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Monthly Variation of Plasma Concentrations of Testosterone and Thyroid Hormones and Reproductive Characteristics in Three Breeds of Iranian Fat-Tailed Rams Throughout One Year

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Abstract: Nine sexually mature rams, three Shall, three Afshari and three Zandi were used in this study. Semen of rams was collected twice per month during the four seasons of the year and blood samples were obtained via jugular vein. Seminal characteristics, scrotal dimension and blood parameters were measured at 2 week intervals. Time of the year significantly ($p<0.05$) affected the volume of semen, sperm concentration, percentage live sperm, percentage abnormal sperm, sperm mass motility, total sperm number, scrotal circumference, relative testis volume and serum levels of testosterone, triiodothyronine and thyroxine. All of semen characteristics, except sperm abnormality, were high in summer months than at other months of the year ($p<0.05$). Sperm abnormality was higher in autumn months ($p<0.05$). Relative testis volume and testicular circumference were higher in autumn and summer months than at other mouth of the year, respectively ($p<0.05$). Scrotal circumference were not significant between Shall and Afshari breeds but comparison with Zandi breed, Scrotal circumference was bigger ($p<0.05$). The largest values for thyroid hormones synchronized with low reproductive performance of rams, but testosterone was highest levels at this time ($p<0.05$). Time×Breed interaction effect was significant for sperm concentration, total sperm number, semen volume, testis circumference, relative testis volume, thyroid hormones and testosterone ($p<0.05$).

Key words: Scrotal circumference, semen, thyroid hormones, testosterone, fat-tailed ram

INTRODUCTION

In Iran, the native sheep breeds are year-round breeders but the intensity of sexual activity varies with seasons of the year. Few studies have been carried out on males to investigate variation in their reproduction but reliable data concerning the reproductive characteristics of Iranian rams are very scare (Zamiri and Izadifard, 1997; Kafi *et al*., 2004; Zamiri and Khodaei, 2005).

Sheep in temperate latitudes are seasonal breeders of the different seasonal cues; photoperiod is the most reliable parameter and is used by animals as an indication of the time of the year to synchronize endogenous annual rhythms of reproduction and physiology (Thiery *et al*., 2002). Rams exhibit seasonal fluctuation in sexual behavior, hormonal activity, gametogenesis and also in testicular weight and volume (Zamiri and Izadifard, 1997; Thiery *et al*., 2002). Thyroid hormones seem to have a key role in regulating seasonal reproduction in sheep. Thyroid activity is affected by season (Hafez, 1959) and the absence of thyroid hormones abolishes normal reproductive endocrine function in rams (Souza *et al*., 2002). Parkinson and Follett (1994) showed that hypothyroidism abolished the seasonal inhibition of reproductive ability and regression of testis in Welsh Mountain rams and suggest that thyroid gland may be involved in seasonal transition of reproductive activity in ram.

In Iran, Zamiri and Khodaei (2005) showed that serum testosterone level was lowest from April to July and increase thereafter reaching highest values in November and remaining high until March in Ghezel and Mehraban rams. Plasma testosterone concentrations decreased in spring (non-breeding season) and were highest at the peak of breeding season in Texel, Suffolk and Ile-de Frence rams (Mandiki *et al*., 1998).

Semen superior quality and quantity and testicular dimensions were especially recorded in late summer and throughout autumn in Karakul rams (Kafi *et al*., 2004) and Ghezel and Mehraban rams (Zamiri and Khodaei, 2005). Relative testis volume, ejaculate volume, sperm concentration, total sperm output, sperm motility and percentage live sperm were higher during summer months than at other months of the year (Taha *et al*., 2000).

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The aim of the present study was to evaluate monthly changes in plasma concentrations of testosterone and thyroid hormones, testicular size and seminal characteristics of three Iranian fat-tailed sheep breeds.

MATERIALS AND METHODS

Nine sexually mature rams, three each of Afshari, Shall and, Zandi (the native breeds in Iran), 3-4 years of age were used in this study. The study was carried out at the Animal Science Research Station (35°56′N, 50°58′E), College of Agriculture, Tehran University from October 2003 to September 2004. All animals were housed in a cover shelter with an open-air run allowed to walk freely. Animals were clinically examined regarding the health of their external genitalia. Rams were fed a mixture of straw-hay and barley according to the recommended requirements of the NRC throughout the year. Water and sheep mineral blocks were available and *ad libitum*. Rams were weighed at monthly intervals.

Seminal characteristics, relative testis volume, testicular circumference and, blood samples were measured two week intervals throughout one year. In terms of sexual performance, scrotal sac volume was measured by the water displacement method described by Evans and Robinson (1980) and then divided by body weight to calculate the relative testis volume. Semen was collected using artificial vagina. Ejaculates were placed in a water bath at 38°C. Mass motility (on a scale of 0 (immotile) to 5 (vigorous motility) was subject estimated at 400×magnification using a light microscope equipped with a warm stage. Sperm concentration was measured by a haemocytometer slid. Total sperm concentration calculated by multiplying semen ejaculate volume and semen concentration. Sperm morphological characteristics were assessed using the nigrosin-eosin staining technique (Evans and Maxwell, 1987). Scrotal circumference was measured with a tape-measure.

Blood samples were collected by heparinized tubes from the jugular vain of each animal, two times during each month of the year and immediately placed on ice. The blood samples were transported to the laboratory and centrifuged at 3000 rpm for 10 min. Blood plasma were collected and stored at -20°C until analysis. Thyroxine, triiodothyronine and testosterone were measured using radioimmunoassay technique. Radioimmunoassay was performed using commercial kits of triiodothyronine (Biosource Co., Belgium; minimum detectable concentration of 0.15 nmol L^{-1} and intra- and inter-assay coefficient of variation was 1.35 and 4.55%, respectively), thyroxine (Biosource Co., Belgium; minimum detectable concentration of 0.4 nmol L^{-1} and intra and inter-assay coefficient of variation was 3.24 and 6.75%, respectively) and testosterone (Radim Co, Italy; minimum detectable concentration of 0.35 nmol L^{-1} and intra and inter-assay coefficient of variation was 5.4 and 7.5%, respectively). Data were analyzed using the procedures of general linear model (GLM) (SAS, 1996). Significant differences among means were detected using Duncan's multiple range tests of SAS (1996).

RESULTS

Overall means values of semen parameters throughout one year in three ram breeds are present in Table 1. Semen volume was significantly affected by month of the year ($p<0.001$) and between breeds ($p<0.001$). Semen volume was greater in Afshari rams than Shall and Zandi rams and greater in September ($p<0.05$) than other months of the year in three breeds (Table 2). Sperm concentration was significantly different between breeds and the months of the year ($p<0.001$) and the highest sperm concentration (418.56×10^7) of three breeds was that in September ($p<0.05$). Mass motility was not significantly different between Shall and Afshari breeds but was significantly different with Zandi breed ($p<0.05$). Mass motility in three breeds was the highest (4.93) in September ($p<0.05$). Total sperm concentration was

Table 1: Monthly overall values of seminal characteristics in three breeds throughout one year*

Months	Semen volume (mL)	Total sperm concentration ($\times10^7$)	Sperm concentration ($\times10^7$)	Mass motility (0-5)	Live sperm (%)	Sperm abnormality (%)
October	1.13^{b}	438.80^{b}	376.60^{b}	4.69^{ab}	64.62^{d}	18.25^{a}
November	1.06^{bc}	385.20^{cdef}	317.65^{ef}	47.31^{b}	61.43^{g}	12.50^{f}
December	0.95^{cd}	291.80^{fgh}	303.64^{fg}	3.75^{c}	68.93^{b}	10.43^{e}
January	0.95^{cd}	275.06^{gh}	261.38^{hi}	3.06^{de}	63.56^{f}	8.93^{f}
February	0.90^{d}	228.07^{h}	248.31^{i}	3.37^{cd}	58.06^{k}	12.00^{d}
March	0.87^{d}	236.46^{h}	291.63^{fgh}	2.81^{e}	60.37^{h}	11.75^{d}
April	1.06^{bc}	374.20^{bcde}	347.40^{bcd}	2.93^{de}	63.62^{f}	11.75^{d}
May	1.21^{b}	383.13^{bcd}	313.71^{defg}	3.06^{de}	64.31^{e}	11.87^{d}
June	1.15^{b}	326.75^{def}	281.32^{ghi}	4.30^{b}	59.81^{i}	13.62^{b}
July	1.03^{bc}	312.88^{efg}	336.37^{cde}	4.25^{b}	66.37^{c}	8.87^{f}
August	1.12^{b}	415.13^{bc}	366.36^{bc}	4.50^{ab}	58.31^{j}	11.68^{d}
September	1.39^{a}	567.06^{a}	418.54^{a}	4.93^{a}	76.18^{a}	5.56^{g}
SEM	0.022	6.61	5.16	0.069	0.42	0.291

*Mean for each effect in the column, with different superscript differ significantly ($p<0.05$)

Table 2: Breed overall mean values of seminal characteristics in three ram breeds throughout one year*

Breed	Semen volume (mL)	Total sperm concentration ($\times 10^7$)	Sperm concentration ($\times 10^7$)	Mass motility (0-5)	Live sperm (%)	Sperm abnormality (%)
Shall	0.980^{b}	329.680^{b}	328.220^{b}	3.910^{a}	63.250^{c}	11.540^{a}
Afshari	1.320^{a}	442.320^{a}	347.910^{a}	3.980^{a}	63.560^{b}	11.580^{a}
Zandi	0.900^{c}	268.860^{c}	292.650^{c}	3.630^{b}	64.400^{a}	11.220^{b}
SEM	0.031	11.296	5.846	0.079	0.699	0.357

*Mean for each effect in the column, with different superscript differ significantly ($p<0.05$)

Table 3: Monthly overall values of testicular measurements in three breeds throughout one year*

Months	Relative testis volume ($cm^3\ kg^{-1}$)	Scrotal circumference
October	10.720^{b}	36.620^{a}
November	11.340^{a}	34.870^{cd}
December	6.800^{e}	31.120^{h}
January	6.060^{g}	29.870^{i}
February	5.910^{g}	29.750^{i}
March	6.710^{e}	31.680^{g}
April	6.230^{fg}	31.620^{g}
May	7.220^{de}	32.870^{f}
June	7.600^{d}	33.560^{e}
July	6.750^{e}	34.420^{d}
August	8.820^{c}	35.120^{c}
September	10.260^{b}	35.930^{b}
SEM	0.904	0.336

*Mean for each effect in the column, with different superscript differ significantly ($p<0.05$)

Table 4: Breed overall mean values of testicular measurements in three ram breeds throughout one year*

Breed	Relative testis volume ($cm^3\ kg^{-1}$)	Scrotal circumference
Shall	8.210^{b}	33.680^{b}
Afshari	9.710^{a}	35.450^{a}
Zandi	6.230^{c}	30.430^{c}
SEM	0.252	0.308

*Mean for each effect in the column, with different superscript differ significantly ($p<0.05$)

Table 5: Mean monthly values of plasma thyroxine, triiodothyronine and testosterone in three breeds throughout one year*

Months	Triiodothyronine (nmol L^{-1})	Testosterone (nmol L^{-1})	Thyroxine (nmol L^{-1})
October	15.270^{a}	1.450^{g}	19.540^{g}
November	6.510^{d}	1.430^{gh}	17.170^{h}
December	5.190^{ef}	2.330^{b}	24.810^{cd}
January	4.250^{efg}	1.430^{gh}	23.350^{ef}
February	4.040^{fg}	1.360^{i}	24.660^{cd}
March	2.290^{h}	1.800^{c}	20.150^{g}
April	4.370^{efg}	2.350^{a}	33.680^{b}
May	3.420^{g}	2.290^{c}	29.280^{c}
June	5.280^{e}	2.030^{d}	36.190^{a}
July	4.120^{efg}	1.890^{e}	25.720^{d}
August	8.720^{c}	1.420^{h}	22.780^{f}
September	13.720^{b}	1.070^{j}	18.830^{g}
SEM	0.308	0.054	0.736

*Mean for each effect in the column, with different superscript differ significantly ($p<0.05$)

Table 6: Breed overall mean values of testicular measurements in three ram breeds throughout one year*

Breed	Testosterone (nmol L^{-1})	Triiodothyronine (umol L^{-1})	Thyroxine (nmol L^{-1})
Shall	5.590^{c}	1.510^{c}	22.220^{c}
Afshari	6.410^{b}	1.920^{a}	24.650^{b}
Zandi	7.010^{a}	1.700^{b}	26.130^{a}
SEM	0.190	0.033	0.193

*Mean for each effect in the column, with different superscript differ significantly ($p<0.05$)

significantly between breeds ($p<0.001$) and months of the year ($p<0.001$). The lowest and highest total sperm concentration were recorded in September (567.06×10^7) and February (228.04×10^7), respectively (Table 1) ($p<0.05$).

Percentage of live sperm was not significantly different between breeds (Table 2), but significantly affected by months of the year ($p<0.01$) (Table 1). Percentage of live sperm was the highest in September (76.18) and was the lowest in March (58.00) ($p<0.05$). Percentage sperm abnormality was not significantly between breeds (Table 2) but was significantly between months of the year (Table 1) ($p<0.05$).

Relative testis volume varied significantly ($p<0.001$) between breeds (Table 4) and months of the year (Table 3). The month of November showed higher (36.62 kg cm^{-3}) and the month of February showed a lower (29.75 kg cm^{-3}) relative testis volume. Also the Zandi rams showed a lower ($p<0.05$) mean value for relative testis volume (6.23 kg cm^{-3}) than those in the Shall and Afshari rams. Mean scrotal circumference varied significantly ($p<0.001$) between breeds and months of the year (Table 3 and 4). The lowest and highest mean value was recorded in October (36.62 cm) and February (29.35 cm), respectively.

Plasma testosterone, triiodothyronine and thyroxine concentrations were significantly different ($p<0.001$) between breeds (Table 6) and months of the year (Table 5). Interaction effect of breed with sampling time on plasma testosterone and thyroid hormones was significant ($p<0.001$). The highest concentration of thyroxine and triiodothyronine (36.19 and 2.35 nmol L^{-1}, respectively) were seen in the months with long days (April, May and June) and the lowest concentration (17.17 and 1.07 nmol L^{-1}, respectively) were after day length had declined (September, October and November) (Table 5). Plasma testosterone concentration declined ($p<0.05$) in October and reminded low until May with the lowest concentration (2.29 nmol L^{-1}) recorded in March. A significant increase was observed in the mean concentration of testosterone from May to August ($p<0.05$) (Table 5).

DISCUSSION

The results reported in this study showed that Afshari, Shall and Zandi rams are continuous breeders as

they produce semen all the year round. Patterns of changes in semen characteristics were similar in three breeds.

A high ambient temperature, particularly in association with increasing day length during spring months has been demonstrated to result in reduction in semen quality in rams (Perez-Clariget *et al*., 1998; Karagiamdis *et al*., 2000). In present study semen quality and quantity of Afshari, Shall and Zandi breeds increase significantly from mid summer to early autumn. The improvement in semen quality and quantity during breeding season on semen volume, sperm concentration, sperm mass motility, sperm normality and percentage live sperm has been reported (Lincoln and Davidson, 1997; Ibrahim, 1997; Mandiki *et al*., 1998; Karagianidis *et al*., 2000; Taha *et al*., 2000; Kafi *et al*., 2004; Zamiri and Khodaei, 2005).

In northern breeds, Maximum testicular activity has been observed to occur in late summer to mid autumn (Lincoln *et al*., 1990). In this study maximum relative testis volume and scrotal circumference were recorded in late summer to mid autumn months. Scrotal circumference, a high heritable trait, is considered as an excellent index of sperm production in ram (Toe *et al*., 2000).

In the present study plasma concentrations of testosterone were highest at end of summer. At the beginning of October, the scrotal circumference attained its maximum size, corresponding to the highest serum testosterone level during same period. This relationship was also reported in other breeds of rams (Lincoln *et al*., 1990; Perez *et al*., 1997; Kafi *et al*., 2004; Zamiri and Khodaei, 2005). Mandiki *et al*. (1998) indicated that the better quality of semen and the higher reproductive capacity observed during the breeding season may be related to high levels of testosterone and LH secretions. Inversely, the decrease in pulsatility of these hormones may contribute to the low efficiency of spermatogenesis observed in spring.

In the present study plasma thyroxine and triiodothyronine concentrations was significantly higher in winter and spring months than in summer and autumn months in Afshari, Shall and Zandi breeds. 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 responsiveness to oestradiol negative feedback that causes termination of the breeding season. In Corridale and Merino rams, minimal concentrations of testosterone were found in late autumn and maximal levels were noted in mid-summer and early autumn and thyroxine concentration was high at the end of winter and in spring and was low from the end of summer to mid-autumn. In another study with Corriedale rams kept under extensive rearing condition, Perez-Clariget *et al*. (1997) observed minimal thyroxine levels during late summer and early autumn when testosterone concentrations were high. Maximal thyroxine concentrations were found in late autumn when scrotal circumference was decreasing.

Thyroid hormones were also reported to be essential for changes in Gn RH neurosecretory systems that are fundamental to reproductive seasonality. Response to thyroxine is lost gradually during mid-to late anoestrous season (Billings *et al*., 2002). In Iran, when thyroidal activity of Ghezel and Mehraban rams was low, the testicular activity and serum concentration of testosterone was at the highest level (Zamiri and Khodaei, 2005). These finding could be extrapolated to those of the present study since the highest thyroxine and triiodothyronine level was associated with the shortest photoperiod and the lowest values for ambient temperature, plasma testosterone concentration, relative testis volume and other semen parameters.

In conclusion, the present finding confirmed the parallelism between seasonal changes in gonadic function and in variations in testosterone but not in thyroid hormones concentrations. Semen quality and quantity parameters in Afshari, Shall and Zandi rams were better during late summer to mid-autumn (breeding season) than during other months of the year.

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