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REPRODUCTIVE ACTIVITY OF THE HEAT-STRESSED RABBIT BUCKS AND ITS IMPROVEMENT USING VITAMIN E AND SELENIUM UNDER SUBTROPICAL EGYPTIAN CONDITION

(With 5 Tables and 4 Plates)

By

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النشاط التناسلى فى ذكور الأرانب المعرضة للإجهاد الحرارى وتحسين أدائها باستخدام فيتامين E والسيلينيوم تحت الظروف الشبه الاستوائية المصرية

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أجريت هذه الدراسة في موسم الصيف وتحت ظروف الإجهاد الحرارى على عدد أربعون ذكر وعدد واحد وخمسون أنثى من الأرانب النيوزيلاندى الأبيض. قسمت الذكور إلى أربعة مجاميع فى كل مجموعة ١٠ ذكور. استخدمت المجموعة الأولى كمجموعة مقارنة، والمجاميع الثانية، الثالثة والرابعة كمجموعة معامله. حيث حقنت المجموعة الثانية عضلياً بفيتامين E (١٠٠ وحده دولية/ رأس)، حقنت المجموعة الثالثة بالسيلينيوم (٠,١ مللى/كجم من وزن الجسم الحى) وحقنت المجموعة الرابعة بفيتامين E مع السيلينيوم بنفس الجرعات السابقة. تم قياس التنظيم الحرارى للجسم، مقاييس الخصية، الرغبة الجنسية للذكور، صفات السائل المنوى الطبيعية والكيميائية، معدل الإخصاب والحالة الهستولوجية للخصية. أوضحت النتائج انخفاض درجة حرارة المستقيم معنوياً (على مستوى ٠,٠٥)، فى حين انخفضت درجة حرارة الجلد ودرجة حرارة صوان الإذن بدرجة غير معنوية فى ذكور الأرانب النيوزيلاندى الأبيض المجهدة حرارياً فى فصل الصيف والتى حقنت بفيتامين E أو السيلينيوم بمفرده أو فيتامين E مع السيلينيوم مقارنة بمجموعة الكنترول، بينما انخفض معدل التنفس بدرجة معنوية (على مستوى ٠,٠٥) فى الذكور التى حقنت بفيتامين E مع السيلينيوم فقط . زيادة الرغبة الجنسية وحجم قذفه السائل المنوى والنسبة المئوية لحيوية الحيوانات المنوية وتركيز الحيوانات المنوية وحجم القذفه الكلية بدرجة معنوية (على مستوى ٠,٠٥)، بينما انخفضت النسبة المئوية للحيوانات المنوية الميتة والشاذة وشواذ الأكروسوم بدرجة معنوية (على مستوى ٠,٠٥) فى ذكور

الأرانب النيوزيلاندى الأبيض المجهدة حرارياً فى فصل الصيف والتى حقنت بفيتامينE أو السيلينيوم بمفردة أو فيتامين E مع السيلينيوم مقارنة بمجموعة الكنترول. انخفاض درجة pH السائل المنوى بدرجة غير معنوية، بينما ارتفع تركيز الفركتوز الأولى بدرجة معنوية (على مستوى ٠,٠٥) فى ذكور الأرانب المجهدة حرارياً والتى حقنت بفيتامين E أوالسيلينيوم بمفردة أو فيتامين E مع السيلينيوم عن مجموعة المقارنة. انخفض تركيز الصوديوم فى بلازما السائل المنوى بدرجة معنوية (على مستوى ٠,٠٥)، بينما ارتفع تركيز الكالسيوم، البوتاسيوم والفوسفور الكلى بدرجة معنوية (على مستوى ٠,٠٥) فى الأرانب المجهدة حرارياً والتى حقنت بفيتامين E أوالسيلينيوم بمفرده أو فتيامين E مع السيلينيوم عن مجموعة المقارنة. تحسن وزن الخصية، البربخ والغدد المساعدة، حجم الخصية ومحيط الخصية ودرجة نعومه كيس الصفن بدرجة معنوية (على مستوى ٠,٠٥) فى ذكور الأرانب النيوزيلاندى الأبيض المجهده حرارياً والتى حقنت بفيتامين E أو السيلينيوم بمفرده أو فيتامين E مع السيلينيوم عن مجموعة المقارنة. تحسن الحالة الهستولوجية للخصية فى ذكور الأرانب النيوزيلاندى الأبيض المجهده حرارياً فى فص الصيف والتى حقنت بفيتامين E أو السيلينيوم بمفرده أو فيتامين E مع السيلينيوم عن مجموعة المقارنة. زيادة معدل الإخصاب بدرجة معنوية (على مستوى ٠,٠٥) فى إناث الأرانب النيوزيلاندى الأبيض الملقحة من الذكور المجهدة حرارياً والتى حقنت بفيتامين E والسيلينيوم (٦٠,٨٧%) عن مجموعة المقارنة (٤٢,٨٦%).

SUMMARY

Forty bucks and fifty one New-Zealand White rabbit (NZW) does were used. In the summer, heat- stressed rabbit bucks were divided into four groups (10 each). The first group was kept as control. The second, third and fourth groups (treated), were injected intramuscularly with vitamin E (100 IU/ head), selenium (0.1mg/ Kg body weight) or selenium plus vitamin E weakly at the same dose of the previous groups, respectively. Body thermoregulation, testicular measurements, libido, physical and chemical semen characteristics, fertility rate and histological status of the testis of the summer heat- stressed rabbit bucks in the control and treated groups, were recorded. The results showed that, rectal temperature was significantly ($P<0.05$) lower, while skin temperature and ear lobe temperature were insignificantly lower of the summer heat-stressed NZW rabbit bucks injected with Vit. E or Se alone and Vit. E plus Se than the control group, whereas respiration rate was significantly ($P<0.05$) lower with the bucks injected by Vit. E plus Se only. Libido, semen–ejaculate volume, percentage of sperm motility, sperm-cell concentration and total-sperm output were significantly ($P<0.05$) better, while the percentages of dead spermatozoa, sperm abnormalities and acrosomal damage of spermatozoa were significantly ($P<0.05$) lower of the summer heat-stressed NZW rabbit bucks injected with Vit. E or Se

alone and Vit. E plus Se than the control group. Seminal pH was insignificantly lower, while initial fructose concentration was significantly ($P<0.05$) higher of the summer heat-stressed NZW rabbit bucks semen injected with Vit. E or Se alone and Vit. E plus Se than the control group. Seminal sodium concentration was significantly ($P<0.05$) lower, while calcium, potassium and total phosphorus concentrations were significantly ($P<0.05$) higher of the summer heat-stressed NZW rabbit bucks injected with Vit. E or Se alone and Vit. E plus Se than the control group. Testis weight (gm), epididymis weight (gm), accessory glands weight (gm), testicular volume (cm^3), testis tone firmer score and scrotal circumference (cm) were significantly ($P<0.05$) improved of the summer heat-stressed NZW rabbit bucks injected with Vit. E or Se alone and Vit. E plus Se than the control group. Histological status of the testes of the summer heat- stressed NZW rabbit bucks injected with Vit. E or Se alone and Vit. E plus Se were improved as compared to the control group. Fertility rate of NZW rabbit does mated with the summer heat-stressed bucks injected with Vit. E plus Se was significantly ($P<0.05$) higher (60.87%) than the control group (42.86%).

Key words: *Rabbit semen, heat-stress, vitamin E, selenium, fertility.*

INTRODUCTION

In hot summer, rabbit bucks suffer from disorders in spermatogenesis, libido, semen quality, ejaculatory disturbances and reproductive failure (Zeidan *et al.*, 1997). Undoubtedly, bucks are the basis of the reproductive success in the rabbit farms, but they have not received the attention they should have, mainly if we consider that single buck is affecting the fertility and prolificacy of about one-hundred does, especially with artificial insemination programme (Castellini, 1996).

Alpha-tocopherol acts as a protective agent against polyunsaturated fatty acids peroxidation, the basic action mechanism of vitamin E is focused on the scavenging of peroxyl radicals yielding a non-radical product and the tocophyroxyl radical an unusually stable phenoxyl radical that dose not propagate the radical chain (Liebber, 1993). Vitamin E acts also as a non-specific biological antioxidant (Liu, 1988). Its deficiency causes reproductive failure in rabbit and as supplementation produces favorable adaptive and reproductive responses either in thermoneuteral conditions or when exposed to heat-stress (Hassanein *et al.*, 1995). More specific action of vitamin E is associated

with selenium, in which it protects vital phospholipids from peroxidative change. Combination of vitamin E and sodium selenite have a synergistic effect on enhancement of cell-mediated immunity in rabbit (Liu, 1988). In addition, Vit. E increases animal immunity and Se plays an important role in the immune system functional regulates the metabolism of thyroid hormones (Gore and Qureshi, 1997). Administrated rabbits by Vit. E and Se may play an essential role in correcting the negative nutritional balance of rabbits, during hot summer months (Gore and Qureshi, 1997 and Hughest, 1999). However, such studies in rabbit testicular measurements, semen characteristics and fertility rate during summer season are mostly out of the attention and still obscure.

The present work aimed to study the effects of selenium, vitamin E or both together on the physiological and reproductive performance of the heat-stressed rabbit bucks, under Egyptian hot summer conditions.

MATERIALS and METHODS

The present study was carried out in the Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig, Sharkeiya Province, Egypt (30° N). Forty mature NZW rabbit bucks and fifty-one multiparous lactating does and 3.0- 3.5 Kg of body weight (12 months of age), were used in the present work. The rabbit bucks were healthy and clinically free of external and internal parasites and were raised in flat deck batteries with universal specifications. The batteries were accommodated with feeders and automatic fresh water drinkers and were efficient for hygienic control. Feeding was carried out according to NRC (1977), recommendations. Mean values of air temperatures, percentages of relative humidity, temperature- humidity index (THI) and length of day light (hrs) values in the Rabbitry during the experimental were recorded and shown in Table 1.

The temperature- humidity index (THI) was estimated according to Livestock and Poultry Heat-Stress Indices, Agricultural Engineering Technology Guide, Clemson University, Clemson SC 29634, USA, using the following formula: THI= db° F- (0.55- 0.55 RH) (db°F – 58.00), where: db° F = dry bulb temperature in Fahrenheit and RH = relative humidity (RH % ÷ 100). The obtained values of THI were classified as follows: less than 82 = absence of heat-stress, 82 to < 84 = moderate heat-stress, 84 to < 86 = severe heat-stress, and over 86 = very severe heat –stress.

The rabbit bucks were divided into four groups (10 each) nearly equal in average 3.0 – 3.5 Kg of the body weight. The first group was kept as control. The bucks in the second, third and fourth groups (treated) were injected intramuscularly weekly with vitamin E (100 IU/head as dl-α tochopherol acetate: Cairo Company for Medicine) dissolved in soybean oil, selenium (0.1mg selenium/Kg body weight as sodium selenite) and selenium plus vitamin E at the same dose of the previous groups, respectively.

Before slaughter, testicular volume (cm^3), scrotal circumference (cm) and testis tone firmer score, were recorded. Testes, epididymis and accessory glands were weighed to the nearest gram by an ordinary balance after slaughter. Testicular volume (cm^3) was calculated by multiplying length X breadth X depth of the testis by ordinary caliper as described by Weibel (1989). Scrotal circumference was measured with a flexible cloth measuring tape around the largest diameter of the testis and scrotum placed after pushing the testis firmly into the scrotum (Mickelsen *et al.,* 1982). Testis tone firmer score was determined via manual palpation (scored from very soft and 9: very firm) as described by Wildeus and Hammond (1993).

Rectal temperature, ear lobe temperature, skin temperature and respiration rate were measured at 12.00 a.m. three times weekly during the experimental period. Rectal temperature was obtained gently by inserting the clinical thermometer for 2-3 cm in the rectum for two minutes. Skin temperature (between neck and loin, medial dorsal surface) was measured from one location on the body surface. The thermometer was fixed on the bare skin and on fur which was combed back into place by finger. Ear lobe (in the central area of auricle) temperature was measured by a clinical thermometer. The thermometer was placed into direct contact with the central area of the auricle. Respiration rate was determined by counting the frequency of flank movements per one minute. All possible precautions were taken in consideration to avoid disturbing the rabbit bucks, including counting the respiration breaths just before measuring the body temperature.

Semen was collected from rabbit bucks twice weekly by means of an artificial vagina between 08:00 and 10:00 a.m. Libido, physical semen characteristics (semen- ejaculate volume, percentage of sperm motility, dead spermatozoa and sperm abnormalities, acrosomal damage, sperm- cell concentration and total-sperm output) and chemical semen characteristics (hydrogen-ion concentration, initial fructose concentration, sodium, potassium, calcium and total phosphorus

concentrations), were determined. Libido was recorded as described by Chenoweth (1981). Physical semen characteristics were estimated according to Salisbury *et al.* (1978). Acrosomal damage of spermatozoa was examined by staining films according to Watson (1975). Seminal hydrogen-ion concentration (pH) was measured by Universal Indicator Paper and Standard Commercial Stains according to Karras (1952). Initial fructose concentration was measured according to Barakat and El-Sawaf (1964). Total phosphorous, sodium, potassium and calcium concentrations in the seminal plasma were determined colourimetrically according to the method described by Kuttner and Liechtenstein (1930), Trinder (1951), Sunderman Jr and Sunderman (1958) and Gindler (1972), respectively.

In the fertility trial, fifty-one multiparous lactating NZW rabbit does transferred to the bucks cage to be mated and returned back to its cage after mating. Each doe was subjected to two services from the heat – stressed rabbit bucks (control group) and bucks treated with Vit. E plus Se.

Pregnancy was diagnosed by abdominal palpation at day 10 from the date of service. All does were mated one day after kindling (day of kindling =0). Does were failed to conceive were immediately re-mated after pregnancy testing. Fertility rate at birth was recorded.

After slaughter, five randomly samples of the testes in each group were removed and then fixed in Bouins solution. Representative samples were washed, dehydrated in ascending grades of ethyl-alcohol, cleared and embedded in paraffin –wax. Thereafter, the samples were sectioned at 5 microns thickness and stained with haematoxylin and stained with eosin then examined using 400 X objective of a phase contrast microscope. Histological studies of the testis were recorded.

Data were subjected to analysis of variance according to Snedecor and Cochran (1982). Percentage values were transformed to Arc-sin values before being statistically analyzed. Duncan's new multiple rang test was used for the multiple comparisons (Duncan, 1955). The fertility rates were analyzed using Chi-square test.

RESULTS

Temperature – humidity index (THI)

The temperature- humidity index (THI) estimated in Table 1 indicated exposure of the rabbit bucks to severe heat- stress during summer season.

Body thermoregulation:

Table 2 shows that, the effect of the hot summer season on the rectal temperature of the heat-stressed NZW rabbit bucks injected with Vit. E or Se alone and Vit. E plus Se was significantly ($P<0.05$) lower, whereas respiration rate was significantly ($P<0.05$) lower with the bucks injected by Vit. E plus Se only. While, skin temperature and ear lobe temperature was insignificantly lower in the treated groups than the control group. Results indicated that, rectal temperature, ear lobe temperature and skin temperature were insignificantly difference of the summer heat – stressed NZW bucks injected with Vit. E or Se alone and Vit. E plus Se. While, respiration rate was significantly ($P<0.05$) lower of the bucks injected with Vit. E plus Se than Vit. E or Se alone. The highest ($P<0.05$) value of the rectal temperature and respiration rate of the summer heat-stressed rabbit bucks was recorded in the control group and the lowest ($P<0.05$) value was recorded for the heat-stressed rabbit bucks injected with Vit. E plus Se. The lowest values of the skin temperature and ear lobe temperature were recorded of the summer heat-stressed rabbit bucks injected with Vit. E plus Se and the highest value with the non – injected bucks (control group).

The change rates of the rectal temperature, skin temperature, ear lobe temperature and respiration rate of the summer heat–stressed NWZ rabbit bucks injected with Vit. E or Se alone and Vit. E plus Se were insignificantly negative difference.

Libido and physical semen characteristics

Table 3 shows that, the effect of the hot summer season on libido was significantly ($P<0.05$) better of the heat-stressed NZW rabbit bucks injected with Vit. E or Se alone and Vit. E plus Se than the control group. The longest ($P<0.05$) time of libido was recorded of the summer heat-stressed rabbit bucks (control group) and the shortest ($P<0.05$) time with the rabbit bucks injected with Vit. E plus Se.

Significantly ($P<0.01$) negative change rate in the libido was recorded of the summer heat-stressed NZW rabbit bucks injected with Vit. E or Se alone and Vit. E plus Se.

The effect of the hot summer season on semen-ejaculate volume, percentage of sperm motility, sperm-cell concentration and total-sperm output was significantly ($P<0.05$) higher of the heat-stressed NZW rabbit bucks injected with Vit. E or Se alone and Vit. E plus Se than the control group. However, the percentages of dead spermatozoa, sperm abnormalities and acrosomal damage were significantly ($P<0.05$) lower of the treated heat-stressed bucks than the control group. The highest

(P<0.05) values of semen-ejaculate volume, percentage of sperm motility, sperm –cell concentrate and total-sperm output were recorded of the summer heat-stressed rabbit bucks injected with Vit. E plus Se and the lowest (P<0.05) value with the control group. While, the lowest (P<0.05) values of the percentages of dead spermatozoa, sperm abnormalities and acrosomal damage were recorded with the treated bucks and the highest (P<0.05) values with the control group. Libido, percentages of dead spermatozoa, sperm abnormalities and acrosomal damage decreased significantly (P<0.05), while semen- ejaculate volume, percentage of sperm motility, sperm–cell concentration and total sperm – output increased significant (P<0.05) of the summer heat – stressed bucks injected with Vit. E plus Se as compared to Vit. E or Se alone. The differences between bucks injected with either Vit. E or Se alone were insignificantly for all the previously measurements.

Significantly (P<0.01) positive change rates in the semen-ejaculate volume, percentage of sperm motility, sperm–cell concentration and total-sperm output were recorded of the summer heat-stressed rabbit bucks injected with Vit. E or Se alone and Vit. E plus Se. While, significantly (P<0.01) negative change rate was detected in the percentages of dead spermatozoa, sperm abnormalities and acrosomal damage.

Chemical semen characteristics

Data obtained in Table 4 shows that, the effect of the hot summer season on of the pH value was insignificantly lower of the heat-stressed NWZ rabbit bucks semen injected with Vit. E or Se alone and Vit. E plus Se than the control group. The highest value of pH was recorded of the summer heat-stressed rabbit bucks in the control group and the lowest value in the rabbit bucks injected with Se alone. The pH value insignificantly decreased of the summer heat–stressed bucks injected with Vit. E plus Se as compared to Vit. E or Se alone. The differences between bucks injected with either Vit. E or Se alone were insignificantly for the previously measurement.

Significantly (P<0.01) negative change rates were recorded in seminal pH of the summer heat-stressed NZW rabbit bucks injected with Vit. E or Se alone and Vit. E plus Se.

The effect of the hot summer season on seminal initial fructose concentration was significantly (P<0.05) higher of the heat-stressed NZW rabbit bucks injected with Vit. E or Se alone and Vit. E plus Se than the control group. The highest (P<0.05) value of seminal initial fructose concentration was recorded of the summer heat-stressed rabbit

bucks injected with Vit. E plus Se and the lowest (P<0.05) value was recorded with the control group. The differences between bucks injected with either Vit. E or Se alone were insignificantly for the previously measurement.

Significantly (P<0.05 or P<0.01) positive change rates were recorded in seminal initial fructose concentration of the summer heat-stressed NZW rabbit bucks injected with Vit. E or Se alone and Vit. E plus Se.

Seminal electrolytes concentration (mg/100ml):

Table 4 showed that, the effect of the hot summer season on seminal potassium, calcium and total phosphorus concentrations were significantly (P<0.05) higher of the heat- stressed NZW rabbit bucks semen injected with Vit. E or Se alone and Vit. E plus Se than the control group. However, seminal sodium concentration was significantly (P<0.05) lower of the treated heat-stressed bucks than the control groups. The highest (P<0.05) values of potassium and total phosphorus concentrations were recorded with the summer heat-stressed rabbit bucks semen injected with Vit. E plus Se and Vit. E alone with the calcium and the lowest (P<0.05) value was recorded with the control group. While, the lowest (P<0.05) value of the seminal sodium concentration was recorded with the treated bucks with Vit. E. plus Se and the highest (P<0.05) value was recorded with the control group. The differences between bucks injected with either Vit. E or Se alone were insignificantly for all the previously measurements

Significantly (P<0.01) positive change rates were recorded in seminal potassium, calcium and total phosphorus concentrations, while significantly (P<0.01) negative in seminal sodium concentration of the summer heat-stressed NZW rabbit bucks injected with Vit. E or Se alone and Vit. E plus Se.

Testicular and gonadal measurements:

Data presented in Table 5 shows that, the effects of the hot summer season on the testis weight (gm), epididymis weight (gm) and accessory glands weight (gm) were significantly (P<0.05) heavier of the heat-stressed NZW rabbit bucks injected with Vit. E or Se alone and Vit. E plus Se than the control group. The highest (P<0.05) values of the testis weight (gm), epididymis weight (gm) and accessory glands weight (gm) were recorded with the summer heat-stressed bucks injected with Vit. E plus Se and the lowest (P<0.05) values were recorded with the control group.

The effect of the hot summer season on the testicular volume, testis tone firmer score and scrotal circumference were significantly ($P<0.05$) higher of the heat-stressed NZW rabbit bucks injected with Vit. E or Se alone and Vit. E plus Se. The highest ($P < 0.05$) values of the testicular volume, testis tone firmer score and scrotal circumference were recorded of the summer heat-stressed rabbit bucks injected with Vit. E plus Se and the lowest ($P<0.05$) value was recorded with the control group. Testes weight, epididymis weight, accessory glands weight, testicular volume, testes tone firmer score and scrotal circumference increased significantly ($P<0.05$) of the summer heat–stressed bucks injected with Vit. E plus Se as compared to Vit. E or Se alone. The differences between bucks injected with either Vit. E or Se alone were insignificantly for all the testicular and gonadal measurements.

Significantly ($P<0.01$) positive change rates in the testis weight (gm), epididymis weight, accessory glands weight, testicular volume, testis tone firmer score and scrotal circumference were recorded of the summer heat-stressed NZW rabbit bucks injected with Vit. E or Se alone and Vit. E plus Se.

Fertility rate

Table 6 shows that, the fertility rate was significantly ($P<0.05$) increased of the NZW does that mated with the summer heat- stressed bucks injected with Vit. E plus Se (60.87 %) as compared to the control group (42.86 %).

Histological status in the testes

In the hot summer after the intramuscular injection of the heat–stressed NZW rabbit bucks with Vit. E (Plate 1), Se (Plate 2) and Vit. E plus Se (Plate 3), there is an increase in the activity of the testis as compared with that in the summer without any treatment (Plate 4). So, the injection of the heat–stressed NZW bucks with Vit. E or Se alone improved the activity of the testis, function of spermatogenesis stages, increased the number of the normal, healthy and motile spermatozoa during summer. In addition, intramuscular injection of the heat–stressed NZW rabbit bucks with Vit. E plus Se revealed more activity of the testis in the summer than those injected by Vit. E or Se alone and the control group as shown in Plate 3. The variations among groups may be due to treatment by Vit. E or Se alone and Vit. E plus Se or due to the differences in testosterone hormone levels between bucks.

Table 1 : Mean air temperature (°C), daylight length, relative humidity (%) and temperature-humidity index (THI) values, during the different seasons of the year.

Seasons of the year	Air temperature (°C)		Relative humidity (%)		Temperature-humidity index (THI)		Length of daylight (hours)
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	
Winter	8.86±0.21	19.15±0.35	48.62±0.35	64.33±1.15	45.12	64.78	11.55
Spring	13.60±0.18	24.16±0.18	37.41±0.43	52.64±1.21	64.40	70.93	14.13
Summer	20.84±0.32	34.30±0.46	38.83±0.48	53.66±0.95	65.64	84.63	15.24
Autumn	15.43±0.12	28.62±0.42	42.67±0.62	58.42±1.32	59.21	77.68	13.00

Table 2 : Mean values of the body thermoregulation of the summer heat-stressed NZW rabbit bucks and its amelioration using vitamin E, selenium or vitamin E plus selenium .

Items		Control (Summer)	Treatments					
			Vitamin E	Change (%)	Selenium	Change (%)	Vitamin E + Selenium	Change (%)
Rectal temperature	(°C)	40.12±0.09[a]	39.90±0.08[b]	-0.55	39.91±0.08[b]	-0.52	39.79±0.08[b]	-0.82
Ear lobe temperature	(°C)	36.56±0.13	36.25±0.22	-0.85	36.03±0.61	-1.45	35.80±0.28	-2.08
Skin temperature	(°C)	39.88±0.17	39.32±0.11	-1.40	39.59±0.10	-0.73	39.30±0.13	-1.45
Respiration rate	(r.p.m.)	101.58±0.51[a]	100.11±0.24[a]	-1.45	101.28±0.41[a]	-0.30	99.80±0.47[b]	-1.75

Means bearing different letters within the same classification, differ significantly (P< 0.05).

Table 3 : Mean values of libido and physical semen characteristics of the summer heat-stressed NZW rabbit bucks and its amelioration using vitamin E, selenium or vitamin E plus selenium .

Items	Control (Summer)	Treatments					
		Vitamin E	Change (%)	Selenium	Change (%)	Vitamin E + Selenium	Change (%)
Libido (seconds)	27.45±0.42^{a}	6.18±0.16^{b}	-77.49**	6.10±0.12^{b}	-77.78**	4.12±0.11^{c}	-84.99**
Semen-ejaculate volume (ml)	0.40±0.05^{c}	0.57±0.02^{b}	42.50**	0.61±0.04^{b}	52.50**	0.82±0.04^{a}	105.00**
Sperm motility (%)	54.13±1.03^{c}	62.48±1.25^{b}	15.43**	62.84±1.16^{b}	16.09**	71.40±1.13^{a}	31.90**
Dead spermatozoa (%)	32.17±1.06^{a}	24.05±1.05^{b}	-25.24**	23.86±1.08^{b}	-25.83**	18.16±1.10^{c}	-43.55**
Sperm abnormalities (%)	20.72±1.14^{a}	16.22±1.02^{b}	-21.72**	16.18±1.12^{b}	-21.91**	12.26±1.05^{c}	-40.83**
Acrosomal damage (%)	15.64±0.82^{a}	11.06±0.24^{b}	-29.28**	10.85±0.35^{b}	-30.63**	5.14±0.52^{c}	-67.14**
Sperm-cell concentration (x10^{6}/ml)	127.23±25.18^{c}	240.25±24.17^{b}	88.83**	245.17±29.18^{b}	92.70**	286.28±22.16^{a}	125.01**
Total-sperm output (x10^{6}/ ejaculate)	50.89±16.14^{c}	134.54±13.20^{b}	164.37**	147.70±18.16^{b}	190.23**	234.75±17.24^{a}	361.29**

Means bearing different letters within the same classification, differ significantly ($P < 0.05$).
** ($P < 0.01$)

Table 4 : Mean values of some chemical semen characteristics of the summer heat – stressed NZW rabbit bucks and its amelioration using vitamin E, selenium or vitamin E plus selenium .

Items	Control (Summer)	Treatments					
		Vitamin E	Change (%)	Selenium	Change (%)	Vitamin E + Selenium	Change (%)
Hydrogen-ion concentration (pH)	7.24±0.18^{NS}	7.02±0.15^{NS}	-3.04**	6.80±0.14^{NS}	-6.08**	6.86±0.14^{NS}	-5.25**
Initial fructose (mg/100ml)	253.86±8.12^{c}	278.19±8.15^{b}	9.58*	286.14±7.28^{b}	12.72*	308.16±10.15^{a}	21.39**
Sodium (mg/ 100ml)	125.74±6.31^{a}	114.62±5.84^{b}	-8.84**	116.15±6.04^{b}	-7.63**	112.27±6.13^{b}	-10.71**
Potassium (mg/ 100ml)	5.92±0.82^{c}	7.65±0.81^{b}	29.22**	7.14±0.75^{b}	20.61**	9.82±0.68^{a}	65.88**
Calcium (mg/ 100ml)	5.23±0.17^{c}	6.82±0.28^{a}	30.40**	6.78±0.19^{a}	29.64**	6.12±0.24^{b}	17.02**
Total phosphorus (mg/100 ml)	29.36±1.14^{c}	34.15±1.18^{b}	16.31**	34.28±2.04^{b}	16.76**	38.65±1.27^{a}	31.64**

Means bearing different letters within the same classification, differ significantly ($P< 0.05$).

** ($P < 0.01$) * ($P<0.05$) NS: Not significant

Table 5 : Mean values of the testicular measurements of the summer heat-stressed NZW rabbit bucks and its amelioration using vitamin E, selenium or vitamin E plus selenium .

Items		Control (Summer)	Treatments					
			Vitamin E	Change (%)	Selenium	Change (%)	Vitamin E + Selenium	Change (%)
Testis weight	(gm)	5.86±0.35^{b}	6.72±0.13^{a}	14.68**	6.76±0.12^{a}	15.36**	6.84±0.17^{a}	16.73**
Epididymis weight	(gm)	2.54±0.18^{b}	3.10±0.15^{a}	22.05**	3.14±0.13^{a}	23.62**	3.61±0.17^{a}	24.41**
Accessory glands weight	(gm)	2.08±0.16^{c}	2.82±0.12^{b}	35.58**	2.85±0.14^{b}	37.02**	3.26±0.15^{a}	56.73**
Testicular volume	(cm^3)	4.52±0.18^{c}	5.78±0.15^{b}	27.88**	5.82±0.16^{b}	28.76**	6.54±0.19^{a}	44.69**
Testis tone firmer	(Score)	5.82±0.29^{c}	6.81±0.10^{b}	17.01**	6.86±0.13^{b}	17.87**	7.68±0.11^{a}	31.96**
Scrotal circumference	(cm)	6.32±0.35^{c}	7.58±0.12^{b}	19.94**	7.65±0.10^{b}	21.04**	8.11±0.18^{a}	28.32**

Means bearing different letters within the same classification, differ significantly ($P< 0.05$).

** ($P < 0.01$)

Table 6: Fertility rate of NZW rabbit does mated with the summer heat-stressed rabbit bucks injected with vitamin E plus selenium.

Items	Summer	
	Control	Vitamin E plus selenium
No. of does mated	28	23
No. of does conceived	12	14
Fertility rate	42.86[b]	60.87[a]

Means bearing different letters within the same classification, differ significantly ($P < 0.05$).

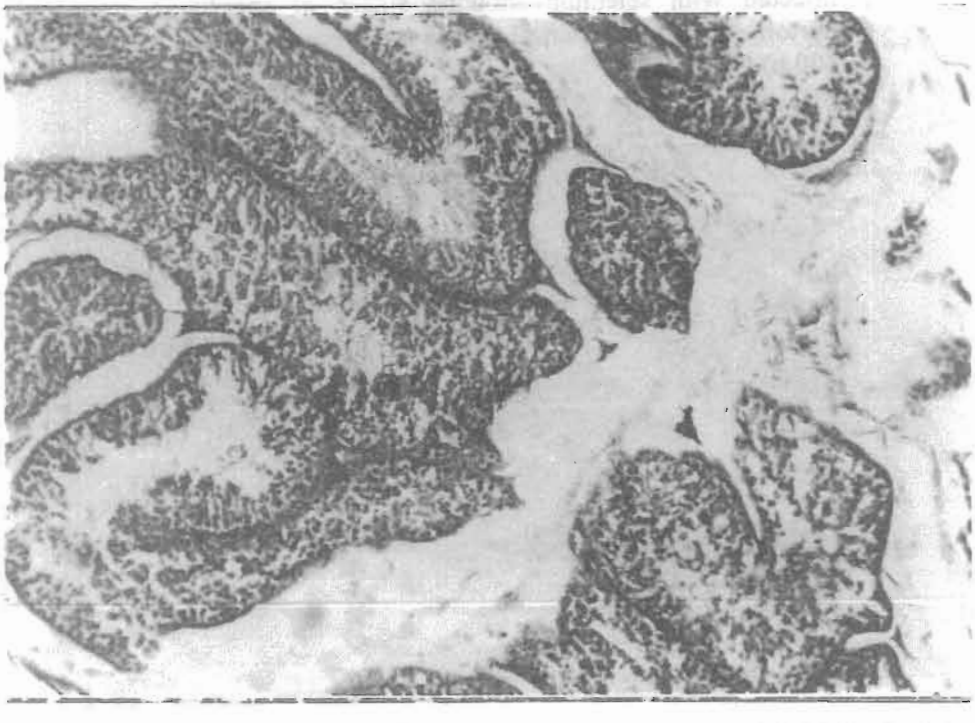

Plate 1: A histological section in the testis of the heat-stressed rabbit bucks injected with vitamin E, showing slightly improvement of the testes and spermatogenesis are present (Stained by H & E at 400 p).

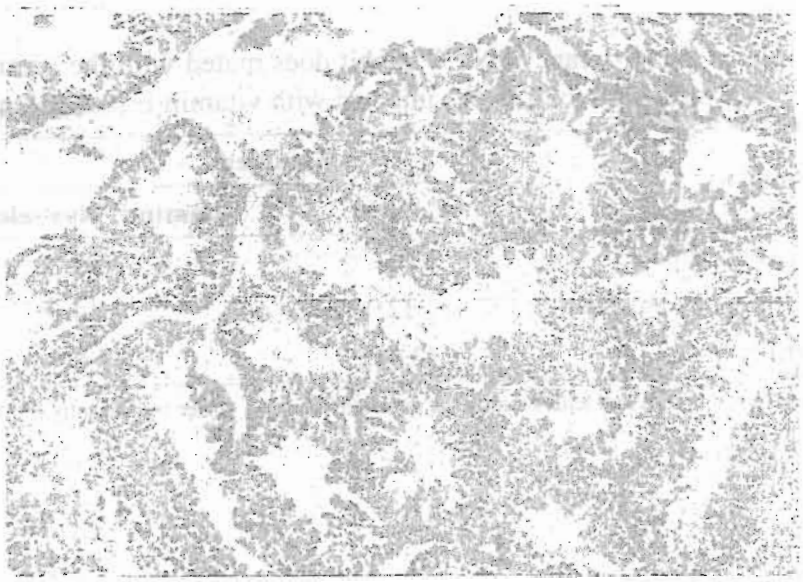

Plate 2: A histological section in the testis of the heat-stressed rabbit bucks injected with selenium showing stages of spermatogenesis and Sertoli cells and interstitial tissues are present (Stained by H & E at 400 p).

Plate 3: A histological section in the testis of the heat-stressed rabbit bucks injected with vitamin E plus selenium showing very clear spermatogenesis stages, active testis and Sertoli cells and interstitial tissues are present (Stained by H & E at 400 p).

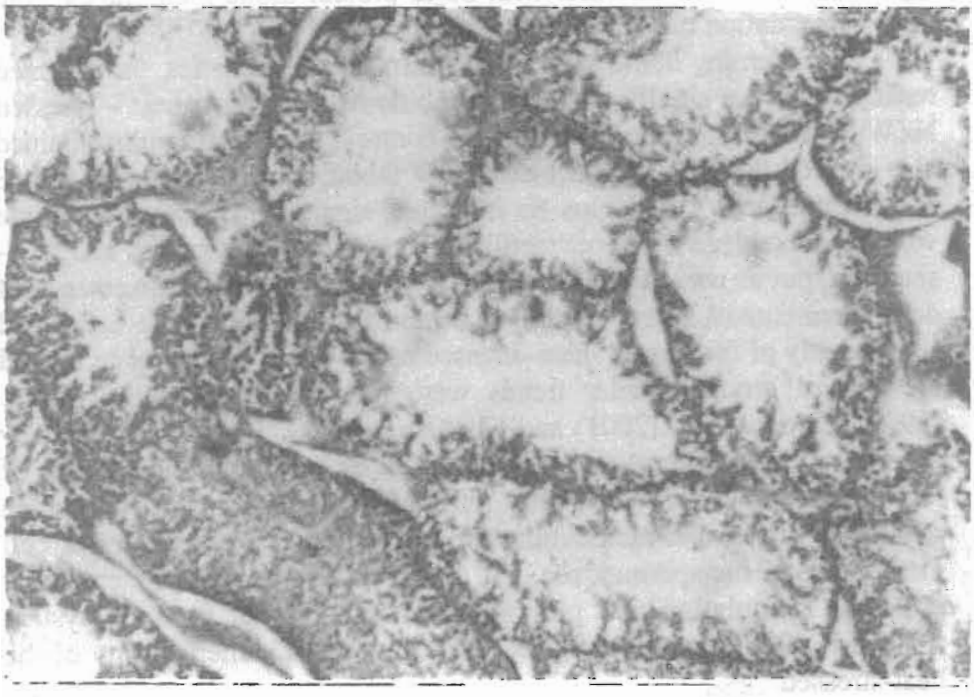

Plate 4: A histological section in the testis of the rabbit bucks during summer revealed inactive testis, no spermatozoa and Sertoli cells (Stained by H & E at 400 p).

DISCUSSION

The results of the present work revealed that, the rectal temperature decreased significantly of the summer heat–stressed NZW rabbit bucks at all treatment groups as compared to the control group, while skin temperature and ear lob temperature decreased insignificantly. Respiration rate decreased significantly in the bucks treated with Vit. E plus Se as compared to the control group. Similar trends were reported by Zeidan *et al.* (2001) and Ghoname (2004). Tizard (1995), Bedmorek *et al.* (1996) and Hamdy and El-Malt (2000) who reported that selenium has beneficial effect on thyroid hormones metabolism and immunity and consequently, improved the biological effect of the physiological traits on the heat-stressed bucks. Furthermore, Vit. E interacts with Se to prevent the oxidative breakdown of cell membranes associated with the hydroproxides of polyunsaturated fatty acids. Gore and Qureshi (1997) and Hamdy and El-Malt (2000) also

reported that Vit. E interacts with Se to protect tissue membranes from lipid peroxidation caused by free radical attack.

Concerning libido and physical semen characteristics, the obtained results showed a significant better in libido of the treated heat – stressed NZW bucks groups as compared to the control group. The results obtained also showed that, the heat–stressed NZW rabbit bucks treated with Vit. E or Se alone and Vit. E plus Se increased significantly semen–ejaculate volume, percentage of sperm motility, sperm–cell concentration and total-sperm output as compared to the control group. While, the percentages of dead spermatozoa, sperm abnormalities and acrosomal damage decreased significantly of the treated heat–stressed rabbit bucks groups as compared the control group. Similar trends were reported by Castellini *et al.* (1999), Zeidan *et al.* (2001) and Ghoname (2004). These findings may be due to the important role of Vit. E and Se on the epithelial cells of reproductive tract of bucks that responsible for acquiring the semen quality or due to the maintaining the viability and permeability of cell membranes of the spermatozoa. Vit. E and Se also have complementary effect on stimulating and promoting the function of immune system (Hughest, 1999). In addition, the improvement of semen quality of the heat-stressed

rabbit bucks also may be due to the biological stimulating and promoting the function of immune system (Hughest, 1999). Hamdy and El–Malt (2000) found that the improvement of semen quality of the heat-stressed may be due to the biological effect of Vit. E on enzymatic oxidation and reduction, nucleic acid metabolism and promoting the activity of oxidized substances such as vitamin A and carotenoids.

With regard to chemical semen characteristics, the heat – stressed NZW rabbit bucks treated with Vit. E or Se alone and Vit. E plus Se decreased insignificantly seminal pH value, while increased significantly seminal initial fructose, potassium, calcium and total phosphorus concentrations as compared to the control group. In contrast, seminal sodium concentration decreased significantly of the treated bucks groups as compared to the control group. These results may be due to the higher sperm-cell concentration of the rabbit bucks treated with Vit. E and Se than the control group. Similar trends were reported by El-Masry *et al.* (1994), Zeidan *et al.* (2001) and Ghoname (2004). The low pH value recorded with the rabbit bucks injected with Vit. E plus Se could be due to the high sperm-cell concentration reflected the important role of Vit. E and Se in semen quality. Castellini *et al.* (1999) and Zeidan *et al.*

(2001) found that fructose concentration was significantly ($P<0.05$) increased of the male rabbits treated with Vit. E alone.

Regarding testicular and gonadal measurements, testis weight, epididymis weight, accessory glands weight, testicular volume, testis tone firmer score and scrotal circumference were tended to be better of the heat–stressed NZW rabbit bucks treated with Vit. E or Se alone and Vit. E plus Se than the control group. Similar trends were reported by Tengerdy *et al.* (1984), Tizard (1995), Zeidan *et al.* (2001) and Ghoname (2004). The reduction in testis weight during summer may be due to exposure of the bucks to heat-stress which due to degeneration in the germinal epithelium and to a partial atrophy in the seminiferous tubules (Chou *et al.,* 1974). In addition, the reduction in the testicular and gonadal measurements may be due to the quantity of spermatozoa or testicular and epididymal fluids which may affect the weight of tail epididymis or may be due to the differences in the development of spermatogenesis (Fujii, 1976).

The results obtained also revealed that, the fertility rate of the NZW does that mated with the heat – stressed bucks treated with Vit. E plus Se was tended to be higher than the control group. Similar trends were reported by El-Masry *et al.* (1994) and Zeidan *et al.* (2003). Castellini *et al.* (1999) confirmed that Vit. E dietary addition for male rabbits insignificantly increased fertility rate. These results may be due to the increase activity of Leydig cells and spermatogenesis process and consequently, improved of semen quality which may be due to the biological effects of Vit. E and Se on enzymatic oxidation and promoting the activity of oxidized substances such as Vit. A or may be due to interaction between Vit. E and Se to prevent the oxidative breakdown of sperm cell membrane.

With regard to histological status, intramuscular injection of the summer heat – stressed NZW rabbit bucks with Vit. E or Se alone and Vit. E plus Se revealed more activity of the testes and spermatogenesis than those without injection. Adamopoulos *et al.* (1990) concluded that in the pre-pubertal rabbit testosterone plays an important role in development and maturation of the testis. Also, Berger *et al.* (1976) found that testosterone may play a role in differentiation and maturation of the rabbit germ cells. These results are in agreement with those obtained by Zeidan *et al.* (1997) and Ghoname (2004). In addition, testes in the rabbit bucks during summer season showed inactive testis, no spermatozoa or sertoli cells and mild testicular degeneration represented by pyknotic nuclei of

the spermatogonia and the lumen of the seminiferous tubules with low spermatocytes (Zeidan *et al.*, 1997).

In conclusion, body thermoregulation, libido, semen quality, fertility rate, testicular activity, histological status of the testes of the summer heat-stressed NZW rabbit bucks injected with Vit. E or Se alone and Vit. E plus Se were better than the un-injected group. Therefore, under Egyptian hot summer conditions, the amelioration of the heat-stressed rabbit bucks using Vit. E plus Se could be used for improving their testicular activity, semen quality and fertility rate as a therapy, simply and applicable techniques for temporary summer sterility which commonly occurs for mostly male rabbits during exposure to hot summer conditions.

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