# Semen Characteristics and Oxidative/Antioxidative Status in Semen and Serum of Male Rabbits Supplemented with Antioxidants during Heat Stress

**Research Article**

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## ABSTRACT

Heat stress (HS) often blamed for suboptimal reproductive efficiency and is a worldwide problem, which inflicts heavy economic losses reflected in limiting the breeding season of rabbits to be normally from September to May in northern hemisphere and subtropical regions. The present experiment was conducted to study the effect of summer heat stress and the alleviating effect of some antioxidants on semen characteristics and the serum and seminal plasma oxidative/antioxidative status of New Zealand White (NZW) rabbit bucks. Forty-eight fertile NZW white bucks were randomly divided into six equal groups (n=8), first two groups served as control (summer heat stressed and winter) groups, the others, orally administrated ascorbic acid 40 mg/kg bw/d, zinc methionine 10 mg/kg bw/d, coenzyme $Q_{10}$ 10 mg/kg bw/d and L-carnitine 40 mg/kg bw/d. Blood and semen samples were collected once weekly among experimental period. Routine spermogram was performed. Lipid peroxidation, total antioxidant capacity and catalase activity in both serum and seminal plasma were assayed. The climatic data were continuously recorded among the experimental period and the weekly average temperature-humidity index (THI) was calculated. Summer HS adversely affected both qualitative and quantitative traits of spermogram but bucks remain within fertile limit. Thiobarbiric acid reactive substances (TBARS) showed a significant increase while total antioxidant capacity (TAC) and catalase showed significant decreases. Contrary to our expectation, selected antioxidants did not restore these parameters to their winter levels. It was concluded that zinc and L-carnitine were found to be the most beneficial antioxidants in the relief of spermogram of HS-induced effects. Serum and seminal plasma oxidative/antioxidant statuses followed an identical pattern in response to heat stress. Taking the current results into consideration, it can be stated that an adequate reproductive performance in NZW rabbit bucks can be achieved in summer and from the scientific point of view, it is not obligatory to stop breeding in summer months.

**KEY WORDS** ascorbic acid, $CoQ_{10}$, heat stress, L-carnitine, zinc.

## INTRODUCTION

Ambient temperature is associated with other climatic factors. Relative humidity (RH) seems to be the most important of those factors. The feeling of warmth under hot ambient temperature increases with high relative humidity.

Such relationship induced by Lphsi (1990) to propose a measurement of the level of severity of heat stress (HS) by using both factors and was termed temperature–humidity index (THI).

In Egypt, the heat stress is aggravated with the high relative humidity, which is normally over 85% during the day

and can reach 100% during night in hot months (Marai *et al.* 2001). THI in mild (from January until May) and hot months (from June until October) were 20.2 and 30.1, respectively (Marai *et al.* 2002; Marai *et al.* 2005). Several studies have suggested that heat exposure get energy to all cell functions. $CoQ_{10}$ is an antioxidant that has great importance against free radicals, protects the stability of the cell membrane, DNA from free radicals induced oxidative damage and helps recycling of vitamin E and maintain healthy energy levels. Function of L-carnitine may further improve the survival of spermatozoa and increase the total number of sperm that are ejaculated (Kozink *et al.* 2004). L-Carnitine has been implicated in buffering the cell against high concentrations of mitochondrial acetyl-CoA by converting it into acyl carnitine (Jeulin and Lewin, 1996). Excess acetyl-CoA inhibits the activity of pyruvate dehydrogenase, a key enzyme in mitochondrial energy metabolism (Jeulin and Lewin, 1996).

Coenzyme $Q_{10}$ ($CoQ_{10}$) plays a crucial role in the production of cellular adenosine triphosphate which provides modulating antioxidants defense system.

Ascorbic acid is essential to the body as both an antioxidant and as a nutritional supplement. Luck *et al.* (1995) reported that ascorbic acid should be considered as an essential biochemical in the reproductive process and as a potentially significant factor in fertility. Ascorbic acid has antioxidant activity when it reduces oxidizing substances such as hydrogen peroxide (Duarte and Lunec, 2005), however, it will also reduce metal ions that generate free radicals through the Fenton reaction (Valko *et al.* 2005).

Zinc is involved intimately in many aspects of sperm morphology, physiology, and biochemistry, although a considerable controversy exists over mechanisms of action of zinc in the male reproductive system (Lord and Averill, 2002).

Intracellular zinc can function as a temporary inhibitor for sperm lipid peroxidation, sperm oxygen uptake, sperm nuclear chromatin decondensation, sperm capacitation, acrosome reaction and for the *in vitro* fertilizing ability of spermatozoa (Stephenson and Brackett, 1999). A mechanism by which Zn may function as cellular antioxidant, is through its involvement in synthesis of metallothionein (Zn-mt), a metal binding protein that may scavenge hydroxide radicals and provides effective protection against lipid peroxidation (LPO) (Prasad *et al.* 2004). Therefore, Zn ions act in terms of membrane stabilization, as well as the protective role of Zn-mt as a cellular antioxidant. Zinc intake has been reported to produce an antiatherogenic effect as it may have a protective effect on lipid metabolism consisting in its ability to prevent hyperlipidemia, including especially hypercholesterolemia, and to protect from LPO (Joanna Rogalska *et al.* 2009). Various factors facilitate the accumulation of free radicals, but common stressors (heat stress, high physiological demands) increase both the cell's metabolic rate and the accumulation of free radicals (Lohrke *et al.* 2005). If the antioxidants that prevent the accumulation of free radicals are absent, or present at suboptimal levels within the cell, or not available at the precise place within the cell where free radicals are formed, therefore, damage can occur (Andrieu, 2008).

Studies have shown that antioxidants are uniquely different from each other and each have a specific function in the body. The present study selects antioxidants that have a wide range of metabolic activities. It has been suggested that reactive oxygen species (ROS) and LPO products in various clinical diagnoses of infertility are associated with high oxidative stress and whether any group of infertile animal is more likely to have high seminal oxidative stress. ROS play an important role in sperm physiological functions, but elevated levels of ROS or normal cell functions and integrity of cell structures may be broken via considerable reactivity of ROS. The organism has enzymatic (e.g. superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic (e.g. vitamin E) antioxidant mechanisms that work as scavenger for this harmful ROS. Radical-scavenging antioxidants are consumed by the increased free radical activity associated with several conditions, and the total antioxidant response has been used to indirectly assess the free radical activity.

The effects of various antioxidants in plasma are additive and the cooperation of antioxidants in serum provides protection of the organism against attacks by free radicals. The specific aims of the present study are to determine the efficacy of ascorbic acid, zinc, coenzyme Q10 and L-carnitine to protect rabbits against HS, improve reproductive performance of rabbits and to determine whether treatments with these antioxidants decrease oxidative stress parameters.

## MATERIALS AND METHODS

The experiment was carried out under non-controlled environmental conditions at the experimental rabbitry in lab animal house of the National Research Center, Dokki, Giza-Egypt.

### Animals and experimental design

Forty-eight sexually mature NZW male rabbits, which proven fertile, were purchased from the same herd in a commercial farm, for the purposes of this experiment. The rabbits were of 26-30 ($28\pm2$) weeks age and of 2.371-2.917 ($2.644\pm0.273$) kg initial weight. Bucks were individually housed in metal wire mesh cages provided with separate facilities for feeding and watering. The experiment was

conducted in two periods, the first period in winter and the second in summer, each period lasted 12 weeks. Rabbits were randomly divided into six equal groups (n=8) as shown in Table 1.

The climatic data was continually recorded during the experimental period using a thermometer and a calibrated hygrometer. The weekly averages of ambient temperature and relative humidity values at midday inside the rabbit building were estimated. The temperature–humidity index (THI) was computed using the formula established by Marai *et al.* (2001) for rabbits as follow:

THI= db ˚C – {(0.31–0.31 RH).(db ˚C –14.4)}

Where:
db ˚C = dry bulb temperature in ˚C
RH= relative humidity expressed in percentage.

The values obtained are then classified as follows:
THI<27.8= absence of heat stress.
27.8<THI<28.9= moderate heat stress.
28.9<THI<30.0= severe heat stress.
THI>30.0 very severe heat stress.

### Samples collection

Blood and semen samples were collected once every week for 12 consequent weeks for each season at 8.00 am before offering ration. Blood samples were collected in plain centrifuge tubes from the ear vein using sterile 3 mL syringes for serum separation. Serum samples were divided into aliquots in Epindorf tubes and stored at -80 ˚C until further analysis. Semen was collected from bucks previously trained to serve on artificial vagina (IMV, France) and a teaser doe. Two ejaculates were collected once a week from each buck, one served as row semen sample for spermogram. The other was centrifuged at 12000 rpm for 10 min. at 4 ˚C to separate seminal plasma that will be stored at -80 ˚C until further analyses. Ejaculates containing urine and/or calcium carbonate deposits were discarded.

### Spermogram

Libido is estimated as the time in seconds elapsed between exposing of the female into the male's cage till the completion of ejaculation. The traits recorded for each sample were volume, pH (using pH paper, Spezial-Indikatorpapier pH 5.5-9.0 (MACHEREY-NAGEL. Germany), mass motility–MM graded from 0 to 9, individual motility–IM (evaluated microscopically as the percentage of forward-motile sperm cells), motility index–MI (calculated by multiplying mass motility grade by individual motility), total motile sperm–TMS (calculated by multiplying percentage of motile sperm and total sperm output), sperm concentration–SC

(number of spermatozoa/mL was counted using an improved Neubauer hemo-cytometer chamber (GmbH+Co., Hamburg, Germany), percentages of alive–LS and abnormal spermatozoa–AS (using an eosin-nigrosine staining mixture, 200 spermatozoa/sample were examined for morphology and viability, Blom (1977) packed sperm volume–PSV (using capillary tubes and a microhematocrit centrifuge adjusted at 12000 rpm for 15 min), total sperm output–TSO (calculated by multiplying semen ejaculate volume by semen concentration) and total functional sperm fraction–TFSF (calculated as the product of multiplying total sperm output ($10^6$ by individual motility (%) by normal morphology (%) (Correa and Zavos, 1994).

### Oxidative and antioxidive status

Lipid peroxidation expressed in malondialdehyde concentration (MDA) (Ohkawa *et al.* 1979), total antioxidant capacity (TAC) (Koracevic, 2001) and catalase activities (Aebi, 1984) were assayed using commercially available kits obtained from Bio Diagnostic Research (Giza, Egypt).

### Statistical analyses

All data were subjected to statistical analysis including the calculation of the mean (M), standard error of the mean (SE) and F-test (one way ANOVA) at a confidence limit of 95% ($P<0.05$). Statistical analysis was conducted according to the method of Armitage (1971) using practicing statistical analysis program (SPSS, Edition 11). Duncan's multiple range test was used for testing pairs of means for comparison at a probability of 5% (Duncan, 1955).

## RESULTS AND DISCUSSION

Respective average ambient temperature and relative humidity were 19.06 ˚C ±0.26 and 63.24% ±0.88 during winter and 36.17 ˚C ±0.44 and 73.40% ±1.00 during summer. Average temperature–humidity index was 18.52±0.22 in winter and 34.38±0.46 in summer, indicating absence of heat stress in winter (less than 27.80) and exposure to very severe heat stress in summer (more than 30.00) .

### Spermogram

Table 2 showed the reproductive efficiency parameters of rabbit bucks as affected by season and antioxidants supplementation. Summer control group showed significant increase in reaction time compared to winter control. All antioxidant-supplemented groups significantly decreased in reaction time than summer control values.

Value of L-carnitine supplemented bucks was comparable to the winter group value. Summer control group revealed significant decrease in pH value compared with winter group.

**Table 1** Experimental design used in this study

| Period | Group | | Supplementation |
|---|---|---|---|
| 1st period (Winter) | I. Winter control | | Basal diet only |
| | II. Summer control (heat stress) | | Basal diet only |
| 2nd period (Summer) | Antioxidant supplementations | III. Ascorbic acid | Ascorbic acid 40 mg/kg bw/d |
| | | IV. Zinc | Zinc methionine 10 mg/kg bw/d |
| | | V. Coenzyme $Q_{10}$ | Coenzyme $Q_{10}$ 10 mg/kg bw/d |
| | | VI. L-carnitine | L-carnitine 40 mg/kg bw/d |

**Table 2** Overall means (±SE) of reproductive efficiency parameters of rabbit bucks as affected by season and antioxidants supplementation

| Season | Winter | Summer | | | | |
|---|---|---|---|---|---|---|
| Groups / Parameters | Control | Control | Ascorbic | Zinc | Co enzyme $Q_{10}$ | L-carnitine |
| Reaction time (sec) | $11.49\pm1.12^a$ | $23.39\pm0.47^d$ | $15.14\pm0.81^b$ | $15.62\pm0.63^b$ | $19.07\pm0.45^c$ | $12.11\pm0.66^a$ |
| pH | $7.50\pm0.04^b$ | $7.38\pm0.03^a$ | $7.36\pm0.04^a$ | $7.41\pm0.04^{ab}$ | $7.39\pm0.03^a$ | $7.34\pm0.05^a$ |
| Ejaculate Volume (Ml) | $1.02\pm0.03^b$ | $0.74\pm0.03^a$ | $0.96\pm0.09^b$ | $0.91\pm0.04^b$ | $0.73\pm0.02^a$ | $0.76\pm0.03^a$ |
| Mass Motility | $7.46\pm0.15^b$ | $7.21\pm0.08^{ab}$ | $6.90\pm0.14^a$ | $7.24\pm0.09^b$ | $6.90\pm0.09^a$ | $7.46\pm0.09^b$ |
| Individual Motility (%) | $67.14\pm1.95^b$ | $63.82\pm1.18^{ab}$ | $61.43\pm1.79^a$ | $64.10\pm1.28^{ab}$ | $59.67\pm1.42^a$ | $66.98\pm1.33^b$ |
| Motility Index | $5.20\pm0.22^d$ | $4.67\pm0.14^{abc}$ | $4.41\pm0.19^{ab}$ | $4.73\pm0.15^{bcd}$ | $4.21\pm0.15^a$ | $5.08\pm0.15^{cd}$ |
| Live spermatozoa (%) | $73.52\pm1.09^c$ | $69.92\pm0.65^a$ | $70.62\pm1.01^{ab}$ | $70.94\pm0.79^{abc}$ | $68.35\pm0.84^a$ | $73.10\pm0.88^{bc}$ |
| Abnormalities (%) | $15.40\pm0.60^a$ | $16.79\pm0.62^{ab}$ | $15.40\pm0.57^a$ | $15.72\pm0.61^a$ | $18.14\pm0.62^b$ | $15.54\pm0.63^a$ |
| Concentration ($10^6$/mL) | $282.92\pm3.12^a$ | $276.59\pm1.96^a$ | $275.65\pm2.78^a$ | $282.97\pm2.34^a$ | $277.53\pm1.88^a$ | $290.31\pm2.34^b$ |
| Packed sperm vol. (%) | $16.14\pm0.16^a$ | $15.97\pm0.10^a$ | $15.80\pm0.14^a$ | $16.13\pm0.11^a$ | $15.88\pm0.086^a$ | $16.49\pm0.12^a$ |
| TSO ($10^6$/ejaculate) | $289.03\pm9.04^d$ | $208.92\pm7.90^a$ | $240.70\pm6.75^{bc}$ | $255.79\pm9.48^c$ | $202.18\pm6.36^a$ | $220.64\pm8.62^{ab}$ |
| TMS ($10^6$/ejaculate) | $199.98\pm10.44^d$ | $136.91\pm6.73^{ab}$ | $147.92\pm6.30^{bc}$ | $162.05\pm6.46^c$ | $123.32\pm5.94^a$ | $150.23\pm7.71^{bc}$ |
| TFSF ($10^6$/ejaculate) | $172.86\pm9.93^d$ | $116.16\pm6.30^{ab}$ | $127.02\pm5.95^{bc}$ | $137.62\pm5.91^c$ | $103.16\pm5.65^a$ | $128.85\pm7.22^{bc}$ |

The means within the same row that have at least one common letter, do not have significant difference (P>0.01).

SE= standard error; TSO= total sperm output; TMS= total motile sperm; TFSF= total functional sperm fraction.

Values of different supplementations were comparable to the summer control group, except for zinc supplemented bucks, which comparable to winter group value. There was a significant decrease in ejaculate volume in summer heat stressed bucks compared to winter group expect for values of ascorbic acid and zinc supplemented groups which were similar to that of the winter group. Summer control group showed non significant decrease in mass motility compared to winter group. Ascorbic and co enzyme $Q_{10}$ supplemented bucks showed significant decrease in mass motility, while zinc and L-carnitine showed non significant change in MM in comparison with summer control group. Individual motility percentage of different experimental groups showed similar pattern to that observed in mass motility results. Motility index values were decreased significantly in summer heat stressed bucks compared to value of the winter group. Among different antioxidant supplementations, zinc and L-carnitine supplemented groups revealed motility indices comparable to the winter group value. Summer experimental bucks showed significant decrease in live sperm percentage than that of the winter group. Zinc and L-carnitine supplemented groups showed values similar to those of the winter group. There were non significant changes in sperm abnormalities percentage in summer experimental groups compared to the winter group. There was no difference in sperm concentration between summer and winter groups. Only L-carnitine supplemented bucks showed significant increase in sperm concentration as compared to summer and winter control groups. Significant decrease in TMS was recorded in summer group compared to winter group. TMS in zinc supplemented bucks revealed significant increase in comparison to the summer control group. No significant difference in packed semen volume was noticed in all experimental groups. There was significant decrease in TSO in summer heat stressed bucks compared to bucks reared in winter. Ascorbic and zinc supplemented bucks showed significant increase in TSO compared with the summer control group. Results of TFSF revealed similar pattern to that of total motile sperm. Table 3 showed the serum and seminal plasma oxidative and antio-

**Table 3** Overall means (±SE) of serum and seminal plasma oxidative and antioxidant status of rabbit bucks as affected by season and antioxidants supplementation

| Season | | Winter | Summer | | | | |
|---|---|---|---|---|---|---|---|
| Parameters | Groups | Control | Control | Ascorbic | Zinc | Co enzyme $Q_{10}$ | L-carnitine |
| Serum | TBARS (nmol/mL) | $2.14^a$±0.09 | $3.68^b$±0.10 | $3.48^b$±0.18 | $3.54^b$±0.12 | $3.57^b$±0.13 | $3.47^b$±0.13 |
| | TAC (mmol/L) | $1.23^b$±0.10 | $0.68^a$±0.02 | $0.76^a$±0.06 | $0.73^a$±0.03 | $0.82^a$±0.05 | $0.80^a$±0.05 |
| | Catalase (U/Ml) | $19.36^d$±0.75 | $13.76^a$±0.23 | $15.14^{bc}$±0.39 | $14.33^{ab}$±0.36 | $15.90^c$±0.33 | $15.61^{bc}$±0.46 |
| Seminal plasma | TBARS (nmol/ML) | $1.21^a$±0.09 | $1.93^d$±0.04 | $1.62^{bc}$±0.09 | $1.82^{cd}$±0.08 | $1.64^{bc}$±0.07 | $1.58^b$±0.05 |
| | TAC (mmol/L) | $1.71^c$±0.05 | $1.12^{ab}$±0.03 | $1.07^a$±0.04 | $1.07^a$±0.07 | $1.26^b$±0.09 | $1.24^b$±0.04 |
| | Catalase (U/mL) | $24.84^c$±0.66 | $13.55^a$±0.27 | $15.11^b$±0.62 | $15.03^b$±0.39 | $16.26^b$±0.59 | $16.09^b$±0.49 |

The means within the same row that have at least one common letter, do not have significant difference (P>0.01).
TBARS= thiobarbituric acid-reactive substances; TAC= total antioxidant capacity; SE= standard error.

xidant status of rabbit bucks as affected by season and antioxidant supplementation. There was significant increase in serum TBARS level in all summer groups compared to the winter group. Antioxidant supplemented bucks showed no change in serum TBARS levels when compared with each other or with summer control bucks.

**Total antioxidant capacity**
Summer heat stressed bucks showed significant decrease in serum TAC levels than those of the winter group. There was no change among values of antioxidant supplemented groups.

**Catalase**
There was a significant decrease in serum catalase activity in summer heat stressed bucks compared to the winter group. Ascorbic, coenzyme $Q_{10}$ and L-carnitine supplemented bucks showed significant increase in serum catalase activities compared with summer control bucks.

The present study was performed to investigate the influence of heat stress on male rabbit health and reproductive performance in an attempt to alleviate the effect of heat stress using selected antioxidants.

The season's averages of temperature–humidity indices (THI) as heat stress index, were 18.52±0.22 in winter and 34.38±0.46 in summer, indicating absence of heat stress in winter (less than 27.80) and exposure to very severe heat stress in summer (more than 30.00). These values were similar (very close) to that estimated by Marai *et al.* (2002). In addition, Marai *et al.* (2005), estimated similar THI in winter and slightly lower values in summer. The decrease in bucks' libido with increasing ambient temperature may be due to delay in sexual urge (Lebas *et al.* 1986) and/or low physical performance of bucks (Hussein, 2007). In the light of the present results, the delayed reaction time in summer with the observation of constant serum testoster-

one level seems to be due to low body activity in attempt to minimize metabolic activity and consequently, heat production. All antioxidant supplemented groups significantly decreased reaction time compared with summer control values. Only L-carnitine supplemented bucks restored this parameter to winter group values. Seminal plasma is usually an isotonic neutral medium and it is a detrimental factor to sperm cell survival (White, 1976). Contrary to previous literature (Hussein, 2007), results of the present study showed that semen pH values significantly decreased in heat stressed bucks in comparison with winter group (7.50 vs. 7.38). Other authors (Nizza *et al.* 2003) reported nonsignificant effect of heat stress on hydrogen ion concentration. Marder *et al.* (1990) reported that after rabbit's exposure to heat stress and development of mild hyperthermia, the first process to develop is metabolic acidosis prior to the metabolic alkalosis that occurs in advanced stage. The progressive metabolic acidosis strongly indicates a shift to anaerobic metabolism and suggests the existence of severe metabolic complications (Kazemi and Johnson, 1986) followed by an increased production of lactic acid in different body tissues. Taking these findings and the current result into consideration, the significant decrease in semen hydrogen ion concentration may be explained. Different antioxidant supplementations did not affect pH values compared to summer control group, except for zinc supplemented bucks, which restored pH to its winter group values. A significant effect of season on ejaculate volume of NZW rabbits was observed in the current study. The lower buck's ejaculate volume in summer is similar to the results of Nagwa *et al.* (2006) and contradicts the results of Nizza *et al.* (2003). Meanwhile, Rastimeshin (1979) in rabbit and Chen *et al.* (2003) in human, reported that season of the year had no significant effects on semen ejaculate volume. The changes in ejaculate volume may be due to a low sperm concentration and a decrease in the volume of seminal plasma

(Macirone and Walton, 1983) as a result of hypoactivity of the accessory glands and the testes due to the adverse effect of high ambient temperature (Zeidan *et al.* 1997). Ascorbic acid and zinc supplemented groups restored ejaculate volume to winter group values. A remarkable and significant decrease in motility parameters (MM, IM and MI) was observed in bucks suffered from summer heat stress. The present results showed a significant increase in individual motility percentage in case of L-carnitine and zinc in comparison to summer control bucks. Considering our results and the others it is obvious that L-carnitine has a role in conserving the potentiality of sperm motility.The present study showed that summer heat had no significant effect on mass motility compared to winter group. Kafi *et al.* (2004) reported similar result in ram. Ascorbic and coenzyme $Q_{10}$ supplemented bucks showed significant decrease in mass motility, while zinc and L-carnitine showed non significant increase in MM in comparison to summer control group. There were insignificant decreases in individual motility percentage in summer control bucks compared to winter group. This finding matches the result of Nizza *et al.* (2003) in rabbits, Nichi *et al.* (2006) in bull and Chen *et al.* (2003) in human. Another investigations, such as Hussein (2007) in rabbit, Janett *et al.* (2003) in stallion**,** showed that the decrease in motility percentage was remarkable and significant. Ascorbic acid and coenzyme $Q_{10}$ supplementations significantly decreased IM, while L-carnitine supplemented group showed non significant increase in individual motility percentage in comparison with summer control group. In spite of the insignificant changes in mass and individual motility, the motility index values were decreased significantly in summer heat stressed bucks compared to winter ones. Zinc and L-carnitine supplemented groups restored motility index to winter group values. A significant adverse effect of summer heat stress on live sperm percentage was observed in the present study. This is in agreement with the results of Hussein (2007) in rabbits and Janett *et al.* (2003) in stallion. In contrast there was the report of Kafi *et al.* (2004) in ram, meanwhile, Nizza *et al.* (2003) reported no change in rabbits live spermatozoa in relation to heat stress. The increase in dead sperm percentage in semen of bucks reared in summer may be due to spermatozoa susceptibility to oxidative stress that increased during summer heat stress (Aitken and Fisher, 1994). In addition, it may be due to the adverse effect of heat stress on epididymal function, which is under the control of testosterone that is affected negatively by heat stress (Marai and Habeeb, 1998). Ascorbic acid supplemented bucks showed non significant increase in live sperm percentage, while zinc and L-carnitine supplementation restored this parameter to winter group values. There were non significant increases in sperm abnormalities percentage as affected by summer heat stress. This

result is congruent with Hussein (2007) in rabbits and Janett *et al.* (2003) in stallion. On the other hand, numerous literatures showed significant increase in sperm abnormalities as affected by high ambient temperature Nichi *et al.* (2006) in bull, and Chen *et al.* (2003) in human). The increase in total number of defective sperm during summer is consistent with the well known effects of increased temperature on spermatogenesis process, which could lead to generation of high percentages of deformed spermatozoa (Zeidan, 1989). In addition, the harmful effect of the heat stress-induced oxidative stress on early spermatogenesis stages (Nichi *et al.,* 2006) may be another tool of heat stress in this concern. On the contrary, in stallion, Janett *et al.* (2003) and in rabbits, Nizza *et al.* (2003) reported that heat stress did not affect sperm morphology. All antioxidant supplemented groups showed non significant decrease in sperm abnormalities in sperm abnormalities in comparison with summer control group percentage.

Summer heat stress has no effect on sperm concentration in comparison with winter group. The present result is in harmony with the finding of Hussein (2007) in rabbits and Chen *et al.* (2003) in human and dissimilar to the results of Nizza *et al.* (2003) in rabbits, and Janett *et al.* (2003) in stallion. The apparent stability in sperm concentration may be due to the low ejaculate volume, therefore, the total sperm output could be a real parameter as quantitative index. L-carnitine supplementation significantly increased sperm concentration compared to the summer control group. Non significant differences in packed semen volume were noticed in all experimental groups. There were significant decreases in total sperm output in summer heat stressed bucks compared to bucks reared in winter. The present findings correspond to the results of Marai *et al.* (1998, 2002). Contrary to the current results was recorded by Janett *et al.* (2003) in stallion. Chen *et al.* (2003) in human, Kafi *et al.* (2004) in ram and Hussein (2007) in rabbits reported that heat stress showed no change in TSO. The observed sperm output decrease in heat stressed bucks may be due to direct interaction of heat stress-induced ROS with the sperm cell membrane, resulting in impairment of membrane fluidity and permeability and damage of germ cells, spermatozoa and mature sperms (Mishra and Acharya, 2004). Ascorbic acid and zinc supplemented bucks showed significant increases in TSO in comparison with summer control group. Notable significant decrease in total motile sperm was recorded in summer control group compared to winter group. Hussein (2007) reported similar result in rabbits. Meanwhile, Chen *et al.* (2003) found non-significant effect in this concern in human.

Zinc supplementation significantly increased TMS in comparison with summer control group. Summer control group showed significant decrease in total functional sperm

fraction compared to winter group. Zinc supplementation significantly increased TFSF in comparison with summer control group. Literature regarding oxidative stress characteristics in semen in response to heat stress is very limited. The objective of most experiments was to evaluate the effects of prooxidants or antioxidants on the concentrations of TBARS, or on semen quality (Nichi *et al.* 2006). Summer control group showed significant increase in seminal plasma TBARS levels compared to winter ones. In bull, Nichi *et al.* (2006) reported similar result. The high semen concentrations of TBARS in summer than winter suggest that there was more lipid peroxidation in semen during the summer months, presumably due to increased production in the testes (Gil-Guzman *et al.* 2001). Furthermore, the increased TBARS may be due to increased incidence of dead sperms. A note that may proposes an association between lipid peroxidation and sperm quality. The findings suggest that the higher concentrations of TBARS found in seminal plasma during the summer were apparently related to higher levels of ROS, and not due to a lower antioxidant capacity. Fructose concentration in seminal plasma could falsely affect concentrations of TBARS in semen. This carbohydrate, an important source of energy for sperm cells, can react with thiobarbituric acid, falsely increasing TBARS concentrations (Sobenin *et al.* 1998). Initial fructose concentrations in the present results were not affected by heat stress and showed non significant difference between the two seasons.

Therefore, the increased TBARS concentrations during the summer were apparently not due to higher fructose concentrations. All antioxidants supplemented groups showed decrease in seminal plasma TBARS levels than summer control group, which was significant in ascorbic, coenzyme $Q_{10}$ and L-carnitine supplemented groups.

Seminal plasma total antioxidant capacity level showed significant decrease in summer control bucks compared to those reared in winter. Surai *et al.* (1998) in poultry, Zini *et al.* (2000) in human and Nichi *et al.* (2006) in bull, reported no change in seminal plasma TAC. None of the antioxidant supplemented groups showed significant effect on seminal plasma TAC levels in comparison with summer control group. The imbalance between (ROS) production and (TAC) in seminal fluid indicates oxidative stress and is correlated with male infertility. A composite ROS-TAC score may be more strongly correlated with infertility than ROS or TAC alone scientific evidence revealed that a condition known as "oxidative stress" may be in fact a common factor in some of the causes of male infertility. Oxidative stress in the semen occurs when the level of ROS is greater than the TAC. Although, low levels of ROS are needed to normal sperm function, high levels of ROS clearly impair fertility. Antioxidants are substances that protect cellular compo-

nents from damaging oxidative reactions by reaction with the free radicals and other reactive oxygen species. Catalase activity in semen remains a matter of debate, presumably owing to varying degrees of sample purity. Most species have little protective catalase in their semen, but rabbit semen contains substantial amounts of catalase (Foote and Hare, 2000). In the present results, seminal plasma catalase activity showed marked decline in summer control group compared to winter ones. All antioxidant supplemented bucks showed significant increase in seminal plasma catalase activities in comparison with summer control bucks.

## CONCLUSION

The examined semen traits were not much inferior in summer compared to winter, though seasonal variations in semen characteristics were detected. Protective administration of zinc and L-carnitine caused significant improvement in rabbit sperm characteristics; meanwhile, ascorbic acid and coenzyme $Q_{10}$ administration had no considerable enhancement in such concern. Taking the current results into consideration, it can be stated that an adequate reproductive performance in NZW rabbit bucks can be achieved in summer and from the scientific point of view, it is not obligatory to stop breeding in summer months.

## REFERENCES

Aebi H. (1984). Catalase *in vitro*. *Methods Enzymol*. **105,** 121-126.

Aitken R.J. and Fisher H. (1994). Reactive oxygen species generation and human spermatozoa: the balance of benefit and risk. *Bioassays*. **16,** 259-267.

Andrieu S. (2008). Is there a role for organic trace element supplements in transition cow health. *Vet. J.* **176,** 77-83.

Armitage P. (1971). Statistical Methods in Medical Research. 1st Ed., Blackwell Scientific Publications, Oxford.

Blom E. (1977). Sperm morphology with reference to bull infertility. Pp. 1-6 in Proc. 1st All-India Symp. Anim. Reprod. Ludhiana, India.

Chen Z., Thomas T., Linda G., Nelta M., Isaac S. and Russ H. (2003). Seasonal variation and age-related changes in human semen parameters. *J. Androl*. **24(2),** 226-231.

Correa J.R. and Zavos P.M. (1994). The hypoosmotic swelling test: its employment as an assay to evaluate the functional integrity of the frozen-thawed bovine sperm membrane. *Theriogenology*. **42,** 351-360.

Duarte T.L. and Lunec J. (2005). When is an antioxidant not an antioxidant? A review of novel actions and reactions of vitamin C. *Free Radic*. Res. **39,** 671-686.

Duncan D.B. (1955). Multiple ranges and multiple F-tests. *Biometrics*. **11,** 1-41.

Foote R.H. and Hare E. (2000). High catalase content of rabbit semen appears to be inherited. *J. Androl*. **21,** 664-668.

Gil-Guzman E., Ollero M., Lopez M.C., Sharma R.K., Alvarez J.G., Thomas A.J.J. and Agarwal A. (2001). Differential production of reactive oxygen species by subsets of human spermatozoa at different stages of maturation. *Hum. Reprod.* **16,** 1922-1930.

Hussein A.S.M. (2007). Enhancing rabbit performance reared in newly reclaimed areas by alliating the environmental stress. Ph.D. Thesis. Fac. Agri. Cairo University.

Janett F., Thun R., Niederer K., Burger D. and Hassig M. (2003b). Seasonal changes in semen quality and freezability in the Warmblood stallion. *Theriogenology.* **60,** 453-461

Jeulin C. and Lewin L.M. (1996). Role of free L-carnitine and acetyl-L-carnitine in postgonadal maturation of mammalian spermatozoa. *Hum. Reprod. Update.* **2,** 87-102.

Joanna Rogalska J., Brzóska M.M., Roszczenko A. and Moniuszko-Jakoniuk J. (2009). Enhanced zinc consumption prevents cadmium-induced alterations in lipid metabolism in male rats. *Chemico-Biological Interactions.* **177,** 142-152.

Kafi M., Safdarian M. and Hashemi M. (2004). Seasonal variation in semen characteristics, scrotal circumference and libido of Persian Karakul rams. *Small Rumin. Res.* **53,** 133-139.

Kazemi H. and Johnson D.C. (1986). Regulation of cerebrospinal fluid acid-base balance. *Physiol. Rev.* **66,** 953-1024.

Koracevic D. (2001). Method for the measurement of antioxidant activity in human fluids. *J. Clin. Pathol.* **54,** 356-361.

Kozink D.M., Estienne M.J., Harper A.F. and Knight J.W. (2004). Effects of dietary L-carnitine supplementation on semen characteristics in boars. *Theriogenology.* **61,** 1247-1258.

Lebas F., Coudert P., Rouvier R. and de Rochambeau H. (1986). The Rabbit. Husbandry, Health and Production. FAO, Animal Production and Health Series.

Lohrke B., Viergutz T., Kanitz W., Losand B., Weiss D.G. and Simko M. (2005). Hydroperoxides in circulating lipids from dairy cows, implications for bioactivity of endogenous-oxidized lipids. *J. Dairy Sci.* **88,** 1708-1710.

Lord S. and Averill D.A. (2002). Heat shock inactivates cellular antioxidant defenses against hydrogen peroxide: Protection by glucose. *Free Radic. Biol. Med.* **32(8),** 752-765.

Lphsi A. (1990). Livestock and Poultry Heat Stress Indices, Agriculture Engineering Technology Guide. Clemson University, Clemson, SC. USA.

Luck M.R., Jeyaseelan I. and Scholes R.A. (1995). Ascorbic acid and fertility. *Biol. Reprod.* **52,** 262-266.

Macirone C. and Walton A. (1983). Fecundity of male rabbits as determined by 'Dummy mating'. *J. Agric. Sci.* 122-134.

Marai I.F.M., Abd El-Kariem M.A., Zeidan A.E.B. and Selem T.S.T. (1998). Reproductive performance of heat-stressed low fertile male rabbits as affected by types of gn-rh injection. Pp. 423-431 in Proc. International Conference on Animal Production and Health in Semi-Arid Areas, El-Arish, North Sinai, Egypt.

Marai I.F.M. and Habeeb A.A. (1998). Adaptability of *Bos taurous* cattle under hot arid conditions. *Annals of Arid Zone.* **37(3),** 253-281.

Marai I.F.M., Ayyat M.S. and Abd El-Monem U.M. (2001). Growth performance and reproductive traits at first parity of New Zealand White female rabbits as affected by heat stress and its alleviation, under Egyptian conditions. *Trop. Anim. Health. Prod.* **33,** 1-12.

Marai I.F.M., Habeeb A.A.M. and Gad A.E. (2002). Rabbits' productive, reproductive and physiological performance traits as affected by heat stress: a review. *Livest. Prod. Sci.* **78,** 71-90.

Marai I.F.M., Habeeb A.A.M. and Gad A.E. (2005). Tolerance of imported rabbits grown as meat animals to hot climate and saline drinking water in the subtropical environment of Egypt. *Anim. Sci.* **81,** 115-123.

Marder J., Eylath U., Moskovitz E. and Snarir R. (1990). The effect of heat exposure on blood chemistry of the hyperthermic rabbit. *Camp. Biochem. Physiol.* **97A(2),** 245-247.

Mishra M. and Acharya U.R. (2004). Protective action of vitamins on the spermatogenesis in lead-treated Swiss mice. *J. Trace Elem. Med. Biol.* **18,** 173-178.

Nagwa A.A., Azamel A.A., Barkawi A.H. and Morsy A.S. (2006). Thermo-respiratory response and hormonal profile of male rabbits injected with vitamin C under high and low ambient temperature in Egypt. *Egyptian J. Rabbit. Sci.* **16(1),** 61-75.

Nichi M., Bols P.E.J., Zu¨ge R.M., Barnabe V.H., Goovaerts I.G.F., Barnabe R.C. and Cortada C.N.M. (2006). Seasonal variation in semen quality in *Bos indicus* and *Bos taurus* bulls raised under tropical conditions. *Theriogenology.* **66,** 822-828.

Nizza A., Di Meo C. and Taranto S. (2003). Effect of collection rhythms and season on rabbit semen production. *Reprod. Domest. Anim.* **38,** 436-439.

Ohkawa H., Ohishi N. and Yagi K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95,** 351-358.

Prasad A.S., Bao B., Beck J.R., Kucuk O. and Sakar F.H. (2004). Antioxidant effect of zinc in humans. *Free Radic. Biol. Med.* **37,** 1182-1190.

Rastimeshin S.P. (1979). Reproductive performance of male rabbits. *Krolikovodstvoi Zuerovodstvo.* **4,** 30-31.

Sobenin I.A., Tertov V.V. and Orekhov N. (1998). Optimization of the assay for sialic acid determination in low density protein. *J. Lipid Res.* **39,** 2293-2299.

Stephenson J.L. and Brackett B.G. (1999). Influences of zinc on fertilization and development of bovine oocytes *in vitro. Zygote.* **7,** 195-201.

Surai P.F., Blesbois E., Grasseau I., Chalah T., Brillard J.P. and Wishart G.J. (1998). Fatty acid composition, glutathione peroxidase and superoxide dismutase activity and total antioxida-nt activity of avian semen. *Comp. Biochem. Physiol.* **120,** 527-33.

Valko M., Morris H. and Cronin M.T. (2005). Metals, toxicity and oxidative stress. *Curr. Med. Chem.* **12,** 1161-208.

White I.G. (1976). Reproduction in the male. In Veterinary Physiology. J.G. Phillis Bristol: Wright-Scientechnica.

Zeidan A.E.B. (1989). Physiological studies on Friesian cattle. M.S. Thesis. Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

Zeidan A.E.B., Marai I.F.M. and Abd El-Kariem Z.A. (1997). Effects of intratesticular injection of gonadotropin-releasing hormone on reproductive performance of low fertile male rabbits under Egyptian summer conditions. Pp. 557-566 in Proc.

1<sup>st</sup> International Conference on Animal Production and Health. Dokki, Egypt.

Zini A., Finelli A., Phang D. and Jarvi K. (2000). Influence of semen processing on human sperm DNA integrity. *Urology.* **56,** 1081-1084.