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Asian Journal of Animal and Veterinary Advances

Academic Journals Inc.

www.academicjournals.com

Asian Journal of Animal and Veterinary Advances 7 (8): 693-701, 2012
ISSN 1683-9919 / DOI: 10.3923/ajava.2012.693.701

Study of Testosterone Concentrations during Breeding Season of Two Breeds of Goat Bucks and their Crossbred Under Exogenous GnRH Treatments

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ABSTRACT

Reproduction of goat bucks in the semi-arid area under the semi-extensive system often receives little attention. This study aimed to investigate the effect of goat bucks breeds (Mountain Black, Damascus and Mountain Black-Damascus hybrid) and different months of breeding season on Testosterone concentrations after gonadotropin releasing hormone stimulations. Five from each of bucks (Mountain Black, Damascus and Mountain Black- Damascus hybrid) were used. All of the bucks met the minimum requirements of breeding soundness exam. Testosterone concentrations in response to exogenous gonadotropin releasing hormone stimulations were investigated. Results indicated that there were no significant differences in testosterone concentrations and folds due to breed and months of the breeding season. However, there was a tendency for significant differences ($p<0.1$) between breeds and stages of the breeding season for scrotal circumference. Moreover, there was a positive correlation between scrotal circumference and testosterone folds for all the bucks ($r = 0.1$; $p<0.05$). In conclusion, testosterone secretions under gonadotropin releasing hormone stimulations were not different among breeds of goat bucks and months of their breeding season in the semi-extensive grazing system. Thus, libido was not improved during months of breeding season of the bucks.

Key words: Bucks, testosterone, GnRH stimulations, libido, semi-arid area

INTRODUCTION

Goats in Jordan were estimated to be more than 547 thousand heads (Al-Tamimi, 2005). They are from several native breeds: Mountain Black (MB), Damascus (DG), Dhaiwi, Desert and their crosses (CB) (Zaitoun *et al.*, 2005). MB is highly adapted breed to the arid and harsh environmental conditions of Jordan while its prolificacy and milking ability is moderate (Zaitoun *et al.*, 2004). In comparison, DG is recognized for its prolificacy and milking ability (Guney *et al.*, 2006). Therefore, farmers tend to upgrade their MB herds by crossbreeding with DG bucks.

Success of reproduction in the herd is dependent on both bucks and does. Reproduction of bucks often receives little attention. Bucks fertility is dependent on both semen quality and libido. Jordanian goat herders complain about libido of their bucks during breeding season. Testosterone (T) is responsible for libido in farm animals (Howland *et al.*, 1985; Al-Sobayil *et al.*, 2008). Gonadotropin releasing hormone (GnRH) may be used to stimulate T to higher concentrations. Testosterone (T) concentrations were higher in bucks and rams after exogenous GnRH applications

(Falvo *et al*., 1975; Knight *et al*., 1982; Al-Omari, 2001). Moreover, rams libido and semen quality were improved under GnRH stimulations during non-breeding season (Schanbacher and Lunstra, 1977). Response of T secretions due to exogenous GnRH stimulations may be variable among breeds of bucks and different genotypes. Differences in T concentrations for bucks receiving exogenous GnRH before and after and during breeding season were not tested. Therefore, the objective of this study was to determine the effect of bucks breeds (MB, DG and CB) and different months during breeding season on T concentrations after GnRH stimulations.

MATERIALS AND METHODS

This study was conducted during 2010 breeding season that commence in Jordan from May to September under semi-extensive production system. Goat flocks were located on northern part of Karak Governorate, 31°27'N, 35°74'E and 900 m above sea level.

Animals and feeding program: A total of seventeen bucks were initially examined to be used in this study- five from each of Mountain Black (MB), Damascus (DG) and seven from their crossbred (CB). Goat farmers allow their animals to graze for more than half of the year (May-September) on a permanent base ground and then travel with their animals for long distances (October-April) seeking the warmth at another part of the region (Al-Rousan, 2009). Ample vegetation was available for grazing bucks during the whole breeding season which provided 18 MJ MEday^{-1} and no additional supplements was provided for the bucks. Breeding Soundness Exam (BSE) was applied to all bucks prior breeding season. The general physical examination includes evaluation of the overall animal status, (i.e., feet, legs and eyes). Testicles were examined for scrotal circumference, length, consistency and symmetry. The penis and urethral process were examined during semen collection. Two ejaculates were collected from each buck at two-week-intervals. Bucks were placed on lateral recumbency and a rectal electroejaculator probe lubricated with Vaseline was inserted into the rectum, then semen was collected in a graduated tube (Buckrell *et al*., 1994; Belibasaki and Kouimtzis, 2000). Ejaculate volume, color, consistency, mass motility and forward motility were evaluated immediately in the open yard of the flocks. Both mass motility and forward motility were evaluated as described by Al-Ghalban *et al*. (2004). Sperm concentration, percentages of live-dead spermatozoa and morphology of normal spermatozoa and specific abnormalities were evaluated on the laboratory of animal physiology as described by Dott and Foster (1972). Buck did not meet the minimum requirements for BSE were excluded from the study to have five bucks for each breed group.

GnRH challenge test: All of the three breeds of goat bucks were acted as their own controls. Therefore, T (ng mL^{-1}) were measured under the exogenous GnRH stimulations during three different occasions of their breeding season (Early: May; Mid: July and Late: September). Three small doses of GnRH (5 μg) (Cystorelin®: Ceva, Sante Animal, Libourne Cedex, France) were injected intravenously at 1 h interval and blood samples were collected for 6 h at 30 min interval starting from 06:30 am-12:00 noon. The three GnRH doses were injected after the second, fourth and sixth blood samples. Serum was obtained by allowing the blood to clot at room temperature, centrifuged at 4000x g for 10 min and collected in an Eppendorf tubes. Serum samples were stored at -20°C until assayed for T concentrations using a commercially available enzyme immunoassay test kit (BioCheck Inc, USA). T folds every 30 min post GnRH treatments were calculated by dividing each T concentration after GnRH stimulations over the mean T concentration before GnRH stimulations.

Statistical analysis: The data were statistically analysed using SAS™ program, version 9. Split plot analysis design was applied for the data of scrotal circumference and length (SC and SL) considering breed as a main effect and stage of breeding season as sub plot effect. The data of T and T folds were also analyzed using same design but with time as a sub-sub plot effect. In addition, Pearson correlations among different variables were also calculated using SAS for overall data because of no significant effect among different levels of different factors.

RESULTS

Breeding soundness exam and semen evaluation: Prior the breeding season, fifteen bucks out of seventeen passed the minimum requirements of the BSE. There were no differences in semen measurements among the three breeds of bucks. However, individual motility and normal sperms were numerically higher in CB bucks than the other two breeds (Table 1). Scrotal dimensions were measured for all bucks during different stages of the breeding season. There is a tendency for significant differences ($p<0.1$) between breeds and stages of the breeding season (Table 2).

GnRH challenge test: Testosterone concentrations increased within 60-min of the first GnRH injection in all bucks breeds of during three different stages of breeding season. During Early Breeding Season (EBS), T concentrations continued to increase after the second GnRH dose and maintained high after the third GnRH dose until the end of the collection period for DG and MB bucks. In contrast, In CB bucks, T concentrations continued to increase after the second GnRH dose and maintained high 2 h after the third GnRH dose to rebound at 11:30 and remaintained again (Fig. 1). During mid Breeding Season (MBS), T concentrations for DG, MB and CB bucks declined after the second GnRH dose. Testosterone slightly declined for CB bucks after the third dose of GnRH till the end of collection period. However, T concentrations maintained after the third dose till 10:00 for DG and MB bucks then a slight increase (DG bucks) and decrease (MB bucks) till 10:30, followed by a decline (DG bucks) and maintenance (MB bucks) till 11:30 and eventually rose

Table 1: Mean±SE of semen characteristics in Damascus, Mountain black and hybrid bucks

Items	Damascus	Mountain black	Hybrid
Number	5.0	5.0	5.0
Age (years)	2.8±0.4	3.3±0.4	2.4±0.3
Body weight (kg)	69.0±4.00	69.0±4.80	63.0±3.70
Ejaculate consistency*	3.1±1.10	3.9±1.00	4.6±0.60
Ejaculate volume (mL)	1.3±0.30	1.7±0.30	1.9±0.40
Mass motility (0-5)	2.8±0.40	3.1±0.40	3.7±0.20
Individual motility (%)	64.3±8.50	70.5±8.90	80.7±3.60
Sperm concentration ($\times10^9$ mL^{-1})	0.9±0.20	0.7±0.10	1.0±0.20
Total sperm output/ejaculate$\times10^9$	1.3±0.30	1.3±0.40	2.0±0.63
Live spermatozoa (%)	93.5±1.30	94.3±1.50	87.7±8.80
Normal spermatozoa (%)	47.7±12.4	66.4±12.4	75.0±9.00

*1: Yellow, 2: Yellow watery, 3: White milky, 4: Milky, 5: Thick milky, 6: Creamy and 7: Thick creamy

Table 2: Mean±SE of scrotal dimensions in Damascus (DG), Mountain black (MB) and hybrid (CB) bucks

	Scrotal circumfereuce (cm)		Scrotal length (cm)	
SOV	Mean	SE	Mean	SE
Breeds (B)				
DG	29.5	1.3	14.4	0.7
MB	31.7	1.3	15.7	0.7
CB	28.7	1.3	14.8	0.7
Stages of breeding season (S)				
Early	30.1	0.5	15.0	0.4
Mid	30.4	0.5	15.2	0.4
Late	29.4	0.5	14.7	0.4
B×S				
DG1	28.4	0.8^a	14.4	0.7
DG2	30.4	0.8^a	14.7	0.7
DG3	29.7	0.8^a	14.2	0.7
MB1	32.9	0.8^b	16.1	0.7
MB2	32.4	0.8^b	16.2	0.7
MB3	29.8	0.8^b	14.7	0.7
CB1	29.0	0.8^c	14.6	0.7
CB2	28.5	0.8^c	14.8	0.7
CB3	28.7	0.8^c	15.1	0.7

Within each column, means with different superscript have tendeucy for significant differences (p<0.1)

Table 3: Pearson correlation coefficients between testosterone levels and testosterone folds in response to GnRH stimulations with scrotal dimensions to all of three breeds of bucks

	T	TF
SC	0.02	0.10*
SL	0.10*	0.10*
T		0.04
TF		

*Significant at p<0.05, SC: Scrotal circumference, SL: Scrotal length, T: Testosterone, TF: Testosterone folds

up at 12:00 (DG and MB bucks) (Fig. 1). During Late Breeding Season (LBS), T concentrations maintained after second and third GnRH doses till end of collection period for MB bucks. However, T concentrations slightly decreased after second dose of GnRH for DG and CB bucks. This gradual decline continued for DG bucks after third GnRH dose till 11:00 then followed by a gradual increase for 1 h thereafter. In contrast, In CB bucks, a slight increase in T concentrations obtained after third GnRH dose for 1 h thereafter, followed by a slight decrease and eventually reach maintenance concentrations till end of collection period (Fig. 1).

Testosterone concentrations and folds during stages of breeding season are presented in Fig. 1 and 2. Sampling time showed significant ($p<0.05$) effect on T concentrations. It showed the highest concentrations from 8:00-10:00 a.m. (from 1.5-3.5 h after light on). The interaction of breeds and stages of breeding season was not significant for T concentrations and folds. The highest T folds were recorded during EBS at 8:30, 9:30 and 11:00 am for all the bucks. During LBS, highest T folds were at 8:00 for both DG and CB bucks. In MB bucks, highest T folds during MBS recorded at 8:00 am. Positive correlation was recorded between SL and the other T and T folds. In addition, positive correlation was recorded between SC and T folds ($r = 0.1$; $p<0.05$) (Table 3).

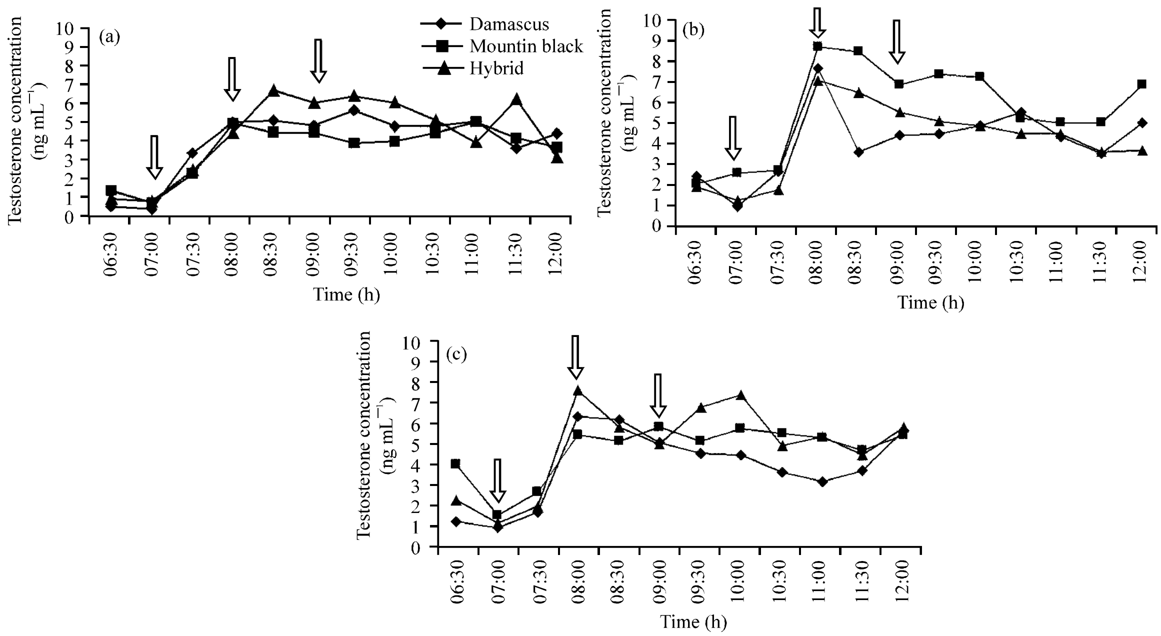

Fig. 1(a-c): Mean testosterone concentrations in response to three doses of GnRH in the three breeds of goat bucks, Arrows indicate the time of GnRH injections, (a) Early breeding season, (b) Mid breeding season and (c) Late breeding season

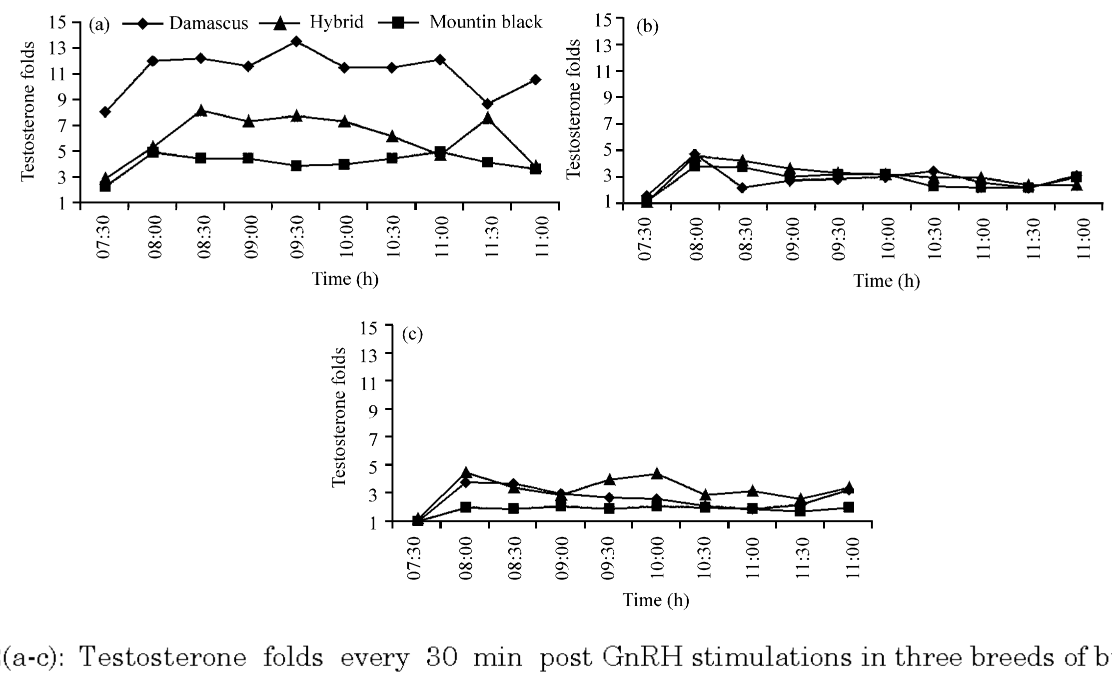

Fig. 2(a-c): Testosterone folds every 30 min post GnRH stimulations in three breeds of bucks, (a) Early breeding season, (b) Mid breeding season and (c) Late breeding season

DISCUSSION

In the present study, semen quality among the three breeds of bucks was similar and met the minimum standards to consider the three breeds as potentially satisfactory breeders. Scrotal Circumference (SC) values of all bucks of the present study were quiet close to those reported in

Zaraibi bucks (Barkawi *et al*., 2006), DG bucks (Al-Ghalban *et al*., 2004) and MB and CB bucks (Kridli *et al*., 2007). In contrast, SC of all bucks of our study were higher than those reported in Nubian goat bucks (Ali and Mustafa, 1986). In the present study, semen quality parameters of the three breeds of bucks passed the minimum requirements for natural breeding of does under the extensive grazing system but did not show any significant difference. Semen quality of DG, MB and CB bucks showed best concentrations during natural breeding of summer and autumn months (Al-Ghalban *et al*., 2004; Kridli *et al*., 2007). Moreover, seasonal semen quality variations are not well recognized in zones between 30° and 40°N but still being higher during summer and autumn. Testicular sperm content obtained highest level in grazing cashmere bucks during late summer and early autumn (Walkden-Brown *et al*., 1994). In this study, semen parameters of DG bucks showed minimum concentrations, however, sperm concentration, total sperm per ejaculate and normal sperms showed higher concentrations in DG bucks raised under intensive system (Al-Ghalban *et al*., 2004). Moreover, previous studies on semen values of DG bucks showed that both ejaculate volume and individual motility were similar to those reported by our present findings (Karagiannidis *et al*., 2000; Al-Ghalban *et al*., 2004). However, in the present study, sperm concentration, total sperm output and mass motility were lower. Both sperm concentration and total sperm output of MB and CB bucks of our study were lower than findings of Kridli *et al*. (2007). In contrast, both individual and mass motility were showed the same level. Ejaculate volume of CB bucks of our study were slightly higher than findings of Kridli *et al*. (2007), although, it showed the same level in MB bucks.

Testosterone concentrations after GnRH stimulations in the present study did not show significant difference between breeds, stages of the breeding season and the interaction between breeds and stages. These concentrations (ng mL^{-1}) were numerically higher during MBS (4.6±0.4) than LBS (4.4±0.4) and EBS (3.9±0.3). Moreover, T concentrations of MB (4.6±0.6) were numerically higher than CB (4.4±0.6) and DG (3.9±0.6) bucks. The above T concentrations were in accordance with other breeds of goat bucks (Georgie *et al*., 1985; Barkawi *et al*., 2006). Ali and Mustafa (1986) reported higher concentrations of T in Nubian goat bucks. In contrast, T concentrations in Pygmy, Zaraibi and DG bucks in Syria during summer and autumn months were higher than those reported in our study (Muduuli *et al*., 1979; Al-Daker and Salhab, 2004; Barkawi *et al*., 2006). The variation in T concentrations may be due to age (Rekwot *et al*., 1997), breeds (Lincoln *et al*., 1990), length of photoperiod, availability of feed and protein content (Clarke, 1981; Wayane and Rissman, 1990; Rekwot *et al*., 1997).

In the present study, T folds every 30 min post GnRH treatments were highly recorded during EBS of all bucks. T concentrations increased 60 min of the first GnRH injection in all bucks and it rose 7-21 folds above mean T concentration before GnRH stimulations. This increase in T concentration in response to GnRH injections is in agreement with that found previously in rats and rams (Falvo *et al*., 1975; Clayton *et al*., 1980; Sharpe, 1982, 1984; Wu *et al*., 1987; Al-Omari, 2001). In contrast, In subfertile stallions, three small doses of GnRH had similar T concentrations compared with fertile stallions (Roser and Hughes, 1992). The increase in T concentration in response to GnRH stimulations might have been due to upregulation of GnRH receptors at pituitary gland where LH stimulates Leydig cell function to secrete more T (Clayton, 1982; Clarke and Cummins, 1987; Clarke *et al*., 1987). In the present study, sampling time showed significant ($p<0.05$) effect on T concentrations after GnRH stimulations. It showed the highest concentrations between 8:00-10:00 a.m. (from 1.5-3.5 h after light on). This is in accordance with distinct episodic peaks of T between 08:00-10:00 a.m. in Bunaji and N'Dama bulls (Sekoni *et al*., 2008). Testosterone peaks of Verata and Malagueña bucks were first observed between 1 and 3 h after dawn, then the second peaks 4-6 h after dawn (Perez and Mateos, 1996). During the whole breeding season, all of the bucks were free access to ample vegetation and they were grazing together with the goats in the same flock, therefore, during this long period all the bucks that met the minimum requirements of BSE will breed sufficient number of goats does, thereafter, semen quality and T concentrations under GnRH stimulations did not show significant differences among breeds, during stages of the breeding season and the interaction of breeds and stages. The tendency for significant differences ($p<0.1$) between breeds and stages of the breeding season for SC and the positive correlation between SC and T folds is probably due to the elevated follicle stimulating hormone in bucks during natural breeding season coincided with an increase in percentage of testicular tissue occupied by seminiferous tubules (Barkawi *et al.*, 2006).

Because measuring T concentrations under GnRH stimulations must be encountered to bucks reared under intensive system, we can't extrapolate, at this point the results derived for bucks reared under semi-extensive production system to be applied on bucks reared under intensive system.

CONCLUSION

Although, T concentrations were higher in bucks after exogenous GnRH application, The results of this study did not meet with the hypothesis that T secretions under GnRH stimulations are variable among breeds of goat bucks and stages of breeding season. GnRH did not improve bucks libido and semen quality during breeding season. Thus, further studies of large number of bucks and more frequent sampling of serum for measuring T concentrations under GnRH and other endocrine stimulations during different stages of breeding season may be required.

ACKNOWLEDGMENTS

The author sincerely thanks the Deanship of Scientific Research at Mu'tah University/Jordan for generously funding this project (S.R. 120/14/237). The author gratefully acknowledges Prof. Mohammad Tabbaa for statistical analysis of data and assistance of Engineer Akef Al-Thenebat for field work of the project.

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