003).

butanoate) is a naturally occurring substance found in most cells of the body, particularly the brain, neural tissues, muscles and heart. Carnitine (CA) is the generic term for a number of compounds that include L-carnitine (LC), acetyl-L-carnitine (ALC), and propionyl-L-carnitine (PLC). It is synthesized primarily in the liver and kidneys from two amino acids, lysine and methionine (Jogl et al., 2004). Carnitine is widely available in animal and sea foods (meat, poultry, fish and diary products), whereas plants have very small amounts. L- Carnitine (LC) and acetyl-l- carnitine (ALC) play several important roles in human body, practically in energy metabolism. These nutrients shuttle acetyl groups and fatty acids into mitochondria for energy production. Without carnitine (CA), fatty acids cannot easily enter into mitochondria (Rahbar et al., 2005). Makowski et al. (2009) recorded the potential value of supplemental carnitine as a therapy for several metabolic disorders. Use of carnitine showed some promise in a controlled trial in selected cases of male infertility improving sperm quality (Lenzi et al., 2003). About 95% of the free carnitine originates from the epididymis in patients with euspermia. The clinical significance of carnitine determination

Carnitine (3- hydroxyl-4N- trimethylammonio-

demonstrated in azoospermia, varicocele, and obstructive azoospermia. L-carnitine supplementation has also shown to have beneficial effects in the treatment of varicocele which is a major cause of male infertility (Seo *et al.*, 2010). These properties of FA might bring about human health problems. Various studies have focused on the harmful effects of FA on the respiratory system and hematological system (Collins, 2004 and Ye *et al.*, 2005). To date, however, reports regarding the effects of FA on male reproduction are still scarce and insufficient. This study designed to examine the effects of subchronic toxicity of formaldehyde and possible protective role of L-carnitine in rabbit testicular function

MATERIALS and METHODS

Animals and mangement

Twenty New Zealand White strain sexually mature male rabbits (Oryctolagus cunniculus) (aged 6 - 7 months) weighing 2.15±0.12 kg (Mean± SEM) were used. Animals obtained from the Experimental Animal House of Faculty of Medicine, Assiut University, Egypt. Bucks housed individually in stainless steel cages (25 X 20 X 16 inch). The animals kept under natural climatic condition (temperature range, 15-25°C) with free access to food and tap water ad libitum. The experiment carried out in accordance with the Animal Experimentation Committee Regulation. The commercial rabbit feed was from pellets (protein 15%, lipid 2.9% and fiber 12.30%). Food consumption and animals weight were measured weekly throughout the experimental period. All rabbits were individually marked for identification and observed daily. Two female were used as teaser for semen collection from the male. All male examined and both testes for each located in the scrotum. Bucks were trained for one month (twice per week) to be familiar with artificial vagina.

Experimental design

The rabbits were divided randomly into 4 equal treatment groups (n = 5 rabbit). In the first group rabbits were received saline peros and left as control (CG). Second group (FA), animals fed on milk with formalin above the allowed level for human consumption (0.026 mg/kg) about 0.06 mg/head/day, for 3 months as a subchronic study (Material safety data sheet). The third group (FA+LC) was fed on market milk containing L-carnitine. The fourth group (CR) ingested L-Carnitine (Carnitene, Santa Farma, Turkey). The L-carnitine dose was 250 mg/kg orally (Stvolinsky and Dobrota, 2000).

Doppler examination

Testicular blood flow was recorded each 15 days using ultrasonographic examinations using a Color-Doppler ultrasound instrument (ESAOTE Pie Medical MyLab30, Vet device- Via di Caciolle, 15 – 50127 Firenze, Italy), equipped with special designed veterinary probe (LV513).

High performance liquid chromatography

Twenty random samples of market milk collected and analyzed to detect the amount of formaldehyde in these samples by high-performance liquid chromatography (HPLC). The system of HPLC (Agilent Technologies, USA), with a VU detector was used. The method for quantitative calculation of formaldehyde was reported earlier (Kaminski *et al.*, 1993 and Li *et al.*, 2007). Milk samples were prepared according to Kaminski *et al.* (1993) and calibration curve was made as previously described (Li *et al.*, 2007)

Ejaculates collection and gross sperm evaluation

The temperature of the lumen of the artificial vagina (AV) ranged from 45 to 50°C at collection (Andrade et al., 2002 and Naughton et al., 2003). Bucks were allowed to do one false mount before semen collection. The AV was properly positioned between the female hindquarters for penis intromission. Bucks have been previously adapted to this routine and no refusals occurred. In all animals, ejaculates collected once each 15 days. The experiment carried out for a period of 16 weeks from the first week of October to the last week of January 2011. Reaction time for each buck calculated as the time needs for mounting a doe until complete ejaculation. It was measured in seconds using a stopwatch. Ejaculates containing urine and calcium carbonate deposits discarded. The color, pH (using pH-paper) and the volume (V) of each ejaculate recorded after removal of the gel mass using graduated conical small collecting tube.

Microscopical evaluation of sperm quality

Sperm motility (SMOT), sperm abnormality (SAB), sperm viability (VSP) and sperm cell concentration (SPCC) evaluated. Semen was diluted (1:8 ratio) in a Tris-buffered extender (Roca et al., 2000) and incubated for 30 min in a warm water bath at 30°C. The percentage of motile spermatozoa (SMOT) were evaluated from three samples of the diluted spermatozoa placed under a cover slide in the centre of a pre-warmed (37°C) slide and transferred to a heated microscope stage set at 37°C. The evaluation was subjectively assessed using phase contrast microscopy (X200 magnification, Leica, Germany). The SMOT was recorded on a five multiple scale of 0 to 5 where 0 is absence of movement and 5 is when all motile spermatozoa are showing progressive head rectilinear linear motility. The proportions of spermatozoa with abnormal morphology (SAB) measured using Giemsa's stain and examined under a phase contrast microscope at the magnification of 1000X. Morphologic abnormalities included head, midpiece (excluding distal cytoplasmic droplets) and tail defects were evaluated after García-Tomás et al. (2006). Assessment of viable sperm percent (VSP) performed using an eosin-nigrosine blue staining mixture (Blom, 1950 and Bamba, 1988). To

determine the SAB and VSP% two hundred spermatozoa were counted from each preparation.

Sperm cell concentration (SPCC) was evaluated in an improved Neubauer haemocytometer (GmbH+Co., Brandstwiete, Hamburg, Germany) after Smith and Mayer (1955) and García-Tomás *et al.* (2006). Dilution rate was 1:400, v/v. An aliquot amount of semen was mixed with 0.3% formaldehyde in phosphate-buffered saline. Few drops of eosin stain were added before counting. The number of total spermatozoa per ejaculate calculated by multiplying semen volume by sperm concentration.

Statistical analysis

All statistical analyses carried out using SPSS statistical software version 16 (2007). All data presented in mean \pm SEM. Distribution analysis conducted using the Shapiro-Wilk test. Data of body weight throughout the experimental period, pH and semen volume analyzed using one-way ANOVA, leaset significant difference (LSD). However, data on sperm parameters among the four groups analyzed by Kruskal-Wallis test, and dual comparisons between groups evaluated using the Mann-Whitney *U*-test. Significance was set at P < 0.05.

RESULTS

The obtained results presented in tables (1-8) and figures (1, 2)

Body weight

Table (1) showed the differences in weight between all groups of animals. Toward the end of the study, the body weight in LC group was heavier (P < 0.01) than the body weight in other groups.

Reaction time

The effect of subchronic toxicity of formaldehyde on sexual behavior as determined by the reaction time and desire showed no significant differences during the experimental period. The range of the reaction times in all groups was between 24 to 42 seconds (Table 2)

Ejaculate volume and pH

There were no significant differences in ejaculate volume among the control, FA and FA + LC groups, while the volume in LC group was higher (P < 0.001) than that in other groups after fourth week to the end of the experiment (Table 3, Fig. 1).

There were no significant differences in the ejaculate pH between the CG, FA + LC and LC groups, while the pH in FA group was alkaline (P < 0.05, Table 4, Fig. 1).

Sperm motility, abnormality, viability and sperm cell count

In general, formaldehyde subchronic toxicity in FA group resulted in impaired motility, viability and sperm morphology at 45^{th} day till the end of treatment (P< 0.001). On the other hand, there were no significant changes in all previous three parameters among CG, FA+LC and LC groups (Tables 5-7).

The total Sperm cell count (SPCC) per ejaculate was remained in all four groups unchanged till the fourth week, then was significantly decreased at the 45^{th} day of treatment in FA group (P< 0.01). The SPCC was increased (P< 0.01) in the LC group at 6^{th} week of treatment to the end of the experiment (Table 8)

Doppler testicular blood flow

Intratesticular arteries and testicular capsular arteries imaged in all 20 rabbits. Blood supply in waveforms from these vessels were similar and consistently showed a low-impedance pattern with high levels of diastolic flow and good testicular blood perfussion in CG and LC group (Fig. 3a and d). The blood vessels in the FA group decrease intratesticular blood perfusion and those changes were nearly normal in FA+LC group (Fig. 2b and c).

Table 1: The body we	ights of rabbits in the control and treatment groups (Mean \pm SEM)

Days after treatment begin	Sperm abnormalities			
	CG*	FR	FR+LC	LC
	(gm)	(gm)	(gm)	(gm)
15 days	2300±10	2575±18	2400±16	2400±14
30 days	2533±22	2600±25	2500±19	2575±21
45 days	2633±18	2650±21	2650±23	2686±15
60 days	2750±11	2653±13	2800±17	2894±10
75 days	2950±19 ^a	2610 ± 16^{b}	2913±18 ^a	3000±21 ^a
90 days	3110±23 ^a	2610 ± 24^{b}	3120±21 ^a	3225±19 ^a

Within rows different superscript indicate significant differences (P<0.01)

Days after treatment begin		Sperm a	abnormalities	
	CG*	FR	FR+LC	LC
	(Sec.)	(Sec.)	(Sec.)	(Sec.)
15 days	33.7±4.3	32.7±4.0	32.7±3.8	31.9±4.1
30 days	34.6±3.3	35.6±2.9	36.5±3.0	35.7±3.2
45 days	29.4±2.4	30.2±2.6	29.7±2.5	30.1±2.1
60 days	34.4±4.3	32.6±3.5	30.4±3.4	30.1±3.8
75 days	32.8±3.8	33.4±3.9	32.0±3.0	31.6±2.7
90 days	40.0±2.2	39.7±2.4	39.8±2.5	39.6±2.1

Cable 2: Duration of the reaction time in the control and treatment groups (Mean ± SEM)

*CG: control group, FA: formaldehyde, FA+LC: formaldehyde+ L-carnitine, LC: L-carnitine only

Days after treatment begin	Sperm abnormalities			
	CG*	FR	FR+LC	LC
	(ml)	(ml)	(ml)	(ml)
15 days	0.3±0.1	0.25±0.08	0.26±0.1	0.37±0.1
30 days	0.28±0.09	0.26±0.1	0.25±0.07	0.34±0.08
45 days	0.26 ± 0.07^{a}	$0.28{\pm}0.09^{a}$	0.27 ± 0.14^{a}	$0.5 {\pm} 0.05^{b}$
60 days	0.3±0.1 ^a	$0.29{\pm}0.1^{a}$	0.26±0.06 ^a	0.5 ± 0.07^{b}
75 days	0.27 ± 0.06^{a}	0.3±0.13 ^a	$0.24{\pm}0.1^{a}$	$0.46{\pm}0.1^{b}$
90 days	$0.28{\pm}0.05^{a}$	$0.27{\pm}0.8^{a}$	0.27 ± 0.06^{a}	0.5 ± 0.09^{b}

Table 3: The volume of the ejaculate of the control and treatment groups (Mean \pm SEM)

Within rows different superscript indicate significant differences (P < 0.01)

*CG: control group, FA: formaldehyde, FA+LC: formaldehyde+ L-carnitine, LC: L-carnitine only

Days after treatment begin	Sperm abnormalities			
	CG*	FR	FR+LC	LC
15 days	6.8±0.1	7.6±0.3	7.1±0.1	7.1±0.1
30 days	7.0±0.1	8.2±0.2	6.9±0.3	7.1±0.2
45 days	6.8±0.09 ^a	8.5±0.1 ^b	7.0±0.2 ^a	7.0±0.3 ^a
60 days	7.1±0.2 ^a	8.1±0.3 ^b	7.1±0.3 ^a	6.9±0.2 ^a
75 days	7.2±0.1 ^a	8.3±0.2 ^b	7.2±0.3 ^a	7.1±0.2 ^a
90 days	7.0±0.3 ^a	8.2±0.3 ^b	7.0±0.4 ^a	6.9±0.1 ^a

Table 4: pH values in the rabbit ejaculate of the control and treatment groups (Mean \pm SEM)

Within rows different superscript indicate significant differences (P<0.05)

Days after treatment begin	Sperm abnormalities			
	CG*	FR	FR+LC	LC
	(%)	(%)	(%)	(%)
15 days	16.7±2.7	16.5±3.3	15.1±2.6	16.6±2.3
30 days	17.3±3.2	22.1±3.1	21.3±3.9	20.2±3.4
45 days	18.4 ± 3.5^{a}	30.0 ± 3.5^{b}	$20.2{\pm}2.6^{a}$	23.3 ± 3.7^{a}
60 days	16.2 ± 3.4^{a}	$29.5{\pm}2.6^{b}$	$17.4{\pm}2.4^{a}$	19.7 ± 3.8^{a}
75 days	18.3±3.6 ^a	31.6 ± 2.4^{b}	19.5±3.1 ^a	18.1 ± 3.0^{a}
90 days	20.2±3.1 ^a	33.4 ± 3.3^{b}	$16.4{\pm}2.3^{a}$	15.6 ± 2.6^{a}

 Table 5: Percentages of sperm abnormalities in rabbit ejaculates of the control and treatment groups (Mean ± SEM)

Within rows different superscript indicate significant differences (P<0.01)

*CG: control group, FA: formaldehyde, FA+LC: formaldehyde+ L-carnitine, LC: L-carnitine only

Days after	Sperm motility			
treatment begin	CG*	FR	FR+LC	LC
	(%)	(%)	(%)	(%)
15 days	80.5±6.4	$75.7{\pm}~3.9$	75.6±4.4	75.5±3.9
30 days	80.0 ± 7.5	70.0 ± 5.3	70.0±5.6	80.4 ± 4.9
45 days	80.3 ± 5.4^{a}	$40.5{\pm}~5.5^{\text{ b}}$	75.5±6.0 ^a	80.5 ± 5.0^{a}
60 days	80.4 ± 6.4^{a}	$40.8{\pm}4.6^{b}$	85.0±5.2 ^a	90.0±6.9 ^a
75 days	$80.1{\pm}~5.5~^{\rm a}$	30.9 ± 4.3 ^b	75.6±5.0 ^a	85.8±5.4 ^a
90 days	80.0 ± 6.4^{a}	$40.5{\pm}5.1^{b}$	70.5±5.5 ^a	80.5 ± 4.8 ^a

Table 6: Percentages of sperm motility in rabbit ejaculates of the control and treatment groups (Mean ± SEM)

Within rows different superscript indicate significant differences (P<0.01)

*CG: control group, FA: formaldehyde, FA+LC: formaldehyde+ L-carnitine, LC: L-carnitine only

Days after treatment begin	Alive spermatozoa			
	CG*	FR	FR+LC	LC
	(%)	(%)	(%)	(%)
15 days	78.3±3.4	80.6±3.1	80.0±3.3	83.1±2.4
30 days	77.9±3.6	70.2±2.7	78.6±3.0	80.2±2.7
45 days	79.2±2.8 ^a	35.8±2.6 ^b	78.9±2.5 ^a	78.3±3.0 ^a
60 days	77.6±2.5 ^a	34.3±2.4 ^b	79.2±2.7 ^a	82.7±2.2 ^a
75 days	80.9±3.1 ^a	45.2±2.5 ^b	78.6±3.1 ^a	83.7±2.8 ^a
90 days	81.4±2.4 ^a	45.3±2.8 ^b	80.5±2.4 ^a	85.4±2.6 ^a

Table 7: Percentages of sperm viability in rabbit ejaculates of the control and treatment groups (Mean \pm SEM)

Within rows different superscript indicate significant differences (P<0.01)

Days after	Total sperm cell count in the whole ejaculate (Million)			
treatment begin	CG*	FR	FR+LC	LC
	n.x10 ⁶ /ml	n.x10 ⁶ /ml	n.x10 ⁶ /ml	n.x10 ⁶ /ml
15 days	248.5±5.6	238.9±7.4	249.3±6.5	246.1±6.6
30 days	246.8±5.2	244.3±6.6	247.9 ± 5.1	246.3±4.1
45 days	313.4±6.1 ^a	216.1 ± 8.0^{b}	315.6 ± 7.3^{a}	319.1±6.2 ^a
60 days	$314.9{\pm}6.4^{a}$	$215.1{\pm}5.4^{b}$	396.0 ± 7.7^{a}	363.3±5.6°
75 days	315.8 ± 5.5^{a}	$219.7{\pm}4.2^{b}$	316.3±5.1 ^a	$385.6 \pm 4.4^{\circ}$
90 days	316.3 ± 7.6^{a}	212.5 ± 5.7^{b}	315.5 ± 6.4^{a}	374.1±6.9 ^c

Within rows different superscript indicate significant differences (P<0.01)



Figure 1: Ejaculate volume and pH of rabbit semen over the period of the study, CG: control group, FA: formaldehyde in milk, FA+LC: formaldehyde in milk + L-carnitine, LC: L-carnitine only (Mean \pm SEM, P < 0.05, P < 0.001).







Figure 2: Sonographic results of different groups at the end of the study, (a) Sonogram from the control group showing moderate blood perfusion,

(b) Sonogram from the formaldehyde group showing low-moderate blood perfusion

(c) Sonogram from the formaldehyde and L-carnitine group showing moderate blood perfusion

(d) Sonogram from the L-carnitine group showing high blood perfusion

DISCUSSION

The formaldehyde affected drastically the weight of the bucks through out the period of study compared with other groups. The exposure to formaldehyde pollutants had a statistically negative significant effect on birth weight (Grazuleviciene et al., 1998). Depression, dullness and anorexia were apparent in quails fed 20 mL formalin/kg feed (Khan et al., 2005). Food intake, body weight, egg production and egg weight together with absolute and relative weight of organs were decreased (Khan et al., 2005). Significant decrease in food consumption, body weight and survival rate was recorded in animals exposed to formaldehyde (Kamata et al., 1997). These findings attributed to decreased triglyceride levels and absolute liver weight, in addition to decrease food consumption. There was a steady decline in body weight and testicular weight with formaldehyde administration (Chowdhury et al., 1992). In the current work, food consumption was not changed, but we think that the reduction in body weight might be result from impairment of the metabolic processes.

The present study showed that almost all semen parameters were dramatically affected by ingesting formaldehyde in milk including decrease in sperm cell count, viabilty and motility; increase in total sperm abnormalities and pH. On contrast, all semen parameters remanied to nearly normal or better than control with addition of L-carnitine to the milk suggesting that L-carnitine antagonize the adverse effects of formaldehyde on rabbit semen quality. The decrease in sperm count and increase in abnormal sperm were consistent with an earlier report on mice (Tang et al., 2003), that the changes in sperm indicate the genotoxicity of formaldehyde. Supporting the present results (Odeigah, 1997) found that there were a significant increase in sperm head abnormalities of formaldehyde treated rats. Although formaldehyde was known to produce DNA protein cross-links in a cell, the precise mechanism by which formaldehyde causes sperm head abnormalities is not yet fully established. In general, damage to the sperm cell by substances may occur by physiological, cytotoxic or genetic mechanisms. In addition there are two mechanisms by which chemicals might indirectly affect sperm cell function and morphology: firstly, exposure to chemicals could produce pituitaryhypothalamic or sex hormonal effects which in turn could affect spermatogenesis and secondly exposure could cause abnormalities in seminal fluid, resulting in functional or structural impairment of sperm (Zhou et al., 2006). Unfortunately, analysis of sex hormones was not performed in this work. Subacute and subchronic formaldehyde exposure can cause growth retardation and altered levels of trace elements including copper, zinc and iron, and on testicular

tissue may induce oxidative damage leading to spermatozoa abnormalities (Ozen *et al.*, 2002).

The recorded results were in agreement with those reported by Zhou *et al.* (2006) and Zhou *et al.* (2011). It was found that the formaldehyde affects the sperm count and motility in addition to decrease the activities of superoxide dismutase and glutathione peroxidase. Zhou *et al.* (2006) and Zhou *et al.* (2011) found that formaldehyde has harmful effects on spermatogenesis; it induces the atrophy of seminiferous tubules with a decrease in sperm density and an increase in abnormal sperm at a dose-dependent manner.

Doppler ultrasonography showed that there is a decrease in blood perfusion of testicles FA group, reflecting high vascular resistance of the testis. These effects not observed in rabbits in carnitine groups, where the testicles were good supplied with blood. Further studies should be done in this point. Early stages of spermatogenesis are sensitive to a moderate, acute reduction in blood flow. Bergh *et al.* (2001) reported that discrete reductions in flow may have a large impact on sperm production in rat.

This study was the first to examine the effects of Lcarnitine as an antioxidant antagonizes the effects of Formaldehyde exposure. Effects of L-carnitine as antioxidant well documented especially on testicular functions but its antagonistic effect to formaldehyde not previously studied. Kanter et al. (2010) cited that L-carnitine attenuate the radiation induced morphological changes and germ cell apoptosis in the irradiated rat testicles. Carnitine estimation in human semen is of diagnostic value for epididymal function (Wetterauer and Heite, 1980). In order to obtain a general picture of the function of all the glands contributing to the formation and composition of seminal plasma we thought not to rely only on fructose estimations but should also take into account those of the citrate and carnitine (Wetterauer and Heite, 1980).

CONCLUSION

Formaldehyde subchronic toxicity has dramatic adverse effects on the rabbit semen. L-carnitine could used to protect against the anticipated adverse effects of formaldehyde used to preserve food and improve the reproductive performance in bucks.

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الكفاءة التناسلبة وخصائص المنى وتدفق دم الخصى بعد التسمم تحت المزمن بالفور مالدهيد وامكانية الدور الوقائى للكارنتين في الأرانب

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أجريت هذه الدراسة بهدف تقييم وظائف الخصى فى الأرانب بعد التعرض للتسمم تحت المزمن بالفور مالدهيد و امكانية استخدام الكارنتين للوقاية والحماية من اثارة. أجريت هذه الدراسة لمدة ثلاثة شهور على ٢٠ ذكر أرنب نيوزيلاندى بالغ وقد قسمت الى اربعة مجموعات متساوية (خمسة أرانب لكل مجموعة). المجموعة الأولى (الضابطه) أخذت محلول ملح عن طريق الفم ، المجموعة الثانية تم اعطائها اللبن والذى يحتوى على نسبة فو مالدهيد أعلى من المسموح بها بعد عمل تحليلا بالكروماتوجرافيا الطيفيه للعديد من الالبان الموجودة بالاسواق المحلية ، المجموعة الثانية تم اعظائها اللبن والذى يحتوى على نسبة فو مالدهيد أعلى من المسموح بها بعد عمل تحليلا بالكروماتوجرافيا الطيفيه للعديد من الالبان الموجودة بالاسواق المحلية ، المجموعة الثالثة وتم اعطائها اللبن به الفور مالدهيد والكارنتين فى نفس الوقت ، المجموعة الرابعة وتم اعطائها الكارنتين فقط تم متابعة ووزن الحيوانات واختبار الرغبة الجنسبة وحساب زمن التفاعل الجنسى الايجابى وتجميع السائل المنوى (استخدمت انثتين للتجميع عليهم) بو اسطة المهبل الإصطناعى وتقييمة كل الجنسبة وحساب زمن التفاعل الجنسى الايجابى وتجميع السائل المنوى (استخدمت انثتين للتجميع عليهم) بو اسطة المهبل الاصطناعى وتقييمة كل وزمن التفاعل الجنبي المجموعات لم تحمي المتخدام الدوبلر لتحديد سريان الدم فى كل المجموعات. أظهرت النائي الرغبة وزمن التفاعل الإينين الموعات لم في من الأوزان الأرانب، حجم القذفة ،نسبة الحيامن المتحركة ، نسبة الحيامن المشوهه فى نفس وزمن التفاعل الإيجابي بين المجموعات لم تختلف كما أظهرت أن أوزان الأرانب، حجم القذفة ،نسبة الحيامن المتحركة ، نسبة الحيامن الحيه وعد وزمن التفاعل الإينبي المجموعات لم تما ملحوم عات لم تحموعة الفورمالدهبد لوحظ أيضا ارتفاع الرقم الهيدر وجيني ونسبة الحيامن المشوهه فى نفس وزمن التفاعل الخري الموجود تما لمامن عربي الرفور الرانب معان المعبوبية الحيامن الحيه وعد وزمن التفاعل الأبخر تم تسبح لموعة الفورمالدهبد لوحظ أيضا ارتفاع الرقم الهيدروجيني ونسبة الحيامن الميوم في معموعات وزمن الرغبة الحيامن المورمالدهب وعد وزمن التفاعل الإيرتين المعمومة قى محموعة قى وران الأرانب، حجم القذفة ،نسبة الحيامن المشومة لمومن المومه وي الحيان يقل بشكل واضح (2000) ، فى محموعة قى حركة ونسبة حويي ونسبة حبوية ويحد الحيمن ونقص ماحومة وى حركة م