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EFFECT OF TESTOSTERONE INJECTION ON PRE- AND POST- SEXUAL MATURITY IN MALE RABBITS

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SUMMARY- Thirty -six weaned male NZW rabbits (35 days of age) were used in the present study. The animals were divided into two experimental groups (18 each group). The animals in the first group were injected bi-weekly with 0.5 ml testosterone during the period from weaning up to 4 months of age. The second group was injected with saline solution (control). Growth performance, gonadal development and reproductive efficiency were studied during summer months. Live body weight, daily gain weight and feed conversion were improved insignificantly following testosterone injection. Histological studies showed full complement of germinal elements in the testosterone injected males as compared to the control. The differences between the two experimental groups in plasma testosterone concentrations were not significant. Sperm motility and sperm concentration increased ($P < 0.01$), while abnormal and dead spermatozoa decreased ($P< 0.05$) in the treated animals as compared to the control. However, the ejaculate volume were insignificantly different between the two experimental groups. The effect of month on each of sperm motility and abnormal spermatozoa was significant ($P< 0.05$). The lowest percentages of sperm motility and the highest percentages of abnormal spermatozoa were recorded during July. The interaction effects between testosterone injection and summer months on semen characteristics were not significant. Conception rate was insignificantly higher in the does mated with testosterone treated bucks. Litter size at birth was significantly ($P< 0.05$) increased in the does mated with testosterone treated bucks, while litter size at 21 and 30 days of age (weaning age) showed insignificant differences between the two experimental groups. Conception rate and litter size at different ages were insignificantly higher during June as compared with July and August months. The interaction effects between testosterone treatment and month of kindling on fertility traits were not significant.

Key words: Testósterone, growth performance, gonadal activity, semen, fertility.

Introduction

Exogenous gonadotrophins are widely used to stimulate the secretion of steroids in the prepubertal rabbits (El-Gaafary *et al.*, 1991 and El-Kelawy, 1993) and sheep (Schanbacher and Lunstra, 1977 and Hochereau-De Reviers *et al.*, 1990). In prepubertal cryptorchid boys, gonadotrophins have been also used to evaluate testosterone secretion and to promote testicular descent into the scrotum (Yoshimoto *et al.*, 1975). However, the effects of using exogenous testosterone in prepubertal male rabbits are lacking. Moreover, the efficiency of buck rabbits and testosterone concentrations are tended to be lower in the summer months than in the winter months (Soad *et al.*, 1991 and Boiti *et al.*, 1992).

The aim of this work was to study the effect of testosterone injection on some productive and reproductive traits of male rabbits during pre- and post-sexual maturity, during summer months.

Materials and Methods

This work was conducted at a Rabbit Farm located in the East of the Nile Delta, Sharkia Province, Zagazig, Egypt, during the period from January, 1993 to September, 1993.

Thirty-six weaned male New Zealand White (NZW) rabbits aged 35 days were used in the present investigation. The animals were divided into two experimental groups (18 each group). The first group was injected intramuscularly bi-weekly with 0.5 ml testosterone (Sustanon produced by The Nile Co. for Pharmaceuticals, Cairo, under licence of N.V. ORGANON, OSS, Holland) during the period from weaning up to 4 months of age. The second group (control) was injected with 0.5 ml saline solution (0.9 n Nacl). The differences between the two groups in the initial body weight were not significant. Live body weight (g) daily gain weight (g), feed conversion and viability (%) were recorded during the experimental period. At 4 months of age, 10 males (5 each group) were sacrificed. Blood samples (10 ml) were taken from the rabbits and centrifuged immediately at 800 g for 20 minutes. Plasma was separated and stored at -20 ^{o}C until assayed for testosterone concentration with a double antibody radioimmunoassay (Diagnostic Products Corporation kits). The gonads (testes) were removed, weighed and fixed in Bouin's solution. Representative samples were later washed,dehydrated in ascending grades of ethyl- alcohol, cleared and embedded in paraffin wax. Thereafter, the samples were sectioned at 5 microns and stained in hematoxylin and eosin and examined using a phase contrast microscope. At 5 months of age, semen was collected once weekly over six weeks from ten bucks (5 treated and 5 control) by means of an artificial vagina. Ejaculate volume (ml), sperm motility (%) and dead spermatozoa (%), abnormal spermatozoa (%) and sperm concentration (x10^{6}) were estimated according to El-Gaafary (1987). In the fertility trial, a number of 38 NZW does individually caged in universal wire

Table 1. Maximum and minimum values of ambient temperature (°C) and relative humidity (%) in the rabbitry, during June, July and August under Sharkia Governorate conditions.

Summer months	Ambient temperature (°C)		Relative humidity (%)	
	Minimum	Maximum	Minimum	Maximum
June	22.97± 0.36	30.28±0.54	30.03±1.35	83.00±1.40
July	25.49±0.15	33.81±0.24	45.00±1.22	86.00±0.98
August	24.52±0.14	31.22±0.20	42.23±1.10	89.10±0.70

cages were mated with the two experimental bucks (i.e. hormonal injected and control) to determine their reproductive efficiency, during summer months. Conception rate and litter size at birth (live, dead and total), 21 and 30 days of age were recorded. The animals were fed *ad libitum* and were provided with clean fresh water all times. All rabbits were kept under the same managerial, hygienic and environmental conditions.

Maximum and minimum values of ambient temperature (°C) and relative humidity (%) in the rabbitry during June, July and August under Sharkia Governorate conditions are shown in Table 1.

Analysis of variance was carried out by using complete random design for growth performance traits and by using factorial design for semen quality and reproductive efficiency as described by Snedecor and Cochran (1982). The models used were as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

(for growth performance)

$$Y_{ijk} = \mu + T_i + M_j + TM_{ij} + e_{ijk}$$

(for semen quality and fertility)

where:

Y_{ij} and Y_{ijk}= An observation, μ = The overall mean, T_i = Effect of treatment groups (i= 1 and 2), M_j = Effect of kindling months (j=1,2 and 3), TM_{ij}= The interaction effect between treatment groups and month of kindling and e_{ij} and e_{ijk}= Random error.

Duncan's New Multiple Range test was used for multiple comparisons. Conception rate and viability (%) were analysed by using Chi- square.

Results and Discussion

Growth performance traits:

Results in Table 2 shows that the values of live body weight at 12 weeks (marketing age) or 16 weeks (breeding age) were insignificantly higher in the testosterone treated bucks than in the control. Moreover, daily weight gain, feed conversion and viability (%) were insignificantly different between the two experimental groups. Schanbacher (1984) observed that subcutaneous implantation of testosterone in beef cattle increased growth rate and feed

efficiency and decreased the time to marketing. The presence of androgen and estrogen receptors in skeletal muscle (Snochowski *et al.*, 1981) suggests that anabolic compounds are able to stimulate protein accretion directly. However, Allen *et al.* (1983) were unable to stimulate consistently cell proliferation and protein accretion *in vitro* in muscle cells treated with testosterone.

Gonadal weight and hormonal activity:

It could be seen from Table 3 that the differences in the live weight and testicular weight between the control and injected groups were not significant. Boiti *et al.* (1992) found a positive correlation between body weight, testicle weight, seminal vesicle weight and testosterone levels in rabbits. Moreover, the activities of the testis in the term of testosterone concentration in the two studied groups did not differ significantly. Berger *et al.* (1981) showed that injection of male rabbits at levels 100, 300, 400 and 500 IU HCG during 30, 60, 80, and 100 days of age, respectively, increased the plasma and testis contents of testosterone as compared to the control.

Histological examination

The study showed that at 4 months of age, the external genitalia were easily observed in both testosterone injected and control male rabbits. However, the testes in the injected bucks consisted of seminiferous tubules with a complete spermatogenic cycle (Plate 1- A). At the same time, the spermatogenic cycle appeared arrested almost uniformly at the spermatocytes stage of development in the adjuvant control males (Plate 1- B). No morphological damage was apparent in any of the tubules in both injected and control bucks. Hypophysectomy of young lambs stopped growth of the testes and prevented onset of spermatogenesis, whereas, luteinizing hormone treatment overcome this effect (Echternkamp and Lunstra, 1984).

Semen characteristics

Treatment of bucks with testosterone significantly increased ($P< 0.01$) sperm concentration ($x10^6$) and sperm motility (%) and decreased ($P< 0.01$) abnormal spermatozoa (%) and dead spermatozoa (%) as compared to the control, whereas, the ejaculate volume was insignificantly different between the two experimental groups (Table 4). These data indicate that testosterone may increase the steroidogenic activity of the interstitial cells which may improve semen quality observed (Schanbacher and Lunstra, 1977 and Echternkamp and Lunstra, 1984). In contrast, injection of 100 mg testosterone propionate daily for 15 days restored mounting activity and increased the strength and frequency pelvic thrusting in male rabbits (Beyer *et al.*, 1980).

The effect of summer months on the percentages of sperm motility and abnormal spermatozoa was highly significant ($P< 0.01$). The lowest sperm motility and highest percentages of abnormal spermatozoa were recorded during July. However, the ejaculate volume, sperm concentration and dead spermatozoa were not affected by summer months. Months' changes in semen characteristics have documented by several investigators (Yan *et al.*, 1985 and Marai *et al.*, 1992). The lower value of semen quality was obtained during

Table 2. Growth performance of growing NZW buck rabbits injected with testosterone from 5 to 16 weeks of age.

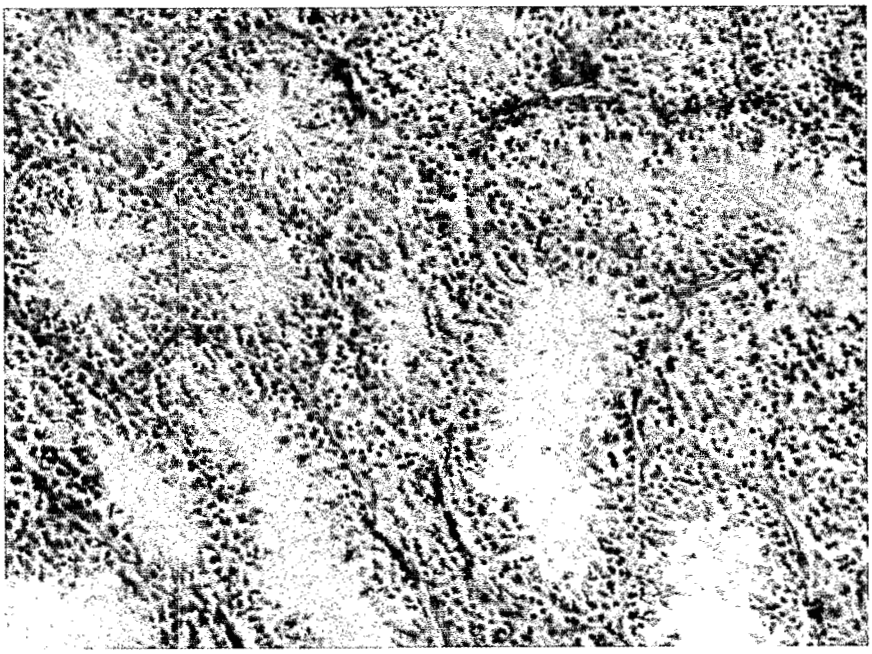
Traits	Control	Injected	Significant test
No. of rabbits	18	18	
Initial weight at 5 weeks (g)	565.28± 29.85	566.94±22.55	NS
Marketing weight at 12 weeks (g)	2058.89±125.70	2061.11±78.07	NS
Breeding weight at 16 weeks (g)	2768.18±76.36	2836.36±62.19	NS
Daily gain weight (g) :			
5 - 12 weeks	26.76±1.56	26.40±0.84	NS
5 - 16 weeks	23.48±1.31	25.49±0.56	NS
Feed conversion (g feed/ g gain) :			
5 - 12 weeks	3.36	3.48	-
5 - 16 weeks	4.47	4.20	-
Viability (%) :			
5 - 12 weeks	94.44	94.44	NS
5 - 16 weeks	94.44	94.44	NS

All values were not significant

Table 3. Effect of testosterone injection on gonadal weight and hormonal concentration of rabbits at 4^{th} month of age (average of 5 rabbits / group).

Traits	Control	Injected
Live weight (g)	2860 ± 6065	2930± 70.50
Tests weight (g)	11.02 ± 0.66	11.70 ± 0.71
(%)	0.39 ± 0.02	0.40 ± 0.02
Testosterone (ng / ml)	3.00 ± 0.06	3.55 ± 0.09

(A)



(B)

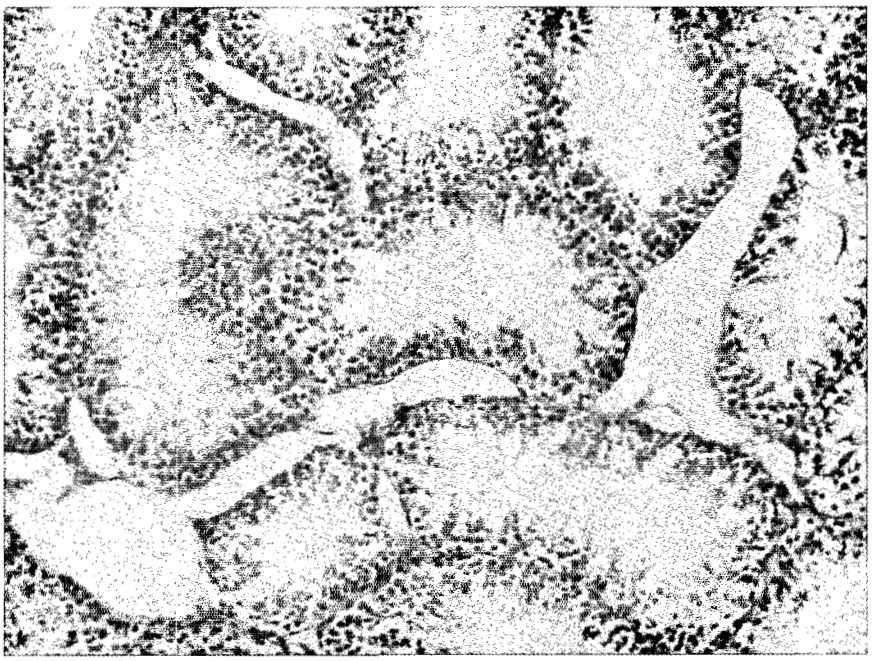


Plate 1. Testis of testosterone injected bucks (A) as compared to the control bucks (B).

Table 4. Semen characteristics of New Zealand White bucks treated with testosterone during summer months

Semen characteristics	Testosterone treatment		Summer months		
	Control	Injected	June	July	August
No. of ejaculates	30	30	20	20	20
Ejaculate volume (ml)	0.40±0.02	0.36±0.02	0.34±0.02	0.40±0.03	0.40±0.04
Sperm concentration (x 10^6)	^{B}177.00±1.40	^{A}192.90±2.10	186.20±3.50	182.30±2.20	186.5±2.6
Sperm motility (%)	^{B}49.60±2.37	^{A}71.30±1.91	^{a}64.90±3.72	^{b}53.70±3.79	^{a}63.4±2.63
Abnormal spermatozoa (%)	^{A}20.10±1.19	^{B}11.90±0.72	^{b}15.30±1.28	^{a}20.20±1.70	^{c}12.2±0.90
Dead spermatozoa (%)	^{A}30.00±1.66	^{B}16.70±0.91	24.10±2.45	24.10±2.32	20.9±1.88

A, B or a, b, c, means in the same row in each classification having different letters, differ significantly ($P< 0.05$).

July month. This may be due to the higher temperature and relative humidity during July than in the other months of the study (Table 1).

The interactions between testosterone treatment and summer months on semen characteristics were not significant.

Fertility traits

Results in Table 5 indicate that conception rate was insignificantly higher in the does mated with testosterone treated bucks as compared to those mated with control bucks. The increase of conception rate in the does mated with testosterone treated bucks may be associated with the improvement of semen quality observed. Similar results were reported by El- Gaafary *et al.* (1991) who observed an increase in conception rate in the does mated with bucks injected with HCG. Litter size at birth increased significantly ($P< 0.05$) does mated with testosterone treated in bucks. However, litter size at 21 and 30 weeks of age showed insignificant differences between the two experimental groups (Table 5).

As shown in Table 5, conception rate and litter size at different ages were insignificantly higher during June as compared to the other months. The better results obtained during June may be due to the lower temperature and relative humidity than the other months (Table 1). Similar results were obtained by Soad *et al.* (1991). The interaction effects between testosterone treatment and month of kindling on fertility traits were not significant.

In conclusion, injection of bucks with 0.5 ml testosterone improved semen quality and increased fertility during summer months. However, testosterone injection for broiler rabbits as a growth promoter is not recommended.