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Seasonal variation in sexual behavior, plasma testosterone and semen characteristics of Argentine Pampinta and Corriedale rams

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Abstract

Five Argentine Pampinta (dairy breed) and six Corriedale (dual-purpose breed) adult rams were used to study the effect of season on sexual behavior, plasma testosterone (T) and semen characteristics for a period of 1 year. Scrotal circumference (SC) and T concentrations were determined at 2-week intervals. Each ram was exposed monthly to three ovariectomized ewes in one 20-min test and the total number of mounts and successful matings were recorded. Semen collection was attempted from each ram twice weekly for six consecutive weeks during each season. In both breeds, SC varied seasonally showing the lowest mean values during winter and spring and in the Corriedale rams (32.8 ± 0.3 cm) was smaller ($p < 0.05$) than in Pampinta rams (34.4 ± 0.4 cm). No difference ($p > 0.05$) was recorded in T concentration between breeds and both breeds reached a peak in summer and autumn. Number of matings showed the maximal values in autumn in both breeds. Season had no significant effect on mass motility, sperm concentration and percentage of live sperm. Semen volume, percentage of progressive motility and total sperm per ejaculate were higher during summer and autumn than in other seasons of the year. Pampinta rams had the highest values ($p < 0.05$) of sperm concentration, total sperm per ejaculate and percentage of abnormal sperm. Results of this study show that sexual behavior, SC and T concentrations exhibit seasonal variation in Pampinta and Corriedale rams and that semen of superior quantity and quality is especially collected in both breeds during the summer and autumn.

Additional key words: hormone, rams; semen quality; serving capacity.

Resumen

Variación estacional en el comportamiento sexual, testosterona plasmática y características seminales de carneros Pampinta Argentina y Corriedale

Cinco carneros adultos Pampinta Argentina (raza lechera) y seis Corriedale (raza doble propósito) fueron utilizados para estudiar el efecto de la estación del año sobre el comportamiento sexual, testosterona plasmática (T) y características seminales. La circunferencia escrotal (CE) y las concentraciones de T fueron determinadas cada dos semanas. Mensualmente, cada carnero fue expuesto a tres ovejas ovariectomizadas en una prueba de 20 min y se registró el número total de montas y apareamientos. La colección del semen fue intentada dos veces por semana por seis semanas consecutivas durante cada estación. En ambas razas los menores valores de CE fueron observados en invierno y primavera (Corriedale = 32,8 ± 0,3 cm; Pampinta = 34,4 ± 0,4 cm; $p < 0,05$). La concentración de T alcanzó un máximo en verano y otoño y el número de apareamientos fue máximo en otoño. La estación no tuvo efecto ($p > 0,05$) sobre la motilidad masal, la concentración espermática y el porcentaje de espermatozoides vivos. El volumen del eyaculado, el porcentaje de motilidad progresiva y el número total de espermatozoides fueron mayores en el verano y otoño. La raza del carnero tuvo un efecto ($p < 0,05$) sobre la concentración espermática, el número total de espermatozoides y el porcentaje de espermatozoides anormales, siendo los valores más altos en carneros Pampinta. Los resultados muestran que la actividad sexual, la CE y las concentraciones de T varían en las estaciones del año en ambas razas y que el semen de superior calidad y cantidad es especialmente colectado en verano y otoño.

Palabras clave adicionales: calidad seminal; capacidad de servicio; carneros; hormona.

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Introduction

In sheep, sexual behavior and semen quality are the main factors that limit male reproduction efficiency along the year. These factors could vary according to the breed (Avdi *et al*., 2004), season of the year (Schanbacher & Lunstra, 1976) and nutrition level (Mukasa-Mugerwa & Ezaz, 1992). Rams are seasonal breeders in which photoperiod is the main environmental cue controlling the seasonal reproductive pattern and there are indicators of seasonality in sexual activity as evidenced by the changes of semen quality (Boland *et al*., 1985; Karagiannidis *et al*., 2000), testicular diameter (Colas *et al*., 1986) and level of hormone secretion (Langford *et al*., 1987).

Corriedale breed, a dual-purpose breed (wool and mutton) of mixed origin (50% Merino and 50% British breeds) is present in several countries of South America and it is considered one of the most important breeds of sheep in Argentina. In this country, Almeida *et al.* (1981) reported that Corriedale rams expressed seasonal variation in their reproductive variables (scrotal circumference and semen quality) and Perez *et al.* (1997) found that the testis activity was highest in late summer/early autumn and lowest in winter in Uruguay (similar latitude). Pampinta breed (a cross of ¾ Ostfriesisches Milchschaf, also known as East Friesian, and ¼ Corriedale) was developed in Argentina by Anguil Research Station (National Institute of Agricultural Technology, INTA) since 1970 by cross-breeding and selection for maintain dairy aptitude and prolificity (from East Friesian) and improve hardiness (from Corriedale). Argentinean sheep breeders are showing increasing interest in the highly prolific and milk producing Pampinta breed, which is well adapted to the conditions of Humid Pampa (latitude from 32 to 40º S; 600,000 km^2) and the Pampinta and East Friesian breeds are most important sheep breeds for milk production in Argentina.

Pampinta female reproductive performance has been described in detail (Fort *et al*., 1992). These authors observed that estrus and ovulation rates were greater than those of Corriedale ewes and that the ovarian activity was maintained until November (spring). However, no detailed studies of estimations of seasonal variation in sexual activity, hormonal concentration and semen quality have been performed on rams of the Pampinta breed. Consequently, we hypothesized that the reproductive activity of the Pampinta rams is longer than the Corriedale rams and thus would allow a better reproductive management of the sheep.

In order to establish the best period(s) to perform mating under intensive breeding systems or to implement semen collection for successful assisted reproductive technologies, the present study was designed i) to evaluate the effect of season of the year on sexual behavior, scrotal circumference, plasma testosterone concentration and semen characteristics and ii) to compare these reproductive traits between dairy breed rams (Pampinta) with rams belonging to a dual-purpose (wool and meat) standard breed (Corriedale), which is the lesser genetic component of the Pampinta breed.

Material and methods

Animals and research conditions

This study was carried out at the Experimental Station Balcarce, located in Buenos Aires province (Humid Pampa; 37º 47' S, 53º 18' W and 150 m above sea level). Over a 1 year period (from 21 December 2005 to 20 December 2006), data were collected from 11 sexually mature (4-6 years of age) rams. The year was divided into the four geographical seasons: summer (21 December to 20 March), autumn (21 March to 20 June), winter (21 July to 20 September) and spring (21 September to 20 December). Daylight length varies in this location from about 14 h and 40 min in December to about 11 h and 30 min in June. Average temperatures during the experiment varied from 9.5ºC in winter to 19.2ºC in summer and there were great variations in rainfall (79.4 mm in autumn and 314.9 in summer). Late summer and autumn are considered the breeding season for sheep in Argentina. Five Pampinta with a mean body weight of 105.6 ± 3.5 kg and six Corriedale with a mean body weight of 89.8 ± 3.8 kg at the start of the study were used. These animals were not used for breeding during this period. Each ram was clinically examined with emphasis on the integrity of its genital tract and disease preventions were strictly observed. Animals were allowed to graze every day

Abbreviations used: BCS (Body condition score); LH (Luteinizing hormone); SB (Sexual behavior); SC (Scrotal circumference); SEM (Standard error mean).

between 08.00 and 16.00 h in a 4-ha cultivated pasture (*Festuca arundinacea* and *Trifolium repens*) and then they were housed indoors and hay (*Thynopirum ponticum*) and water were provided *ad libitum*. Rams of both breeds were managed separately throughout the experiment. No concentrate mixture was delivered.

Physical measurements and blood samples

Live weight, body condition score (BCS; scale from 1 to 5, Russel *et al*., 1969), scrotal circumference (SC) and plasma testosterone concentration were determined at 2-week intervals over the time of the experiment. Three SC measurements were taken by one technician by manipulating the testes to the bottom of the scrotum and measuring the greatest circumference with a flexible tape. The average of the three measurements was used for the analysis. A jugular blood sample was collected (08.00 h) from each ram in heparinized tubes and immediately centrifuged at 1500 × *g* for 10 min. The harvested plasma was then stored at –20ºC until hormone analyses. The concentration of testosterone in plasma was measured in all samples by commercial RIA kit (Diagnostic Products Corporation, Los Angeles, CA, USA). The reagents had been previously validated for sheep plasma using a parallelism test. The intra- and inter-assay coefficients of variation were 7.2% and 9.5% respectively for concentrations between 0.2 and 16 ng mL^{-1}. The minimum detectable concentration was 0.2 ng mL^{-1} of plasma.

Sexual behavior

Sexual behavior (SB) test was performed monthly. Each ram was individually exposed to three restrained ovariectomized hormone non-induced estrus ewes in 6 × 6 m pens for 20-min (each test). Each of two experimenters recorded all occurrences of mounts with and without a successful mating (ejaculation). A serve or ejaculation was defined as a mount accompanied by intromission and ejaculation, characterized by a thrust pelvic with the head thrown back, followed by a short period during which the ram showed no interest in the ewes. The number of mounts and successful matings observed in each of four SB tests by season were averaged. All animals in the present study were randomly selected to be tested on the same day. Testing was conducted between 08:00 and 14:00 h.

Semen collection

Six to eight weeks before of the beginning of the study, the animals were trained to serve the artificial vagina. Semen collection was attempted from each ram twice weekly using artificial vagina (42ºC; 08.00 to 12.00 h). An ovariectomized and restrained ewe was used for mounting by the ram. The tubes with the freshly collected semen were immediately transferred to the laboratory and immersed into the water bath at 32ºC. To study the effect of season on semen characteristics, the experimental procedure was conducted for 6 consecutive weeks in each season (24 weeks yr^{-1}), starting from the 4th to the 9th week (middle) of each season.

Semen evaluation

Ejaculated semen volume was recorded after collection using a glass graduated tube. Mass motility (wave motion) in undiluted semen was assessed by examining a drop (5 µL) of semen under a warm stage microscope (×100 magnification) on the basis of an arbitrary scale from 0 to 5 (0 = immotile, 5 = vigorous motility). A 5 µL fresh semen sample was diluted with 4 mL of physiological formalin (1‰) solution for determination of spermatozoa concentration by haemocytometric counts. Total number of sperm per ejaculate was calculated by multiplying sperm concentration and ejaculate volume. Sperm progressive motility (%) was subjectively estimated by diluting a drop of semen with sodium citrate (2.92g/100 mL distilled water; pH 6.7-6.9; Mallinckrodt Chemical Works, NY, USA), transferring it to a warm slide (37ºC), mounting it with a cover slip and examining under a microscope (×400 magnification). Furthermore, a semen smear was stained with eosin-nigrosin to determine the live:dead ratio and percentage abnormal sperm by counting at least 200 spermatozoa under an oil immersion objective (1000X) random fields. All examinations were performed by the same operator.

Statistical analysis

Data were analyzed using PROC MIXED (SAS, 1989). A mixed model analysis of variance was used to determine the effect of season, breed, ram within breed and their interactions on reproductive character-

istics. Season and breed were considered as fixed-crossed effects, whereas ram within breed was considered as a random effect according to the following linear model:

$$Y_{ijk} = \mu + S_i + B_j + R_k(B_j) + (SB)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the dependent variable, μ the general mean, S_i the fixed effect of i^{th} season, B_j the fixed effect of j^{th} breed, $R_k(B_j)$ the random effect of k^{th} ram in the j^{th} breed, $(SB)_{ij}$ the effect of season × breed interaction and ε_{ijk} the associated random error assumed to be $N(0, \sigma e^2)$. Dependent variables in the mathematical model were live weight, BCS, SC, number of mounts, number of successful matings, plasma testosterone concentration and all semen characteristics. Original data were transformed by logarithms (number of mounts and plasma testosterone) and percentage data were transformed to arcsin $\sqrt{x}$ and this allowed the transformed variables to approximate more closely to a normal distribution and hence to stabilize the variance between groups. If a factor was significant, the Tukey's multiple comparisons test was used to determine differences between means and probabilities of < 5% ($p < 0.05$) were considered to be statistically different. All mean values are expressed as the mean ± standard error of mean (SEM). Correlation coefficients (Pearson correlations) between various physical parameters or seminal characteristics were calculated (Steel & Torrie, 1980).

Results

Physical variables and testosterone concentration

No season × breed interactions were detected ($p > 0.05$) for any of the variables measured. Live weight was affected ($p < 0.05$) by season and breed. For the two breeds, live weight decreased continuously from summer (beginning of the experiment) until winter and then increased from the end of winter to spring and summer ($p < 0.05$). Throughout the experiment, Pampinta rams were heavier than Corriedale rams (Table 1). In contrast, BCS was higher ($p < 0.05$) in Corriedale rams than in Pampinta rams. The BCS was also affected ($p < 0.05$) by season of the year, being maximal in spring in both breeds (Table 1). Scrotal circumference of both breeds varied seasonally during the experimental period, with the lowest mean values during winter and spring and the highest values during the breeding season ($p < 0.05$) (Table 1). Scrotal circumference in the Corriedale breed was lower ($p < 0.05$) than in Pampinta breed.

No differences ($p > 0.05$) were recorded in plasma testosterone concentrations between breeds. The effect of season of the year was highly significant ($p < 0.01$) with both breeds reaching a peak in testosterone concentrations in summer (Table 1). A positive coefficient of correlation was calculated between SC and live weight in both breeds (Pampinta: $r = 0.47$; Corriedale: $r = 0.34$; $p < 0.001$). Similarly, significant correlation was detected between SC and plasma testosterone concentration (Pampinta: $r = 0.63$; Corriedale: $r = 0.32$; $p < 0.001$).

Sexual performance test

Data of the effect of season and breed of rams on sexual behavior were examined and results are shown in Table 1. No breed × season interaction was detected for any variables measured in the test. Season affected ($p > 0.05$) the mean number of mounts and the highest values were observed in autumn and winter. Corriedale rams had more mounts ($p < 0.05$) within the allocated time than Pampinta rams throughout the seasons. The mean number of successful matings within 20-min exposure to the three restrained ewes varied ($p < 0.05$) according to the season of the year, being highest in autumn compared with the remaining seasons. Breed did not affect ($p>0.05$) the mean number of successful matings. No correlation existed between number of successful matings and plasma testosterone concentration (Pampinta: $r = 0.10$; $p = 0.27$ and Corriedale: $r = –0.03$; $p = 0.73$). Similarly, no significant correlation was found between number of successful matings and SC (Pampinta: $r = –0.02$; $p = 0.81$ and Corriedale: $r = 0.13$; $p = 0.13$).

Seminal characteristics

In the present study, 528 ejaculate attempts from eleven rams over 6-weeks in each season resulted in 436 (82.6%; Pampinta = 202 and Corriedale = 234) successful ejaculation procedures and were thus evaluated. Season × breed interactions were not detected ($p > 0.05$) for any seminal characteristics. Seminal characteristics of Pampinta and Corriedale rams are presented in Table 1. Significant differences ($p < 0.05$)

Table 1. Mean (± SEM) of the physical characteristics, sexual behavior, seminal characteristics and sperm abnormalities of Corriedale and Pampinta rams

Characteristics	Season				Breed	
	Summer	Autumn	Winter	Spring	Corriedale	Pampinta
Physical characteristics						
Live weight (kg)	97.2 ± 2.4^{a}	96.6 ± 2.3^{ab}	87.3 ± 2.4^{b}	96.2 ± 2.1^{a}	82.6 ± 1.5^{b}	104.1 ± 1.7^{a}
Body condition score	2.7 ± 0.1^{b}	2.7 ± 0.1^{b}	2.6 ± 0.1^{b}	3.1 ± 0.1^{a}	2.9 ± 0.1^{a}	2.6 ± 0.1^{b}
Scrotal circumference (cm)	35.3 ± 0.4^{a}	34.1 ± 0.5^{ab}	33.1 ± 0.5^{b}	32.1 ± 0.4^{b}	32.8 ± 0.3^{b}	34.4 ± 0.4^{a}
Plasma testosterone (ng mL^{-1})	8.3 ± 0.6^{a}	5.0 ± 0.4^{b}	2.7 ± 0.5^{c}	2.3 ± 0.4^{c}	3.9 ± 0.3	5.3 ± 0.4
Sexual behavior						
No. of mounts	5.1 ± 1.4^{b}	10.4 ± 1.8^{a}	9.7 ± 2.5^{a}	5.8 ± 0.9^{b}	8.7 ± 1.2^{a}	6.8 ± 1.3^{b}
No. of matings	2.4 ± 0.2^{b}	3.4 ± 0.3^{a}	2.0 ± 0.2^{b}	2.4 ± 0.2^{b}	2.6 ± 0.2	2.5 ± 0.2
Seminal characteristics						
Volume (mL)	1.0 ± 0.1^{a}	1.0 ± 0.1^{a}	0.8 ± 0.1^{b}	0.7 ± 0.1^{b}	0.8 ± 0.1	0.9 ± 0.1
Mass motility (0-5)	4.4 ± 0.1	4.3 ± 0.1	4.2 ± 0.2	4.2 ± 0.1	4.3 ± 0.1	4.3 ± 0.1
pH	7.5 ± 0.1^{c}	8.2 ± 0.1^{a}	7.9 ± 0.1^{b}	7.9 ± 0.1^{ab}	8.0 ± 0.1^{a}	7.8 ± 0.1^{b}
Progressive motility (%)	82.1 ± 1.5^{a}	79.8 ± 0.8^{a}	74.0 ± 2.0^{ab}	72.7 ± 1.7^{b}	77.3 ± 1.1	77.0 ± 1.2
Concentration (×10^{9} mL^{-1})	3.8 ± 0.1	3.8 ± 0.1	4.0 ± 0.2	4.2 ± 0.2	3.5 ± 0.1^{b}	4.3 ± 0.1^{a}
Total output (×10^{9})	4.0 ± 0.1^{a}	3.9 ± 0.2^{a}	3.3 ± 0.3^{b}	3.0 ± 0.1^{b}	3.0 ± 0.1^{b}	4.1 ± 0.1^{a}
Live sperm (%)	79.9 ± 1.2	79.1 ± 0.8	75.4 ± 2.1	82.2 ± 1.3	79.4 ± 1.0	78.8 ± 1.1
Abnormality (%)	5.9 ± 0.1^{b}	5.5 ± 0.1^{b}	16.5 ± 0.2^{a}	16.0 ± 0.2^{a}	8.8 ± 0.1^{b}	12.7 ± 0.2^{a}
Sperm abnormalities						
Shape head (%)	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Detached head (%)	0.9 ± 0.1	0.9 ± 0.1	2.0 ± 0.3	1.5 ± 0.3	1.3 ± 0.1	1.4 ± 0.2
Reflex distal midpiece (%)	1.0 ± 0.2	0.9 ± 0.1	1.2 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	0.7 ± 0.1
Proximal droplet (%)	2.0 ± 0.2^{c}	1.9 ± 0.3^{c}	9.0 ± 2.0^{a}	6.7 ± 0.8^{b}	3.3 ± 0.3^{b}	7.0 ± 0.2^{a}
Coiled/bent principal piece (%)	1.6 ± 0.3^{c}	1.3 ± 0.1^{c}	4.0 ± 0.6^{b}	6.9 ± 1.0^{a}	2.5 ± 0.1^{b}	4.5 ± 0.5^{a}

Different superscripts (a, b, c) in the same row within season or breed indicate differences ($p < 0.05$).

among rams within each breed were found in most characteristics examined. There was a significant difference ($p < 0.05$) in semen volume among different seasons. Rams of both breeds produced semen with a greater mean volume in reproductive season (summer and autumn). Breed of ram had no effect ($p > 0.05$) on the semen volume. Percentage of sperm progressive motility and total sperm output (volume × sperm concentration) were affected ($p < 0.05$) by season of the year in both breeds, being highest results in summer and autumn compared with all other seasons. Pampinta rams produced semen which had a greater sperm concentration, total sperm output and percentage of abnormal sperm ($p < 0.05$). The highest values ($p < 0.05$) of abnormal sperm were recorded in winter and spring for both breeds (Table 1). There were significant correlations between mass motility and progressive motility, sperm concentration and live sperm in Corriedale ($r = 0.53$, 0.43 and 0.39, $p < 0.001$). However, mass motility was only highly correlated with progressive motility and live sperm ($r = 0.62$ and 0.51, $p < 0.001$).

Discussion

The present study is the first to report on the seasonal changes in reproductive variables of Pampinta (dairy breed) and Corriedale (dual-purpose breed) rams reared in the Argentine Humid Pampa. As it was expected, live weight and body condition of both Pampinta and Corriedale rams varied seasonally and the reduction in both variables was mostly determined by the nutritional influences (winter drop in pasture production) because the experiment was conducted under nutritionally non-controlled conditions rather than intensive production system (grain supplementation). For the two breeds, live weight increased continuously from winter to summer and Pampinta rams were heavier than Corriedale rams throughout the experiment. Similarly, a decrease in live weight in winter was found in Suffolk rams (Mandiki *et al*., 1998a). However, Gastel *et al.* (1995) observed that the body weight did not decrease during the winter in Corriedale rams (15 to 18 months of age) in Uruguay. Over the time of

the experiment, body condition was lower in Pampinta rams than in Corriedale rams, probably because of increased body size of that breed as genetic influence of East Frisian breed. These seasonal variations confirm that the two breeds have similar sensitivity to environmental factors.

A significant effect of season on SC of Pampinta and Corriedale rams was observed in the current study. This is in agreement with the results of Dufour *et al.* (1984), Mandiki *et al*. (1998a) and Kafi *et al*. (2004), who demonstrated seasonal variation in SC in Suffolk, Texel and Persian Karakul rams, respectively. Seasonal fluctuation in testicular size has been shown to be under the influence of serum luteinizing hormone (LH) concentration (Schanbacher & Lunstra, 1976) and specially submitted to the frequency of LH pulses (Sanford *et al*., 1977). In the present study, the greatest SC was recorded in summer and autumn in both breeds and it was positively correlated with the body weight. Similar information has been reported in other studies performed in Vendean and Texel rams (Colas *et al*., 1986) and in Greek breed rams (Avdi *et al*., 2004).

In the present study, SC measurements followed approximately the same profile as testosterone concentration, with breed differences being more pronounced in autumn, winter and spring in favor of the Pampinta rams. Similarly, Gastel *et al*. (1995) in a study with Uruguayan Corriedale rams found that SC was significantly correlated with testosterone levels and with seminiferous tubule diameter. However, Perez *et al*. (1997) observed that plasma testosterone concentration decreased at the end of autumn in Corriedale rams under extensive rearing and one month later the SC decreased markedly. The time for the initiation of testosterone secretory activity of the testes depends also on the latitude at which animals are located (Barrell & Lapwood, 1979). In our case (at latitude 37ºS) and for both breeds testosterone levels began increase after summer solstice (22 December), which agrees with Sanford *et al*. (1978). Breed differences in plasma testosterone concentration were not observed in the present study, possibly because one sample every two weeks could not be sufficient to detect them. The positive correlation between SC and plasma testosterone concentration found in the present study, was also observed by Dufour *et al*. (1984) and Kafi *et al*. (2004). The latter report indicates that the highest plasma testosterone concentration was found in summer in Karakul rams. In contrast, serum testosterone levels were recorded to be higher during the decreasing daylenght period (autumn months) in Daglic and Chios (Turkish breeds) rams (Gündoğan, 2007) and in Texel, Suffolk and Ile-de-France rams (Mandiki *et al*., 1998b). The latter authors found that testosterone concentration was not related to testicular mass.

The results of this study showed that Pampinta and Corriedale rams have seasonal variations of the sexual behavior as evidenced by the sexual behavior test. Significant differences were observed in the mean number of mounts among seasons, being the highest values in autumn and winter and they progressively declined in spring and summer in both breeds. However, the highest number of completed matings was observed only in autumn in both breeds. Corriedale rams showed a higher mounting activity but were less effective in their mating ability compared to Pampinta rams. There was no difference between breeds in the number of completed matings, therefore, it appears that Pampinta rams have a higher ratio of successful matings to mounts compared to Corriedale rams. Plasma testosterone concentrations were highest at the middle and end of summer; however, two months later, approximately at the middle of autumn, the number of successful matings attained its maximum value. These results suggest that, on the one hand, the maximum summer temperatures (35 ºC) combined with high relative humidity that usually dominates until end March in the Humid Pampa may have suppressed mating activity without affecting normal endocrine activity; on the other hand, the high sexual activity of the autumn may require several months before maximum testosterone levels. The low libido of Pampinta and Corriedale rams during the winter and spring can possibly be attributed to the low level of circulating plasma testosterone. Similarly, Avdi *et al.* (2004) reported a maximum number of successful matings in Chios rams in November in the northern hemisphere. Evidence of enhanced sexual activity of rams during the short days of autumn and winter (normal breeding season) as evidenced by a greater total number of mounts and services plus a shorter reaction time in a serving capacity test was also reported by Tulley & Burfening (1983). In contrast, Kafi *et al*. (2004) observed that the number of matings of Persian Karakul rams was significantly lower in autumn (2.5 ± 0.6) than in winter (7.0 ± 1.6). However, Boland *et al*. (1985) reported that the month of the year had no significant effect on either the number of mounts or the number of matings in Suffolk and Dorset Horn rams.

In the present study, seasonal fluctuations in seminal characteristics were observed in the rams under investigation. The season of semen collection significantly affected the ejaculate volume, sperm progressive motility, sperm output and percentage of abnormal sperm. The highest ejaculate volume was recorded in summer and autumn and decreased to a minimum in winter and spring seasons. It is consistent with the results observed in Egyptian breeds (at latitude 31º N) (Taha *et al*., 2000) and in the Persian Karakul breed (at latitude 20º N) (Kafi *et al.*, 2004). However, the sperm concentration remained constant throughout of this study, reflecting seasonal variations in the secretion and release of the seminal plasma by the accessory glands, possibly related to the changes in plasma testosterone concentrations. Season and breed had no significant effect on mass motility. However, in other studies semen samples had a higher mass motility in autumn (Kafi *et al*., 2004; Gündoğan, 2007) and differences among breeds were reported (Boland *et al*., 1985). These authors observed that Dorset Horn rams produced semen which had a higher wave motion grading than Suffolk and Texel rams.

Sperm progressive motility was low during winter and spring with a minimum being recorded in spring season. However, Karagiannidis *et al.* (2000) found that the sperm progressive motility was lower during summer compared to the other three seasons of the year. The highest values for total spermatozoa output were recorded in summer and autumn and differences between breeds were observed over the time in the present study.

A significant effect of season on percentage of abnormal sperm was found in the current study. The highest values were recorded in winter and spring, with lower values in the summer and autumn seasons. This is consistent with the results obtained in both Suffolk and Ile-de-France rams (Mandiki *et al*., 1998a). On the other hand, it was also observed that the percentage of abnormal spermatozoa was higher during spring and summer, while winter was a transitional period in Greek breeds (Karagiannidis *et al*., 2000). The high percentage of sperm abnormalities during the winter and spring seasons can possibly be attributed to the low level of circulating plasma testosterone and a reduction in the thickness of the seminiferous tubules and spermatogenic activity (Barkawi *et al*., 2006). Whether the magnitude of the differences between seasons found for sperm abnormal are large enough to affect the fertility results remains to be investigated. Results of the present study also indicated that breed differences exist in the sperm concentration and percentage of abnormal sperm, both being significantly higher in Pampinta than in Corriedale rams.

The findings of the present study show that the Pampinta and Corriedale sexual activity is better during late summer and early autumn (breeding season) than during the other seasons of the year and supported the idea that testicular activity in male sheep recommences in the long days. Rams of both breeds are capable of producing semen all the year round. However, seasonal fluctuations in semen characteristic were observed and semen of the best quality was obtained in summer and autumn. The semen quality of the Pampinta rams, as judged by sperm concentration and sperm total output was comparatively higher than the Corriedale rams. The latter breed showed lower sperm abnormalities. Therefore, these differences in several seminal characteristics must be taken into account for reproductive management and semen collection in areas with similar climatic conditions.

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