Assiut Vet. Med. J. Vol. 58 No. 135 October 2012

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

H.A HUSSEIN* SAFA M. GEORGE** and HEBA A. YASSA**
*Department of Therigenology, Faculty of Vet. Med., Assiut University, 71526 Assiut, Egypt.
**Forensic and Clinical Toxicology Department, Faculty of Medicine, Assiut University, 71526 Assiut, Egypt.

ABSTRACT

Received at: 22/9/2012

Accepted: 18/11/12

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. 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 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 formaldehyde 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).

Carnitine (3- hydroxyl-4N- trimethylammonio-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

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 ± 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 45th 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 45th day of treatment in FA group ($P< 0.01$). The SPCC was increased ($P< 0.01$) in the LC group at 6th 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 perfusion 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 weights of rabbits in the control and treatment groups (Mean ± SEM)

Days after treatment begin	Sperm abnormalities			
	CG* (gm)	FR (gm)	FR+LC (gm)	LC (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)
*CG: control group, FA: formaldehyde, FA+LC: formaldehyde+ L-carnitine, LC: L-carnitine only

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

Days after treatment begin	Sperm abnormalities			
	CG* (Sec.)	FR (Sec.)	FR+LC (Sec.)	LC (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

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

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

Days after treatment begin	Sperm abnormalities			
	CG* (ml)	FR (ml)	FR+LC (ml)	LC (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±0.09^{a}	0.27±0.14^{a}	0.5±0.05^{b}
60 days	0.3±0.1^{a}	0.29±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±0.1^{a}	0.46±0.1^{b}
90 days	0.28±0.05^{a}	0.27±0.8^{a}	0.27±0.06^{a}	0.5±0.09^{b}

Within rows different superscript indicate significant differences (P<0.01)
*CG: control group, FA: formaldehyde, FA+LC: formaldehyde+ L-carnitine, LC: L-carnitine only

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

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}

Within rows different superscript indicate significant differences (P<0.05)
*CG: control group, FA: formaldehyde, FA+LC: formaldehyde+ L-carnitine, LC: L-carnitine only

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

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±2.6[a]	23.3±3.7[a]
60 days	16.2±3.4[a]	29.5±2.6[b]	17.4±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±2.3[a]	15.6±2.6[a]

Within rows different superscript indicate significant differences (P<0.01)
*CG: control group, FA: formaldehyde, FA+LC: formaldehyde+ L-carnitine, LC: L-carnitine only

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

Days after treatment begin	Sperm motility			
	CG* (%)	FR (%)	FR+LC (%)	LC (%)
15 days	80.5±6.4	75.7± 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± 5.5[b]	75.5±6.0[a]	80.5±5.0[a]
60 days	80.4± 6.4[a]	40.8± 4.6[b]	85.0±5.2[a]	90.0±6.9[a]
75 days	80.1± 5.5[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± 5.1[b]	70.5±5.5[a]	80.5±4.8[a]

Within rows different superscript indicate significant differences (P<0.01)
*CG: control group, FA: formaldehyde, FA+LC: formaldehyde+ L-carnitine, LC: L-carnitine only

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

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]

Within rows different superscript indicate significant differences (P<0.01)
*CG: control group, FA: formaldehyde, FA+LC: formaldehyde+ L-carnitine, LC: L-carnitine only

Table 8: Total sperm cell count of the rabbit ejaculates in the control and treatment groups

Days after treatment begin	Total sperm cell count in the whole ejaculate (Million)			
	CG* n.x10^6/ml	FR n.x10^6/ml	FR+LC n.x10^6/ml	LC 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±6.4^a	215.1±5.4^b	396.0±7.7^a	363.3±5.6^c
75 days	315.8±5.5^a	219.7±4.2^b	316.3±5.1^a	385.6±4.4^c
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)
*CG: control group, FA: formaldehyde, FA+LC: formaldehyde+ L-carnitine, LC: L-carnitine only

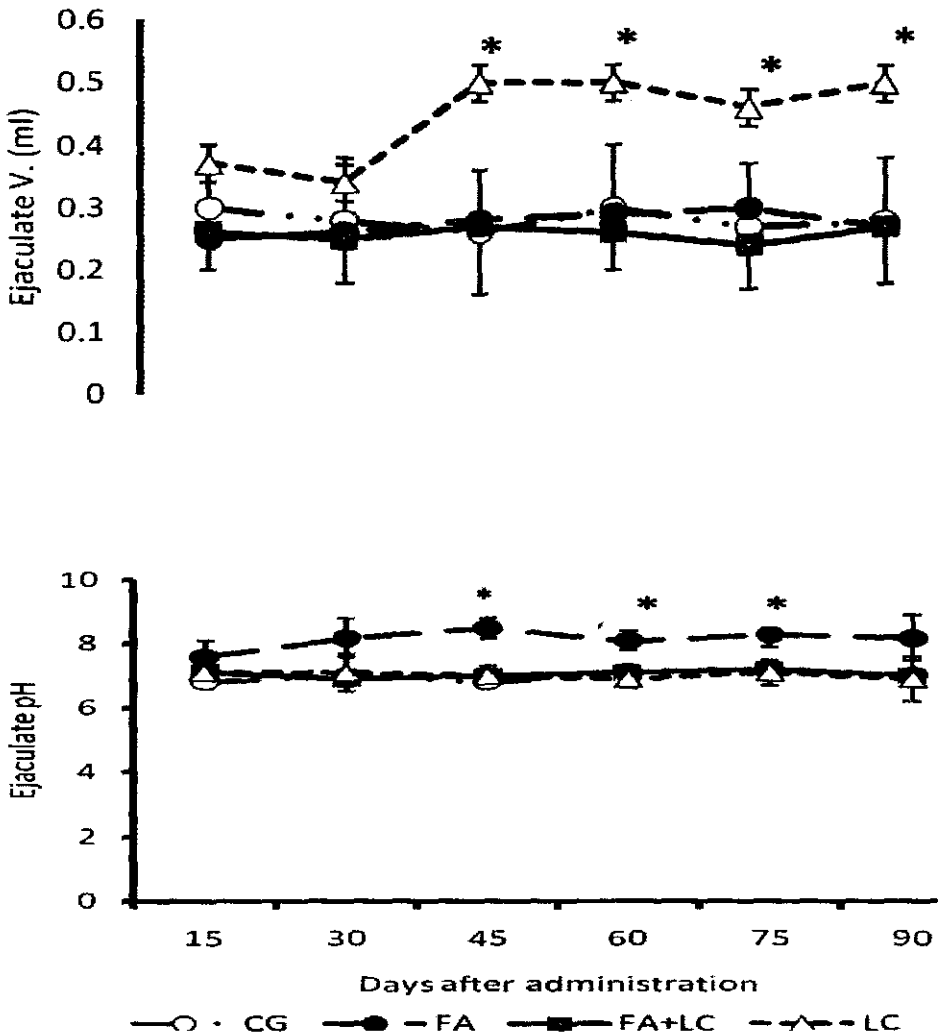

Figure 1: Ejaculate volume and pH of rabbit semen over the period of the study, CG: control group, FA: formaldehyde in milk, FA+LC: formaldehyde in milk + L-carnitine, LC: L-carnitine only (Mean ± SEM, P < 0.05, P < 0.001).

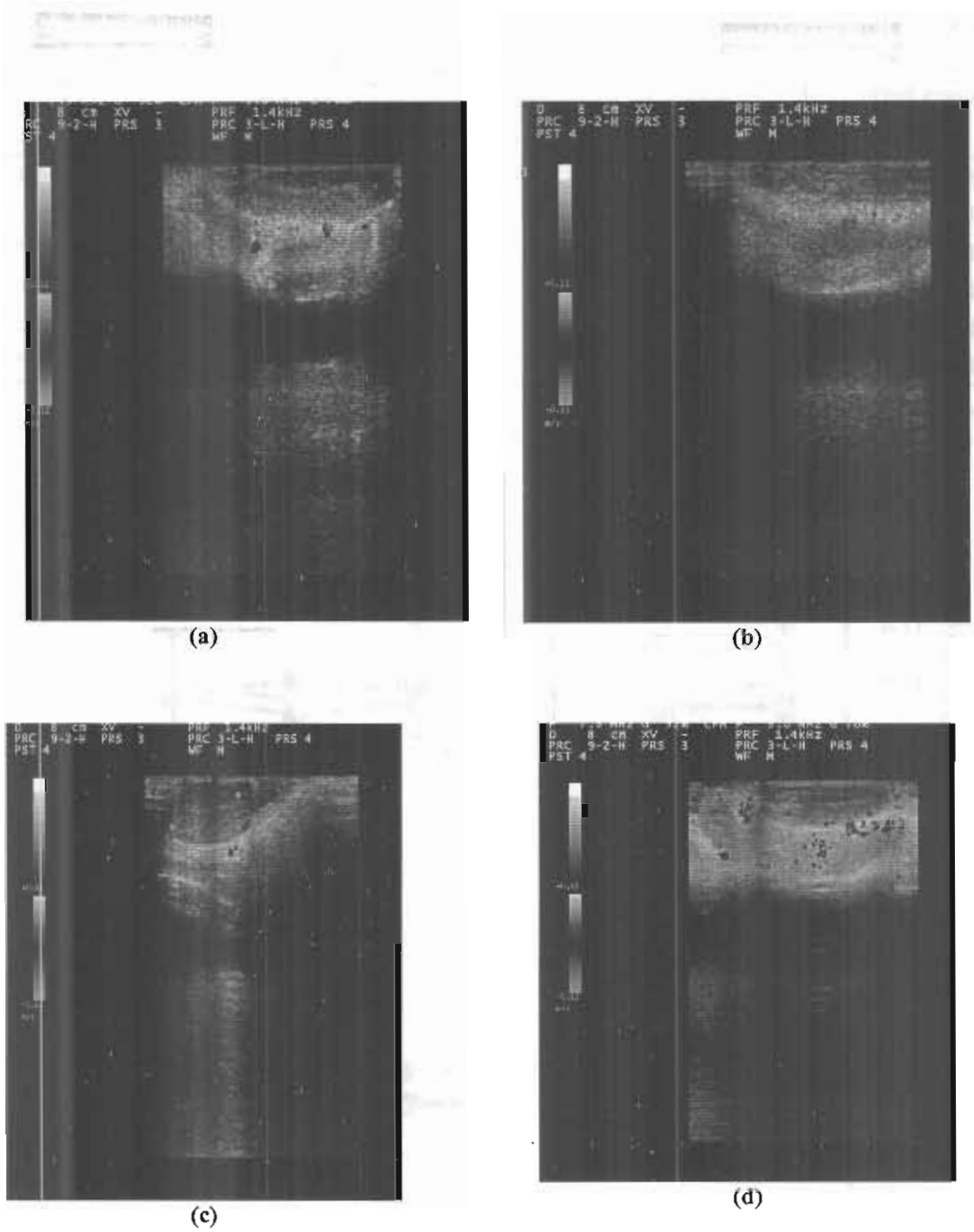

Figure 2: Sonographic results of different groups at the end of the study,
(a) Sonogram from the control group showing moderate blood perfusion,
(b) Sonogram from the formaldehyde group showing low-moderate blood perfusion
(c) Sonogram from the formaldehyde and L-carnitine group showing moderate blood 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 pituitary-hypothalamic 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 L-carnitine 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.

REFERENCES

Andrade, A.F.C.; Yonezawa, L.A.; Celeghini, E.C.C.; Spers, A. and Arruda, R.P. (2002): Um novo modelo de vagina artificial para coelhos. Rev. Bras. Reprod. Anim., 26: 201–204.

Bamba, K. (1988): Evaluation of acrosomal integrity of boar spermatozoa by bright field

microscopy using an eosin–nigrosinstain. Theriogenology, 29: 1245–1251.

Bergh, A.; Collin, O. and Lissbrant, E. (2001): Effects of Acute Graded Reductions in Testicular Blood Flow on Testicular Morphology in the Adult Rat. Biol. Reprod., 64 (1): 13-20

Blom, E. (1950): A one-minute live-dead sperm stain by means of eosin–nigrosin. J. Fertil. Steril., 1: 176–177.

Chowdhury, A.R.; Gautam, A.K.; Patel, K.G. and Trivedi, H.S. (1992): Steroidogenic inhibition in testicular tissue of formaldehyde exposed rats. Indian J. Physiol. Pharmacol., 36(3): 162-8.

Collins, J.J. (2004): Formaldehyde exposure and leukaemia. Occup. Environ. Med., 61: 875–6.

García-Tomás, M.; Sánchez, J.; Rafel, O.; Ramon, J. and Piles, M. (2006): Reproductive performance of crossbred and purebred male rabbits. Livestock Sci., 104: 233–243.

Grazuleviciene, R.; Dulskiene, V. and Vencloviene, J. (1998): Formaldehyde exposure and low birth weight incidence. J. Occup. Health., 40: 61–67.

Gurel, A.; Coskun, O.; Armutcu, F.; Kanter, M.and Ozen, O.A. (2005): Vitamin E against oxidative damage caused by formaldehyde in frontal cortex and hippocampus: biochemical and histological studies. J. Chem. Neuroanat., 29: 173–8.

Jogl, G.; Hsiao, S. and Tong L. (2004): Structure and function of carnitine acyltransferases. Ann. N.Y. Acad. Sci., 1033:17-29.

Kamata, E.; Nakadate, M.; Uchida, O.; Ogawa, Y.; Suzuki, S.; Kaneko, T.; Saito, M. and Kurokawa, Y. (1997): Results of a 28-month chronic inhalation toxicity study of formaldehyde in male Fisher-344 rats. J. Toxicol. Sci., 22(3): 239-254.

Kaminski, J.; Atwal, A.S. and Mahadevan, S. (1993): Determination of formaldehyde in fresh and retail milk by liquid column chromatography. Journal of the Association of Official Analytical Chemists international, 76(5): 1010–1013.

Kanter, M.; Topcu-Tarladacalisir, Y. and Parlar, S. (2010): Antiapoptotic effect of L-carnitine on testicular irradiation in rats. J. Mol. Histol. 41, 6(2-3): 121-128.

Khan, A.; Bachaya, H.; Khan, M.Z. and Mahmood, F. (2005): Pathological effects of formalin (37% formaldehyde) feeding in female Japanese quails (Coturnix coturnix japonica). Human and Experimental Toxicology, 24: 415-422

Lenzi, A.; Lombardo, F.; Sgro, P.; Salacone, P.; Caponecchia, L.; Dondero, F. and Gandini, L. (2003): Use of carnitine therapy in selected cases of male factor infertility: a double-blind crossover trial. Fertil. Steril., 79(2): 292–300.

Li, J.; Zhu, J. and Ye, L. (2007): Determination of formaldehyde in squid by highperformance liquid chromatography. Asia Pac. J. Clin. Nutr., 16 (Suppl 1): 127-130.

Majumder, P. and Kumar, V. (1995): Inhibitory effects of formaldehyde on reproductive system of male rats. Indian J. physiology pharma., 39(1): 80 – 82.

Makowski, L.; Noland, R.; Koves, T.; Xing, W.; Ilkayeva, O.R.; Michael, J.; Muehlbauer, M.J.; Stevens, R.D. and Muoio, D.M. (2009): Metabolic profiling of PPARY mice reveals defect in carintine and amino acid homeostasis that are partially reversed by oral carnitine supplementation. The FASEB journal; 23:586-604. www. Material safety data sheet.com. www.healthlink.com.au/nat_lib/htm-data/htm-supp/supps72.htm

Naughton, C.K.; Nelson, D.R. and Thomas, J.R. (2003): Development of an inexpensive artificial vagina for semen collection from rabbits. J. Andrology, 24 (5): 712-715.

Odeigah, P. (1997): Sperm head abnormalities and dominant lethal effects of formaldehyde in albino rats. Mutation research, 389: 141–148.

Ozen, O.; Yaman, M.; Sarsilmaz, M.; Songur, A. and Kus, I. (2002): Testicular zinc, copper and iron concentrations in male rats exposed to subacute and subchronic formaldehyde gas inhalation. Journal of Trace Elements in Medicine and Biology, 16: 119-122.

Rahbar, R.; Shakerhosseini, R.; Saadat, N.; Taleban, F.; Pordal, A. and Gollestan, B. (2005): Effect of L-carnitine on plasma glycemic and lipidr\emic profile in patients with type II diabetes mellitus. Eur. J. Cin. Nutr., 2: 16-22.

Roca, J.; Martinez, S.; Vazquez, JM.; Lucas, X. and Martinez, E. (2000): Viability and fertility of rabbit spermatozoa diluted in Trisbuffer extenders and stored at 15°C. Anim. Reprod. Sci., 64: 103– 12.

Sari, D.K.; Kuwahara, S.; Tsukamoto, Y.; Hori, H.; Kunugita, N.; Arashidani, K., Fujimaki, H. and Sasaki, F. (2004): Effect of prolonged exposure to low concentrations of formaldehyde on the corticotrophin releasing hormone neurons in the hypothalamus and adrenocorticotropic hormone cells in the pituitary gland in female mice. Brain Res., 1013: 107–16.

Seo, J.T.; Kim, K.T.; Moon, M.H. and Kim, W.T. (2010): The significance of microsurgical varicocelectomy in the treatment of subclinical varicocele. Fertil. Steril., 93(6): 1907–10.

SPSS for Windows (2007): Personal package, version 16.0.

Smith, J.T. and Mayer, D.T. (1955): Evaluation of sperm concentration by the hemocytometer method. Fertil. Steril,. 6: 271–275.

Stvolinsky, S.L. and Dobrota, D. (2000): Anti-ischemic activity of carnosine. Biochemistry (Moscow) 65: 849–855.

Tang, M.; Xie, Y.; Yi, Y. and Wang, W. (2003): Effects of formaldehyde on germ cells of male mice. Weisheng Yanjiu, 32(6): 544–548.

Weng, X.; Chon, C.; Jiang, H. and Li, D. (2009): Rapid detection of formaldehyde concentration in food on a polydimethylsiloxane (PDMS) microfluidic chip, Food Chemistry, 114: 1079–1082.

Wetterauer, 1.U. and Heitel, H. (1980): Carnitine in Seminal Plasma: Its Significance in Diagnostic Andrology. Informa Health care, 4(2): 137-143.

Wong, E.Y.; Ray, R.; Gao, D.L.; Wernli, K.J.; Li, W.; Fitzgibbons, E.D.; Camp, J.E.; Heagerty, P.J.; De Roos, A.J.; Holt, V.L.; Thomas, D.B. and Checkoway, H. (2006): Reproductive history, occupational exposures, and thyroid cancer risk among women textile workers in Shanghai, China. Int. Arch. Occup. Environ. Health, 79: 251–8.

Ye, X.; Yan, W.; Xie, H.; Zhao, M. and Ying, C. (2005): Cytogenetic analysis of nasal mucosa cells and lymphocytes from high-level long-term formaldehyde exposed workers and low-level short-term exposed waiters. Mutat. Res., 588: 22–7.

Zhang, L.; Steinmaus, C.; Eastmond, D.; Xin, X. and Smith, M. (2009): Formaldehyde exposure and leukemia: a new meta analysis and potential mechanisms mutation research, 681(2-3): 150-168.

Zhou, D.; Qiu, S.; Zhang, Z. and Wang, H. (2006): Effect of Formaldehyde on Spermatogenesis and Testicular Morphology in Adult Rats. Journal of US –China. Medical Sci., 3(3): 57-60.

Zhou, D.; Zhang, J.; Wang, H. and Xue, Y. (2011): Effect of formaldehyde exposure on structure and function of epididymis in adult rats: a histological and biochemical study. Toxicological & Environmental Chemistry, 93(1): 134–144.

الكفاءة التناسلية وخصائص المنى وتدفق دم الخصى بعد التسمم تحت المزمن بالفورمالدهيد وامكانية الدور الوقائى للكارنتين فى الأرانب

حسن عبد الصبور على حسين ، صفاء ماهر جورج ، هبة عطية يسى

أجريت هذه الدراسة بهدف تقييم وظائف الخصى فى الأرانب بعد التعرض للتسمم تحت المزمن بالفورمالدهيد وامكانية 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 فورمالدهيد أعلى من المسموح بها بعد عمل تحليلا بالكروماتوجرافيا الطيفيه للعديد من الالبان الموجودة بالاسواق المحلية ، المجموعة الثالثة وتم اعطائها اللبن به الفورمالدهيد والكارنتين فى نفس الوقت ، المجموعة الرابعة وتم اعطائها الكارنتين فقط. تم متابعة ووزن الحيوانات واختبار الرغبة الجنسية وحساب زمن التفاعل الجنسى الايجابى وتجميع السائل المنوى (استخدمت انثتين للتجميع عليهم) بواسطة المهبل الاصطناعى وتقييمة كل ١٥ يوم طوال فترة التجربة. كما تم فحص الخصى باستخدام الدوبلر لتحديد سريان الدم فى كل المجموعات. أظهرت النتائج ان الرغبه الجنسية وزمن التفاعل الايجابى بين المجموعات لم تختلف. كما أظهرت أن أوزان الأرانب، حجم القذفة ،نسبة الحيامن المتحركة ، نسبة الحيامن الحيه وعدد الحيامن يقل بشكل واضح ($P<0.001$) ، فى مجموعة الفورمالدهيد لوحظ أيضا ارتفاع الرقم الهيدروجينى ونسبة الحيامن المشوهه فى نفس المجموعة. على الجانب الأخر تم تسجيل زيادة ملحوظة فى حركة ونسبة حيوية وعدد الحيامن ونقص ملحوظ فى نسبة الحيامن المشوهه ($P<0.001$) فى مجموعة الكارنتين. لوحظ نقص فى تدفق الدم فى الخصى للأرانب بمجموعة الفورمالدهيد فقط. وقد خلصت الدراسة الى أن التسمم تحت المزمن بالفورمالدهيد له تأثير سئ جدا على المنى بذكور الأرانب ويمكن أستخدام الكارنتين للوقاية من تأثيره الضار.