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MONTHLY AND SEASONAL VARIATIONS IN SEMEN CHARACTERISTICS AND SERUM TESTOSTERONE AND TRIIODOTHYRONINE LEVELS IN BARKI, DAMASCUS MALE GOATS AND THEIR CROSSES UNDER SUBTROPICAL CONDITIONS

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SUMMARY

Fifteen sexually mature bucks, 5 each of Barki (B), Damascus (D, imported from Syria) and their crossbred (BD) were used to study semen characteristics and levels of serum testosterone and triiodothyronine (T_3) hormones throughout one year. Results showed that Barki, Damascus, and BD bucks are continuous breeders and produce semen all the year round. Relative testes volume and male sex libido values were better during summer and fall months than those observed in winter and spring months. In addition, levels of serum testosterone hormone were higher in summer followed by fall months than in other months of the year. However, serum T_3 concentrations were higher in winter, spring and fall months than those found during summer months. Goat semen parameters varied between months of the year. Generally, spring and summer months showed higher percentages in abnormal and altered acrosomal spermatozoa than those found in fall and winter months. However, ejaculate volume, sperm output and live sperm showed higher values in summer and fall months, while sperm concentrations were high in spring and summer. The interaction of goat breeds and months of the year was significant in reaction time, sperm motility, ejaculate volume, sperm concentration, sperm output, dead and abnormal sperm and sperm with altered acrosomes.

Keywords: Goats, semen, libido, season

INTRODUCTION

Seasonal variations in the semen of small ruminants have been associated with the weather. Nuti and McWhinney (1987) stated that day length is the most important environmental factor in determining the breeding season and reproductive capacity in goats living in high or mid-latitudes in temperate zones. In temperate areas, hot weather is often associated with low quality semen in goats (Corteel, 1981). However, Roca *et al.* (1992) found that semen quality of Murciano-Granadina goats was highest during summer and fall seasons in Southern Spain (Mediterranean basin). In addition, El-wishy *et al* (1971) reported that Damascus goat semen quality was better during summer months than other months of the year in Egypt. They stated that Damascus goats were largely influenced by changes in relative humidity than by either sunshine or ambient temperature. The aim of the present study is to investigate the monthly variations in reproductive parameters, semen quality and levels of serum testosterone and triiodothyronine hormones in Barki, Damascus and their crossbred goats throughout one year.

MATERIALS AND METHODS

This work was carried out at Bourg El-Arab Research Station, located 50 km west of Alexandria (31^0 15 N and 30^0 10 E), Egypt. Chemical and physical analyses of samples were carried out at the Animal Physiology Laboratory of the Animal Production Department, Faculty of Agriculture, Alexandria University.

Animals and management

In a factorial randomized complete block design experiment, fifteen sexually mature goat bucks were used. Five native Barki breed, weighed between 40-55 kg; 5 Damascus, imported from Syria, weighed between 52-69 kg and 5 crossbred Barki x Damascus, weighed between 52-67 kg were used to study the reproductive performance of these goat groups throughout 12 months and each animal was used as a replicate. All animals were between 2-3 years of age and were kept outdoors under shed during daytime and housed in a semi-open barn at night. Animals were fed roughage and concentrate supplement according to their body weight requirements (Morrison, 1959). Animals were fed Egyptian clover (*Trifolium alexandrinum*) in winter and spring, and chopped green maize in summer and autumn

in addition to hay. Each animal also received 500g/day pelleted concentrate mixture that contained 61% total digestible nutrients (TDN) and 18% crude protein.
Daily temperature, humidity and photoperiod length data were obtained from a Meteorological Station nearby the Research Station.

In terms of sexual performance, testes volume was measured by water displacement method described by Evans and Robinson (1980) then was divided by body weight to calculate the relative testes volume. Sexual behavior of bucks was recorded monthly using the reaction time criterion (the time elapsed between introducing the buck to a female goat at estrus and semen collection).

Semen collection and evaluation

Semen was collected once every month using an artificial vagina. Ejaculates were placed in water bath at 38 ^{0}C. Spermatozoae progressive motility (%) was subjectively estimated at 400X magnification using light microscope equipped with warm stage. Sperm concentration was measured by a haemocytometer slide. Sperm output was calculated by multiplying semen ejaculate volume by semen concentration. The percentages of dead and live spermatozoa were assessed according to Blom's method (1983) and acrosome integrity was evaluated according to the method of Bryan and Akruk (1977).

Blood collection

Blood was collected from the jugular vein of each animal; and was performed four times during the year in February (winter), May (spring), August (summer) and November (fall). Blood samples were centrifuged and sera were collected and stored at –20 °C until analysis.

Hormonal assays

The concentration of triiodothyronine (T_3) in the blood serum was measured using solid-phase enzyme-immunoassay kits obtained from Diatech Diagnostics Inc., Boston, USA. The lower limit of detection (95% B/Bo) was 0.16 ng/ml serum, and the intra- and inter-assay coefficients of variation (CV) were 6.43% and 5.13% respectively based on the mean of low, medium and high quality control samples measured in 12 assays. The concentration of testosterone in sera was determined by solid-phase enzyme immunoassay kits obtained from Medgenix Diagnostics, Fleurus, Belgium. The lower limit of detection (95% B/Bo) was 0.05$\pm$0.02 ng/ml serum, and the intra- and inter-assay coefficients of variation (CV) were 7.17% and 6.87%, respectively based on the mean of low, medium and high quality control samples measured in 20 assays.

Statistical analysis

Statistical analysis was performed using the general linear model procedure of the Statistical Analysis Systems Institute (SAS, 1989). A fixed effect model was assumed to underlay each observation in each trait studied. These effects were breed, month, and the interactions between them. Significant differences among means were evalualted using Duncan's Multiple Range Test of SAS (1989).

RESULTS

Figure 1 shows climatological data during the study. Ambient temperature ranged between 13.3 ^{0}C (in January) and 27.2 ^{0}C in August, while relative humidity ranged between 64.3% in October and 79.0% in March. The range for photoperiod was narrow and was from 10.2 hrs in December and 14.2 hrs in June.

Tables 1, 2 and Figure 2 show the overall mean values of some reproductive and semen parameters in goat breeds throughout one year. Relative testes volume varied significantly ($P<0.01$) between months and between breeds. However, the interaction between months and breeds was insignificant. The months of July and August (summer) showed higher ($P<0.05$) relative testes volume (12.12 and 11.29 cm^3/kg) than those in January, February (winter) or April (spring) (9.10, 8.35 and 8.36 cm^3/kg, respectively). Also Barki showed higher ($P<0.05$) value for testes volume (10.26 cm^3/kg) than those in Damascus or BD crossbred (9.81 and 9.63 cm^3/kg). Generally, relative testes volume showed a decline trend during winter and spring months, then increased during the second half of the year (summer and fall seasons).

Significant ($P<0.01$) differences were found in reaction time between goat breeds, among months and in the interaction between months and breeds (Tables 1&2). Damascus breed showed higher ($P<0.05$) overall mean values in reaction time than those in Barki and their crossbred bucks. It is worthy to note that Damascus bucks showed low sexual desire in January, March and October months. Results of the present study (Table 1) showed that values of ejaculate volume, sperm concentration and total sperm output were generally higher during summer, while percentage of sperm motility tended to be slightly higher during spring. The imported Damascus showed higher overall mean values ($P<0.01$) in ejaculate volume than those in the Barki. Percentages of live, dead and abnormal sperm and those with

altered acrosomes varied significantly (P<0.01) between months of the year (Table 3). However, the effect of goat breed was insignificant in these parameters except for % of spermatozoa with altered acrosomes; the values were higher (P<0.01) in the Damascus breed. On the other hand, the interaction effect between months and breeds was significant (P<0.01) in these parameters (Table 4). The overall mean values for % live spermatozoa were higher during summer and fall. Values of abnormal and dead sperm showed opposite trend. However, high percentage of spermatozoa without acrosomes was found during spring season and during June and July months (Table 3 and Figure 3).

Table 1. Monthly overall mean values of reproductive and semen characteristics in goat breeds throughout one year (least square means ± S.E)

Character	R.Tes.Vol (cm^3/kg)	Reaction time (sec)	Sperm motility (%)	Ejac. Vol. (ml)	Sp. Con. ($x10^9$/ml)	Sperm Output ($x10^9$/ejac)
Month(M)Effect	**	**	**	**	**	**
Dec	9.25 ± 0.27^{c}	7.11 ± 1.50^{bc}	65.33 ± 1.92^{bcd}	0.54 ± 0.09^{d}	3.71 ± 0.28^{de}	1.84 ± 0.28^{f}
Jan	9.10 ± 0.23^{ef}	14.47 ± 3.68^{a}	62.83 ± 4.52^{cd}	1.17 ± 0.15^{a}	5.27 ± 0.46^{b}	6.21 ± 0.97^{ab}
Feb	8.35 ± 0.33^{f}	12.15 ± 1.86^{ab}	61.57 ± 1.80^{cd}	0.69 ± 0.13^{cd}	4.88 ± 0.39^{bc}	2.58 ± 0.46^{ef}
Mar	9.45 ± 0.29^{de}	14.47 ± 2.55^{a}	74.67 ± 2.15^{a}	1.08 ± 0.17^{ab}	6.97 ± 0.48^{a}	7.74 ± 0.13^{a}
Apr	8.36 ± 0.32^{f}	9.94 ± 1.78^{abc}	69.13 ± 1.54^{abc}	0.47 ± 0.05^{d}	5.41 ± 0.42^{b}	2.68 ± 0.43^{ef}
May	9.69 ± 0.22^{de}	8.83 ± 1.72^{bc}	62.50 ± 2.03^{cd}	0.55 ± 0.05^{d}	6.77 ± 0.46^{a}	3.82 ± 0.47^{cdef}
Jun	10.27 ± 0.36^{cd}	11.69 ± 1.93^{ab}	73.00 ± 2.23^{ab}	0.76 ± 0.07^{bcd}	7.16 ± 0.39^{a}	5.92 ± 0.66^{abc}
Jul	12.12 ± 0.48^{a}	9.89 ± 2.42^{abc}	65.56 ± 4.25^{bcd}	0.81 ± 0.09^{bcd}	5.35 ± 0.20^{b}	4.31 ± 0.44^{bcde}
Aug	11.29 ± 0.26^{ab}	4.36 ± 0.80^{c}	51.94 ± 4.80^{e}	0.96 ± 0.18^{abc}	3.41 ± 0.36^{e}	3.88 ± 0.10^{cdef}
Sep	11.11 ± 0.24^{bc}	6.77 ± 0.91^{bc}	64.66 ± 3.63^{bcd}	1.29 ± 0.12^{a}	4.03 ± 0.29^{cde}	5.35 ± 0.77^{bcd}
Oct	10.26 ± 0.23^{cd}	9.78 ± 2.32^{abc}	59.33 ± 4.31^{de}	1.21 ± 0.14^{a}	4.47 ± 0.40^{bcde}	5.62 ± 0.10^{bc}
Nov	9.54 ± 0.21^{de}	8.12 ± 1.51^{bc}	64.66 ± 2.36^{bcd}	0.78 ± 0.18^{bcd}	4.67 ± 0.38^{bcd}	3.25 ± 0.55^{def}

[a-e] Means within each column, with different superscripts differ significantly (P < 0.05).
** P < 0.01;. Means were calculated using 15 male bucks during each month.

Table 2. Breed and month X breed interaction overall mean values of reproductive and semen characteristics in goat breeds throughout one year (least squar means ± S.E)

Character	N	R.Tes. Vol. (cm^3/kg)	Reaction time (sec)	Sperm Motility (%)	Ejac. Vol. (ml)	Sp. Con. ($x10^9$/ml)	Sperm Output ($x10^9$/ejac)
Breed (Br) Effect		**	**	NS	**	NS	NS
Barki (B)	60	10.26 ± 0.17^{a}	7.26 ± 0.80^{b}	62.78 ± 1.72	0.72 ± 0.06^{b}	5.36 ± 0.26	3.86 ± 0.43
Damascus (D)	60	9.81 ± 0.24^{b}	12.96 ± 1.31^{a}	63.94 ± 2.13	0.99 ± 0.09^{a}	4.96 ± 0.23	4.98 ± 0.50
BXD	60	9.63 ± 0.19^{b}	9.17 ± 0.90^{b}	67.08 ± 1.12	0.87 ± 0.05^{ab}	5.20 ± 0.23	4.46 ± 0.32
MXBr effect	180	NS	**	**	**	*	**

[a-e] Means within each column, with different superscripts differ significantly (P < 0.05).
P < 0.05; ** P < 0.01;. NS= Non significant.

Table 3. Monthly overall mean values of sperm morphological characteristics in goat breeds throughout one year (least square means ± S.E)

Character	Live sperm (%)	Dead sperm (%)	Abn sperm (%)	Altered acrosome (%)
Month (M) effect	**	**	**	**
Dec	62.41 ± 5.50^{bc}	15.53 ± 3.66^{bcde}	22.06 ± 2.57^{cd}	8.63 ± 0.74^{de}
Jan	48.90 ± 3.58^{de}	26.84 ± 2.86^{a}	24.26 ± 1.49^{c}	8.71 ± 0.42^{de}
Feb	52.25 ± 3.00^{de}	22.22 ± 3.62^{ab}	27.54 ± 1.82^{c}	10.05 ± 0.71^{cd}
Mar	43.58 ± 3.21^{ef}	20.27 ± 1.81^{abc}	36.15 ± 2.38^{ab}	12.16 ± 1.14^{c}
Apr	37.32 ± 4.37^{f}	22.46 ± 2.42^{ab}	40.32 ± 2.68^{a}	16.64 ± 1.35^{b}
May	47.07 ± 2.90^{de}	22.18 ± 2.71^{ab}	30.74 ± 2.10^{b}	17.65 ± 0.92^{b}
Jun	55.52 ± 2.30^{cd}	10.78 ± 2.13^{e}	33.77 ± 2.16^{b}	20.78 ± 1.04^{a}
Jul	49.87 ± 2.58^{de}	18.07 ± 2.06^{bcde}	32.06 ± 2.41^{b}	20.81 ± 1.55^{a}
Aug	70.56 ± 1.27^{ab}	10.60 ± 1.14^{e}	18.84 ± 1.54^{de}	8.86 ± 0.63^{de}
Sep	72.95 ± 2.29^{a}	11.87 ± 1.78^{de}	15.19 ± 1.08^{ef}	7.43 ± 0.70^{e}
Oct	68.02 ± 3.21^{ab}	19.03 ± 3.65^{bcd}	12.95 ± 1.11^{f}	6.94 ± 0.59^{e}
Nov	73.47 ± 1.96^{a}	13.94 ± 1.96^{cde}	12.59 ± 0.66^{f}	7.03 ± 0.65^{e}

[a-f] Means within each column, with different superscript differ significantly (P < 0.05).
** P < 0.01 , Means were calculated using 15 male bucks during each month.

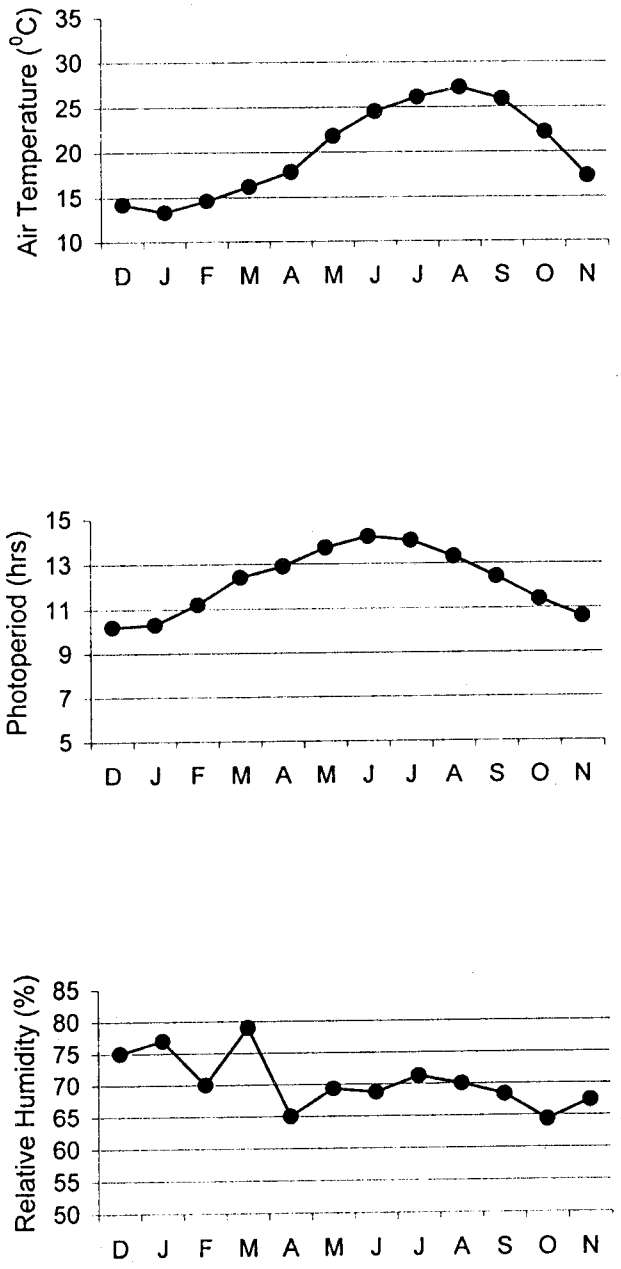

Figure 1. Monthly variations in mean air temperature, photoperiod and relative humidity throughout one year.

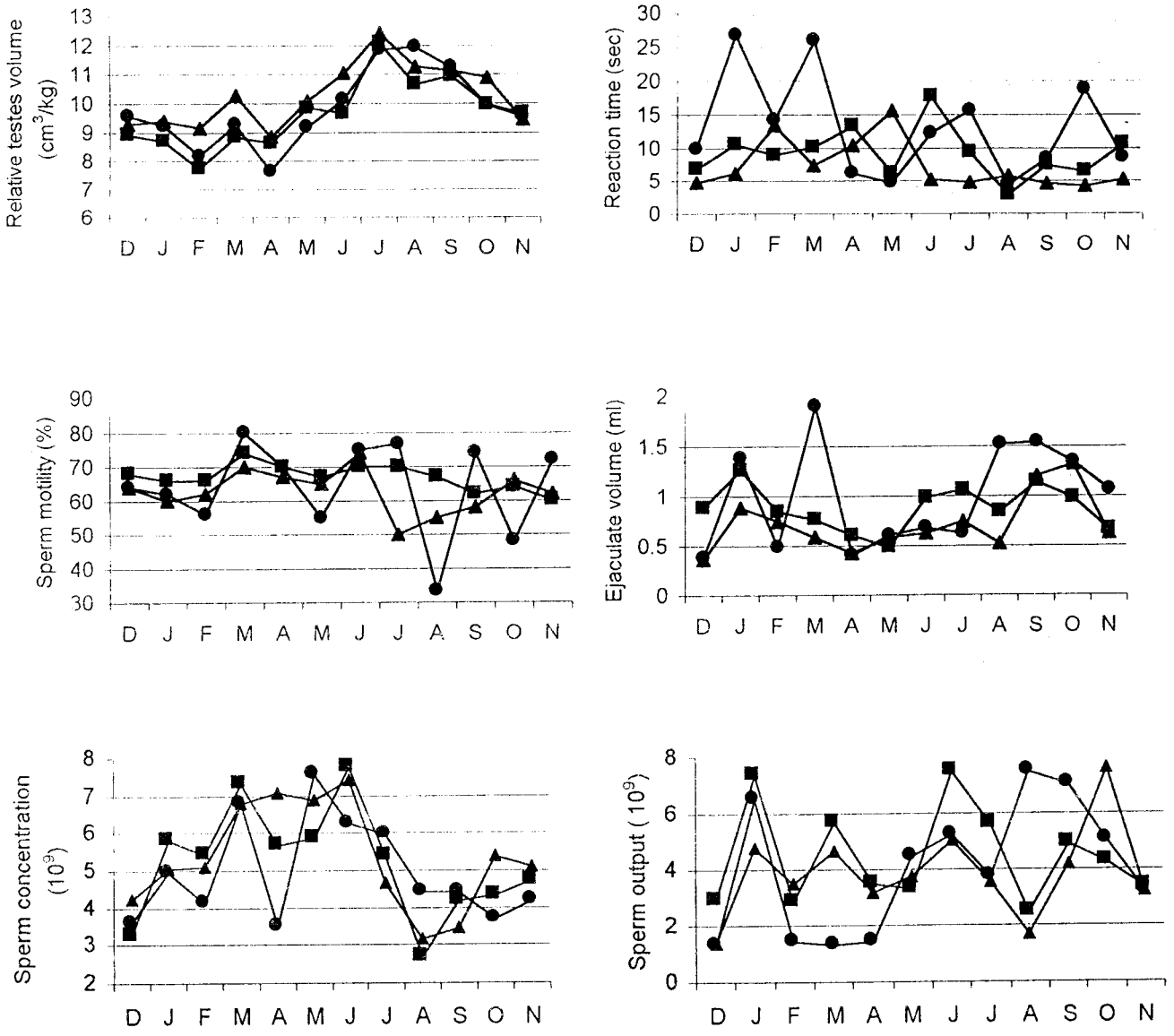

Figure 2. Monthly variations in relative testes volume, reaction time, and semen characteristics in goat bree (Barki ▲ , Damascus •) and their crossbred ■,) throughout one year.

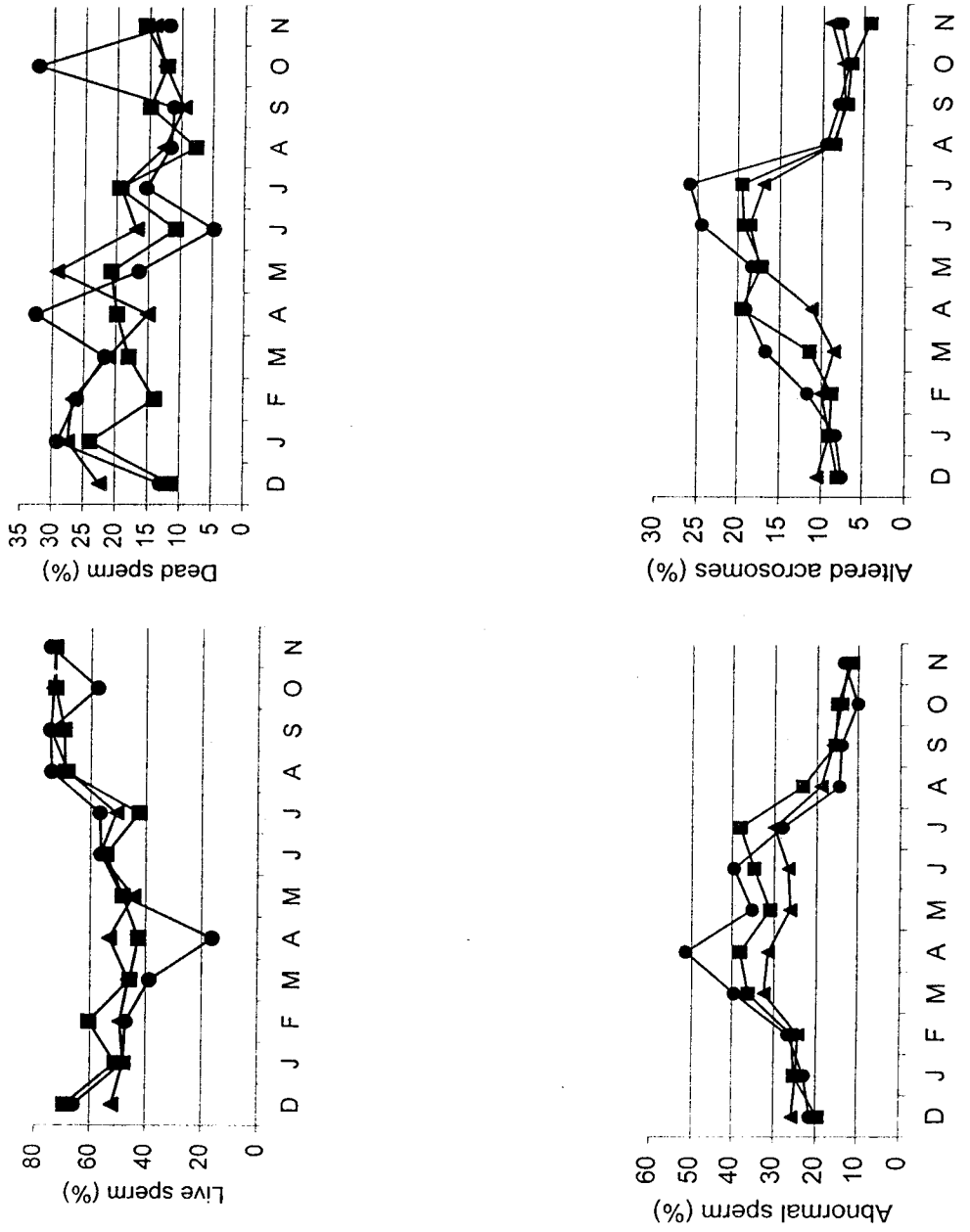

Figure 3. Monthly variations in sperm morphology in goat breeds (Barki ^ , Damascus ?) and their crossbred ¦ ,) throughout one year.

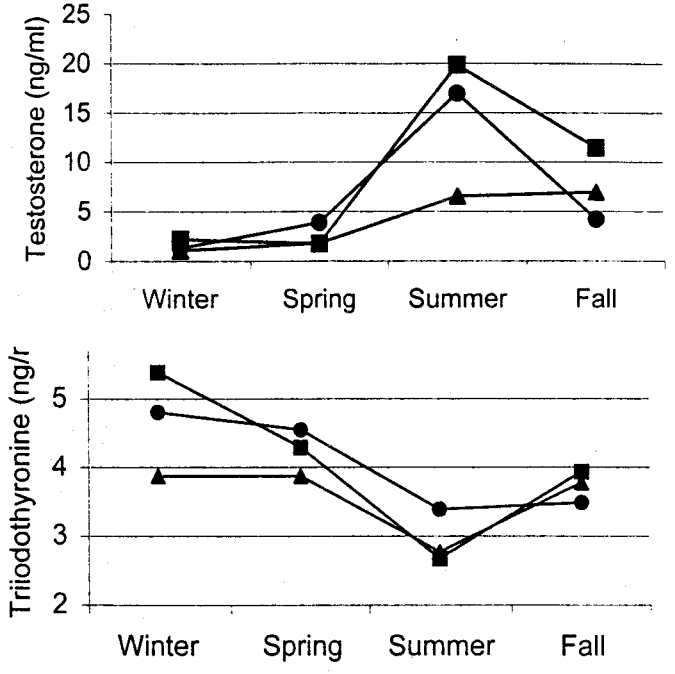

Figure 4. Seasonal values of serum testosterone and triiodothyronine in goat breeds (**Barki ^ , Damascus ?) and their crossbred ¦ ,) throughout one year.**

The hormonal profiles of serum testosterone and triiodothyronine (T_3) hormones during seasons of the year are presented in Table (5) and Figure (4). Season of the year showed significant (P<0.01) effect on testosterone and T_3 levels. However, the effect of goat breed and the interaction between season and breed was significant (P<0.05) only on testosterone values. Testosterone values showed lower (P<0.05) levels in winter and spring than those determined in summer and fall seasons. Summer season showed higher (P<0.05) testosterone values (14.46 ng/ml) than other seasons of the year. On the other hand, serum T_3 values were higher in winter and spring than those observed in summer and fall. BD crossbred bucks showed higher (P<0.05) levels in testosterone than those found in Barki bucks. The interaction between season and breed was significant only (P<0.05) in testosterone hormone values.

Table 4. Breed and month X breed interaction overall mean values of sperm morphology parameters in goat breeds throughout one year (mean ± S.E).

Character	N	Live sperm (%)	Dead sperm (%)	Abn sperm (%)	Altered acrosomes (%)
Breed (Br) effect		NS	NS	NS	**
Barki (B)	60	57.46±2.11	18.93±1.56	23.57±1.14	11.18 ± 0.69^{b}
Damascus (D)	60	54.82±2.40	18.82±1.42	26.38±1.73	13.63 ± 0.89^{a}
BXD	60	58.20±2.00	15.70±1.26	26.17±1.55	11.61 ± 0.79^{b}
MXBr effect	180	**	**	**	**

^{a,b} Means within each column, with different superscript differ significantly (P < 0.05).
** P < 0.01, NS = Non significant.

Table 5. Overall mean values of serum testosterone and triiodothyronine (T_3) in goat breeds throughout seasons of one year (least square means±S.E)

Character	N	Testosterone (ng/ml)	T_3 (ng/ml)
Season (S) effect		**	**
Winter	15	1.54 ± 0.27^{c}	4.69 ± 0.24^{a}
Spring	15	2.48 ± 0.77^{c}	4.24 ± 0.27^{ab}
Summer	15	14.46 ± 2.13^{a}	2.94 ± 0.25^{c}
Fall	15	7.55 ± 1.87^{b}	3.73 ± 0.20^{b}
Breed (Br) effect		*	NS
Barki (B)	20	4.10 ± 1.05^{b}	3.57±0.22
Damascus(D)	20	6.60 ± 1.81^{ab}	4.06±0.26
BXD	20	8.82 ± 1.99^{a}	4.06±0.26
S X Br effect	60	*	NS

$^{a-c}$ Means within each column, with different superscripts differ significantly (P < 0.05).
* P < 0.05; ** P < 0.01; NS = Non significant.

DISCUSSION

The results reported in this study show that Barki, Damascus and their crossbred bucks are continuous breeders as they show sexual libido and produce semen all the year round. However, reproductive parameters and semen characteristics were better during the second half (summer and fall) than those during the first half of the year (winter and spring). This coincided with the high level of testosterone during summer and fall. The opposite trend was found in T_3 level. Damascus bucks were higher (P<0.01) in the overall mean reaction time than that in Barki and their crossbred bucks. El-wishy *et al.* (1971) found that Damascus bucks showed a clear decline in sexual desire during spring and a clear improvement in this parameter occurring during August till November. This is in agreement with the present study. Previous studies on Egyptian local fat-tailed rams (Barki, Ossimi and Rahmani) which were reported by Taha *et al.* (2000); Hafez *et al.* (1955) and El-Fouly *et al.* (1977) showed that semen quality was better during summer months. This trend coincided with the increase in both ambient temperature and photoperiod and relatively low relative humidity in the summer season. Many authors (Folch, 1984; Thimonier *et al.*, 1986) have shown that seasonal variations in goat semen quality is associated with weather. Mukherjee *et al.* (1953) stated that goats have high susceptibility to changes in environment relative humidity. El-Wishy *et al.* (1971) found negative partial correlation coefficient between relative humidity and Damascus bucks' sexual desire and most of the semen characteristics, which indicated the adverse influence of high level of humidity on sexual activity of goats. In addition, Sengupta *et al.* (1963) reported a negative correlation between relative humidity and each of initial motility, concentration of live spermatozoa and storage efficacy of semen of Indian buffalo bulls. Day length is the most important environmental factor in determining the breeding season in small ruminants

within temperate latitudes (Folch, 1984; Thimonier *et al.*, 1986) and the influence of day length on reproductive capacity in goats living in high or mid-latitudes is pronounced (Nuti and McWhinney, 1987). Goat semen output was high during the period of decreasing day length (Corteel, 1981 and Nelson *et al.*, 1987). However, Roca *et al.* (1992) found in the Southern part of Spain (less variation in day length at this latitude) that Murciano-Granadina goat bucks were less affected by day length and Mediterranean temperature and showed better semen quality in summer and fall months. The same observation was found in the present study as the relative humidity was higher during winter and spring months (low semen quality) than that during summer and fall months (high semen quality) (Figure 1). Serum T_3 was significantly higher in winter and spring than in summer and fall. Moenter *et al.* (1991) and Webster *et al.* (1991) reported that thyroid hormones play a key role in the expression of the seasonal reproductive cycle in ewes, because they increase the responsiveness to estradiol negative feedback that causes termination of the breeding season. These findings could be extrapolated to those of the present study since the highest T_3 level was associated with the lowest ambient temperature, photoperiod, serum testosterone level, relative testis volume, and other semen parameters. The high levels of testosterone and T_3 in Damascus and DB crossbred than those in Barki bucks could be due to breed differences. Similar trend to our results was observed in southern sheep breeds which showed maximum testicular activity in summer and minimum activity in winter (Gomes and Joyce, 1975; Lincoln *et al.*, 1990). However, in the northern breeds, the maximum testicular activity occurred in the fall (Lincoln *et al.*, 1990).

In the present study, the maximum sperm motility, sperm concentration, dead and abnormal sperm and high altered acrosome spermatozoa were recorded in the spring season. Summer and fall months showed moderate levels of percentages abnormal and altered acrosome spermatozoa but high ejaculate volume and % live spermatozoa. Barki (subtropical native breed), Damascus (temperate exotic breed) and their crossbred goat semen qualities were better during summer and fall months than during winter and spring months. This may be attributed to the acclimatization of males to elevated ambient temperature with continued spermatogenesis (Sod-Moriah *et al.*, 1974), and coincided with the high level of serum testosterone and the presence of male libido during summer and fall seasons.

In conclusion, the present study showed that semen quality parameters were better during summer and fall months and that Barki, Damascus and their crossbred goats could be arranged to be bred successfully under subtropical conditions in summer.

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