

Effect of season on sexual behavior, testicular measurements and seminal characteristics in "Beni arouss" North Moroccan bucks

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ABSTRACT

Changes in anatomical, behavioural and seminal characteristics were measured throughout the year in seven Beni Arouss bucks maintained at a latitude of 35°N. Testicular size varied significantly. There were the greatest values during the summer and autumn for scrotal circumference and least in the winter for testicular length. Values for most variables related to sexual behavior were not affected by season except the number of mounts before first ejaculation which was least in the summer. No differences were detected between seasons in terms of semen volume. The sperm concentration, viability and percentage of normal sperm, however, were less during the winter period. The size of sperm heads was larger in the autumn and motility of spermatozoa was greater in the summer and autumn. Results from the correlation analysis indicated that the majority of conventional seminal characteristics were correlated with testis measurements ($P < 0.05$; $r < 0.5$). Values for conventional and CASA motility variables indicated there were positive correlations between viability and progressive motility ($r = 0.4$; $P < 0.05$) and between the percentage of normal sperm and straight line and average path velocity ($r = 0.4$; $P < 0.05$). A global reproduction performance score was established for each buck, which allowed for assessment of magnitude of seasonal changes for each individual. These global score values indicated there was a greater reproductive performance for all bucks during summer and autumn. This study described for the first time seasonal variations of reproductive characteristics of Beni Arouss bucks and results indicate that even though there is a maximal capacity for reproductive performance during the summer and autumn, breeding should be possible throughout the year.

1. Introduction

The productivity of small ruminants is affected by seasonality of reproduction. For goats, sexual behaviour, testicular size and semen quantity and quality are the main factors that affect buck reproductive efficiency during the year. This efficiency varies as a result of various factors including breed (Perez and Mateas, 1996; Arrebola and Abecia, 2017), latitude (Karagiannidis et al., 2000; Gómez-Brunet et al., 2012), climate (Zarei et al., 2009), season (Talebi et al., 2009; Zarazaga et al., 2009; Arrebola and Abecia, 2017),

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nutrition (Walkden-Brown and Bocquier, 2000; Martin et al., 2010) and circulating gonadotropin concentrations (Yu et al., 2018).

In the Northern hemisphere (at 30–40 °N) season of the year was reported as the main factor affecting semen characteristics in goats with maximum reproductive functions occurring in the summer and autumn when natural photoperiods exist (Barkawi et al., 2006; Zamiri and Heidari, 2006; Talebi et al., 2009). In the southern hemisphere and for breeds from the lower latitudes in the Mediterranean area (36–38 °N), bucks still have maximum reproductive activity in summer and autumn but variations are not as pronounced as those when animals are located in other parts of the world (Arrebola et al., 2010; Chentouf et al., 2011; Arrebola and Abecia, 2017).

In Morocco, goats account for 6.1 million among the 28.6 million livestock (MAPM, 2017) and are bred in mountainous and isolated areas. In Northern Morocco, the goat herds constitute approximately 788,000 animals. Beni Arouss is a North Moroccan indigenous goat breed that has been officially recognized recently with approximately 8600 animals that have been recorded by the National Association of Sheep and Goats in Morocco (Bouaissa, 2017). This breed is well adapted to extensive management practices and harsh local conditions. Beni Arouss goats are relatively resistant to local pests and diseases. The mean body weight of adult Beni Arouss is 37.5 ± 8.8 kg in females and 41.8 ± 13.6 kg in males with red as predominant coat color for both sexes (Hilal et al., 2013). Goats from this breed are seasonal breeders (Chentouf et al., 2011) with a daily milk production of 0.47 kg/d in extensive systems and 0.51 kg/d when there are intensive management conditions. This difference of productivity seems to be due to the large variability between goats (El Otmani et al., 2014). Because of large-scale dissemination of imported breeds in Morocco, there is a continuous decrease in the number of local breeds of goats. This phenomenon could lead in the future to the total loss of the local breeds and inclusion of the genetics from these autochthonous populations into cosmopolitan breeds (Benjelloun et al., 2015). One of the goals of the Moroccan Agricultural Ministry is to improve herd productivity and preservation of this breed by implementation of a breeding program using artificial insemination. There, however, is little information on the effect of season on buck reproductive characteristics in northern Morocco. The only study of this kind was conducted by Chentouf et al. (2011) and was limited to physical measurements and a few characteristics of semen.

In artificial insemination centers, general sperm characteristics such as concentration, motility, viability and morphology are routinely used to assess semen quality. Inconsistent results regarding the relationship between fertility-related values and these values for conventional sperm quality variables have been reported. Many researchers have reported that there are strong correlations between these types of values (Linford et al., 1976; Rodríguez-Martínez, 2003), while in other studies it was reported there were little or no relationships between ram fertility-based values and those for sperm quality variables (Gadea, 2005; Bishop et al., 2009). Unfortunately, the methods for performing such analyses lack standardization and, therefore, there cannot be comparisons between operators and laboratories. Computer Assisted Sperm Analysis system (CASA) was developed as a more accurate technique to assess sperm motility and morphometry (Mwaanga et al., 2014). In bulls and rams, results indicated that sperm motility and velocity can be used in predicting fertility (Kathiravan et al., 2008; Del Olmo et al., 2013). The CASA variables have been used also to assess seasonal variation of semen quality in species such as goats (Wang et al., 2015), sheep (Bravo et al., 2014), cattle (Valeanu et al., 2015) and horses (Waheed et al., 2015). Such data are not available for Beni Arouss bucks.

The aim of the present study, therefore, was to evaluate in North Moroccan bucks seasonal variations of sexual behaviour, testicular measurements and seminal characteristics during a 12-month period. A general reproduction performance score was developed that took into account values for all reproductive variables as an attempt to provide information about the magnitude of seasonal reproductive changes in bucks of this breed.

2. Materials and methods

2.1. Localization and animal management

This study was conducted at the experimental station of Boukhalef, INRA, Regional center of Tangier, located at the north of Morocco (35°44'N Latitude, 5°54' O Longitude). The climate in this region is Mediterranean sub-humid with an average annual rainfall of 887 mm. The greatest ambient temperature is 27.9 °C and the least is 9.6 °C. The day length ranges from 10 h during the winter solstice to 14 h 45 min in the summer solstice. During a 12-month period (March 2015 to March 2016), data were collected from seven sexually mature Beni Arouss bucks aged between 5 and 8 years of age, at the onset of the study, with a body weight (BW) ranging between 28 and 67 kg. None of the bucks were in physical proximity with other bucks used in the study. The year was divided into four seasons according to the calendar: spring (from 21 March to 21 June), summer (from 22 June to 21 September), autumn (from 22 September to 21 December) and winter (from 22 December to 20 March). During the experimental period, bucks were subjected to the same barn management. They were maintained indoors in individual pens and with natural photoperiodic conditions. Animals were fed basal diets of oat hay and concentrate feed mixture according to the recommended requirements of INRA (1988) and had free access to water.

2.2. Testicular measurements and sexual behaviour

Scrotal circumference (SC), testicular length (TL) and testicular diameter (TD) were determined monthly. Testis measurements were performed by the same operator using a flexible tape and a caliper.

Sexual behavior was evaluated monthly. Before semen collection, each buck was individually exposed to a female goat in which there was an induced estrus. Reaction time (time to ejaculate) was evaluated for three consecutive ejaculates. The time allocated in which each ejaculation had to occur during an assessment period was 5 min. If ejaculation occurred within less than five minutes, a

new time interval was calculated for the next ejaculation. In the absence of ejaculation within 5 min, a short period of 30 to 45 min during which the buck was separated from the estrous female was imposed either once or twice, and a new assessment period was attempted where the previous 5 (or 10) minutes were added to the time for first ejaculation. The number of mounts before each ejaculation was also recorded.

2.3. Semen collection and evaluation

Semen was collected from each buck monthly using artificial vagina and an estrous goat as a mount. For semen evaluation samples from the first ejaculate were analyzed. Immediately after collection, the semen was transported to the laboratory and immersed into a water bath at 37 °C.

General seminal characteristics assessed in the current study represent conventional variables and these included semen volume, sperm concentration, viability and percentage of normal sperm.

Semen volume was measured in a graduated collection tube and a pre-dilution (v:v) using a synthetic extender prepared in the research laboratory (pH = 7.2 and osmotic pressure = 320 mOsm/kg). Sperm concentration was determined by manual counting with a Burkner haemocytometer after diluting an aliquot of semen with a 0.05% formaldehyde distilled water (1:200).

Motility analysis of sperm was performed using a CASA system (ISAS, Proiser R + D SL, Spain). This system consisted of use of an optical phase contrast microscope (UB203i, Proiser R + D SL, Spain) connected to a digital camera and a computer. Prior to analysis, semen sample concentration was adjusted to a 30×10^6 spermatozoa/ml with extender. The images were taken from 3 μ l semen aliquots, which were placed on a 20 μ m deep chamber slide (SpermTrack 20, Proiser R + D SL, Spain) on a pre-warmed stage. At least 200 spermatozoa from six different fields were analyzed in each sample using a negative phase-contrast microscope at 10 \times magnification. Samples were analyzed to determine values for sperm motility variables within 20 min after collection. Variables included in the analysis were total motility (TM, %), progressive motility (PM, %), rapid spermatozoa (%), curvilinear velocity (VCL, μ m/s), straight line velocity (VSL, μ m/s) and average path velocity (VAP, μ m/s).

The sperm viability was evaluated by eosin-nigrosin (minutube, Germany) staining according to the method described by Evans and Maxwell (1987). Smears were prepared by mixing 5 μ l of eosin (2% solution), 5 μ l of nigrosin (4% solution) and 5 μ l of semen (diluted at 800×10^6 spermatozoa/ml) on a warm slide and immediately spread with another slide. A total of 200 cells from different microscopic fields were evaluated using a bright-field microscope using a 60 \times objective. Spermatozoa with a partial or complete purple head were considered dead and only unstained spermatozoa were considered to be viable.

Morphometric analysis of sperm samples was performed using the morphometric module of the ISAS. Prior to analysis, smears were prepared by placing 3 μ l of the sperm sample previously diluted at 800×10^6 spermatozoa/ml on a clean slide. These were subsequently air-dried for 10 min on a warming stage and fixed for 15 s in Diff-Quik (Microptic Automatic diagnostic system, Barcelona, Spain) fixative solution (containing 0.002 g/l of Fast Green in methanol). These samples were subsequently stained with Diff-Quik solution I (contains 1.22 g/l of Eosin Y in phosphate buffer at pH 6.6 and 0.1% (w/v) sodium azide as preservative) for 10 s and then with Diff-Quik solution II (contains 1.1 g/l of Thiazine Dye in phosphate buffer at pH 6.6) for 10 s. After each step, the excess solutions were allowed to drain from the slides by placing them vertically on absorbent paper. Once stained, the slides were rinsed with distilled water, air dried on a warming stage and permanently sealed with Eukitt quick hardening mounting medium (Sigma-Aldrich, Quimica SA., Spain) and a coverslip. Slides were examined using an UB203i microscope, with a $\times 60$ bright-field objective. Sperm heads ($n = 200$ per sample) with no imbrications with other particles were randomly and manually recorded using a software function. The sperm head morphometric dimensions for length (μ m), width (μ m), area (μ m²) and perimeter (μ m) were generated by the system software. Data were compiled and stored for further analysis. After morphometric evaluation, a total of 100 spermatozoa per slide and sample were characterized at 60 \times objective for normal morphology using the procedures previously published by Evans and Maxwell (1987).

2.4. Establishment of a global reproduction performance score for bucks

An individual score taking into account the seasonal changes of the 22 reproduction performance variables was established as follows: The same five previously described reproduction performance categories were considered (i.e., testicular measurements, sexual behaviour, general semen characteristics, sperm motility and sperm morphometry). To assess relative changes due to seasonality, values recorded during the spring were considered as a reference (100%) and values for assessments for each buck during the following seasons were calculated as a percentage of the spring value for that individual. Relative changes were transformed into negative (reduced reproduction performance) or positive (improved reproduction performance) scores: 0 for changes below 10%; -1 or +1 for changes between 10% and 20%; -2 or +2 for changes between 20% and 40%; -3 or +3 for changes between 40% and 60%; -4 or +4 for changes greater than 60%. Scores for each performance category were weighted according to a within-category coefficient with the aim being to take into consideration the greater importance of certain variables. Each performance category score was further weighted using a between-category coefficient that allowed for recognizing the greater importance of these variables to general semen characteristics and sperm motility (see Table 1 for details). This global reproduction performance score ranged between -1 and 1.

2.5. Statistical analysis

Data analysis was performed using SAS 9.0 software (SAS Inst. Inc., Cary, NC, USA). Before analysis, the percentage data were

Table 1

Establishment of a global reproduction performance score aiming to assess seasonal changes of Beni Arouss bucks' reproduction performance.

Between-category coefficient of ponderation	Category	Variables	Within-category coefficient of ponderation
0.10	Testicular measurements	Scrotal circumference	2
		Testicular diameter	2
		Testicular length	1
0.15	Sexual behaviour	Number of ejaculates	1
		Number of mounts before 1 st ejaculation	1
		Reaction time/first ejaculation	1
		Number of mounts before 2 nd ejaculation	1
		Reaction time/second ejaculation	1
0.30	General seminal characteristics	Semen volume	2
		Sperm concentration	3
		Viability	3
		Normal sperm	3
0.30	Sperm motility	TM	1
		PM	1
		Rapid spermatozoa	1
		VCL	1
		VSL	1
		VAP	1
0.15	Sperm morphometry	Length	1
		Width	1
		Area	1
		Perimeter	1

transformed to arcsine and all data were subjected to ANOVA using Proc mixed of SAS. The statistical model included the fixed effect of season or month and the random effect of buck. Body weight of bucks was added as a co-variate to the model. Least squares means for seasons were compared using the PDIF option (a pairwise t-test) of the LSMEANS statement of SAS. Data were expressed as mean \pm SD and the level of significance was set at $P < 0.05$. Pearson correlation coefficients among all the variables were also investigated.

3. Results

3.1. Testicular measurements and sexual behaviour

Changes in values for testicular variables are shown in Table 2 and Fig. 1. Scrotal circumference varied throughout the year and was affected by the bucks' body weight, with the least mean values during the spring and winter (26.0 ± 2.5 cm and 26.0 ± 2.0 cm, respectively) and the greatest values ($P < 0.05$) during summer and autumn (27.5 ± 1.4 cm and 27.5 ± 1.6 cm, respectively). There was a slight increase in the testicular diameter during the summer and autumn (5.6 ± 0.5 cm and 5.7 ± 0.5 cm, respectively) compared with the winter (5.4 ± 0.5 cm; $P < 0.05$). Testicular length was affected by season and month ($P < 0.05$), being least in the winter (10.5 ± 1.5 cm) especially in February (10.0 ± 1.5 cm) compared with all other seasons.

Due to rare occurrences of third ejaculations, only data related to the first and second ejaculation were analyzed (Table 3). Values for most variables related to sexual behavior (number of ejaculates, time for first ejaculation, number of mounts before the second ejaculation and reaction time per second ejaculation) were neither affected by season ($P > 0.05$), nor body weight. In the 3 months of the summer and in September (Fig. 1) there, however, was a decrease in the number of mounts before occurrence of the first ejaculation ($P < 0.001$), where most bucks ejaculated after one mount in comparison with there being two mounts before ejaculation in the other seasons of the year. The greatest libido of the bucks was, therefore, observed in the summer and early autumn.

Table 2Effect of season on Testicular measurements in Beni Arouss bucks ($n = 7$).

Traits	Season			
	Spring	Summer	Autumn	Winter
Scrotal circumference (cm)	26 ± 2.5^b	27.5 ± 1.4^a	27.5 ± 1.6^a	26 ± 2^b
Testicular diameter (cm)	5.5 ± 0.8^{bc}	5.6 ± 0.5^{ab}	5.7 ± 0.5^a	5.4 ± 0.5^c
Testicular length (cm)	11.4 ± 1.8^a	11.7 ± 1^a	11.9 ± 1.1^a	10.5 ± 1.5^b

Data are shown as mean \pm SD; Means with different superscript letters in the same row are different; lowercase letters: $P < 0.05$; uppercase letters: $P < 0.001$.

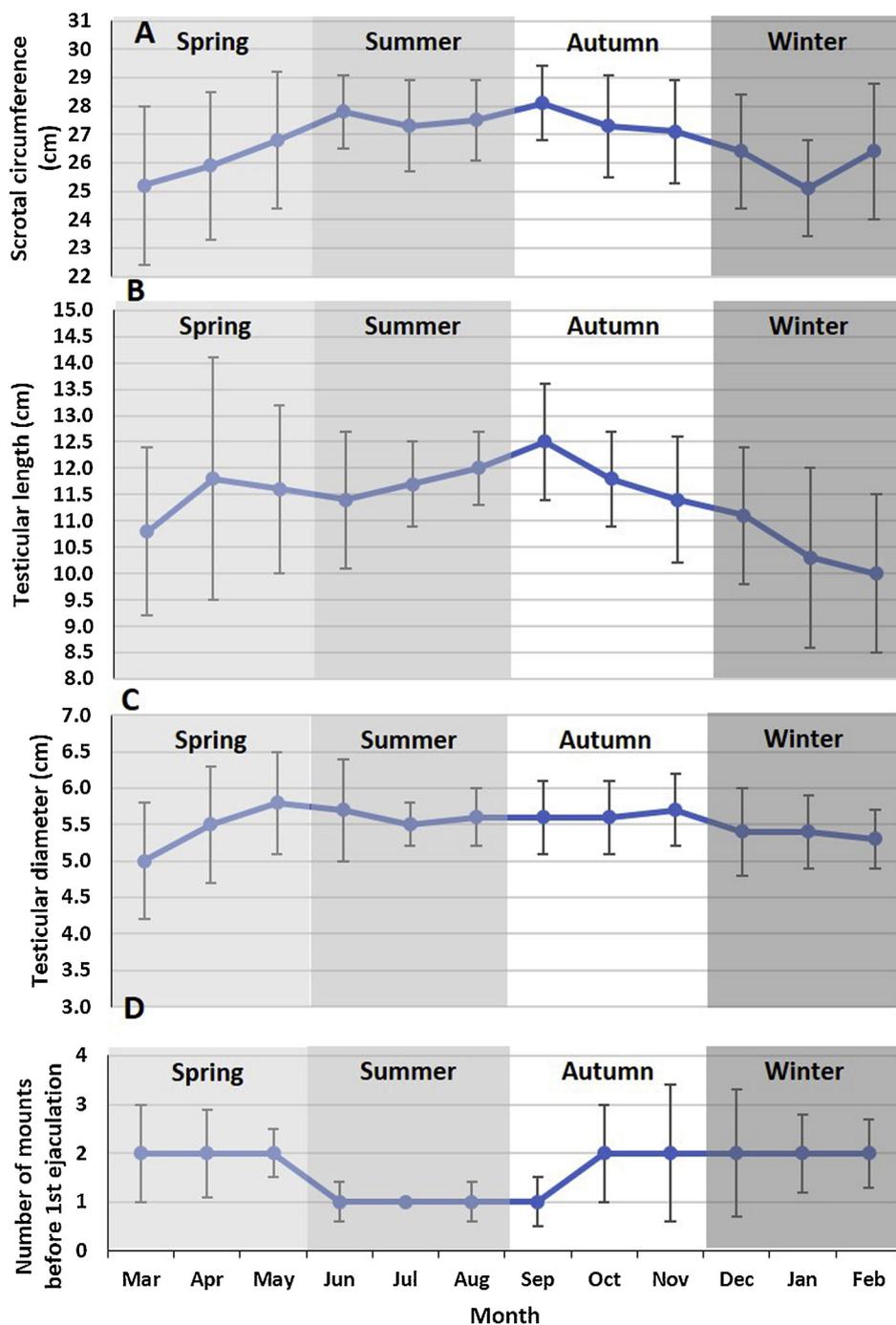


Fig. 1. Monthly variations in scrotal circumference (A), testicular length (B), testicular diameter (C) and number of mounts before first ejaculation (D) in Beni Arouss bucks; Data are shown as mean \pm SD ($P < 0.05$).

3.2. General seminal characteristics

In the present study, a total of 145 ejaculates were collected from seven bucks. There were 84 ejaculates collected from the first ejaculation that were analyzed for general seminal characteristics and CASA sperm motility variables (Tables 4 and 5; Figs. 2–4). Semen volume and concentration were affected by body weight. Except for semen volume, semen characteristics were affected by month or season. An increase in sperm concentration occurred during the summer ($5.5 \pm 2.2 \cdot 10^9/\text{ml}$) compared with the winter ($3.5 \pm 1.0 \cdot 10^9/\text{ml}$; $P < 0.05$). Sperm viability was greater in the spring and summer ($86 \pm 6.8\%$ and $84 \pm 3.9\%$, respectively) than during autumn and winter ($81 \pm 2.3\%$ and $79 \pm 1.3\%$, respectively) with the greatest value being in March ($90.9 \pm 2.5\%$; $P <$

Table 3
Effect of season on sexual behavior in Beni Arouss bucks ($n = 7$).

Traits	Season			
	Spring	Summer	Autumn	Winter
Number of ejaculates	1.6 ± 0.6	1.7 ± 0.6	1.9 ± 0.3	1.7 ± 0.5
Number of mounts before first ejaculation	2 ± 0.8 ^A	1 ± 0.3 ^B	2 ± 1.1 ^A	2 ± 0.9 ^A
Reaction time/first ejaculation (s)	147 ± 223	114 ± 157	75 ± 38	70 ± 61
Number of mounts before second ejaculation	2 ± 1.7	1 ± 0.8	1 ± 0.8	1 ± 0.6
Reaction time/second ejaculation (s)	318 ± 217	336 ± 177	386 ± 80	347 ± 94

Data are shown as mean ± SD; Means with different superscript letters in the same row are different ($P < 0.001$).

Table 4
Effect of season on general seminal characteristics in Beni Arouss bucks ($n = 7$).

Variables	Season			
	Spring	Summer	Autumn	Winter
Semen volume (ml)	0.8 ± 0.3	0.7 ± 0.4	0.8 ± 0.3	0.6 ± 0.3
Sperm concentration ($\times 10^9$ /ml)	4.8 ± 1.4 ^{ab}	5.5 ± 2.2 ^a	4.4 ± 1.2 ^{bc}	3.5 ± 1 ^c
Viability (%)	86 ± 6.8 ^A	84 ± 3.9 ^A	81 ± 2.3 ^B	79 ± 1.3 ^B
Normal sperm (%)	73 ± 6.7 ^C	82 ± 4.9 ^A	78 ± 3.9 ^B	75 ± 3.2 ^C

Data are shown as mean ± SD. Means with different superscript letters in the same row are different; lowercase letters: $P < 0.05$; uppercase letters: $P < 0.001$.

Table 5
Effect of season on values for sperm motility and morphometry variables in Beni Arouss bucks ($n = 7$).

Variables	Season			
	Spring	Summer	Autumn	Winter
TM (%)	88.4 ± 5.7 ^b	89.5 ± 4 ^b	92.2 ± 3.3 ^a	90.8 ± 5.9 ^{ab}
PM (%)	75.1 ± 5.6 ^a	77 ± 3.5 ^a	77.1 ± 2.6 ^a	72.3 ± 5.3 ^b
Rapid spermatozoa (%)	85.1 ± 7.5	86.2 ± 5	89.5 ± 3.9	86.1 ± 8.8
VCL (μ m/s)	140.1 ± 18.2 ^b	145.9 ± 20 ^b	159.7 ± 16.8 ^a	139.6 ± 12.6 ^b
VSL (μ m/s)	86.1 ± 15.6 ^B	95.9 ± 17.2 ^A	100.4 ± 12.3 ^A	77.7 ± 7.4 ^C
VAP (μ m/s)	110.5 ± 19.7 ^c	126.1 ± 22.2 ^b	137 ± 14.9 ^a	112 ± 11.4 ^c
Length (μ m)	8.5 ± 0.1 ^B	8.5 ± 0.2 ^B	8.8 ± 0.1 ^A	8.5 ± 0.1 ^B
Width (μ m)	4.2 ± 0.1 ^B	4.2 ± 0.1 ^B	4.3 ± 0.1 ^A	4.2 ± 0.1 ^B
Area (μ m ²)	30.6 ± 0.9 ^B	30.5 ± 1.3 ^B	32.4 ± 1.1 ^A	30.4 ± 0.8 ^B
Perimeter (μ m)	22.8 ± 0.3 ^B	22.8 ± 0.5 ^B	23.4 ± 0.3 ^A	22.8 ± 0.3 ^B

TM, total motility; PM, progressive motility; VCL, curvilinear velocity; VSL, straight line velocity; VAP, average path velocity.

Data are shown as mean ± SD. Means with different superscript letters in the same row are different; lowercase letters: $P < 0.05$; uppercase letters: $P < 0.001$.

0.001). The greatest percentage of normal sperm was in the summer ($82 \pm 4.9\%$) while the least was in the winter and spring ($75 \pm 3.2\%$ and $73 \pm 6.7\%$, respectively; $P < 0.001$).

3.3. Sperm motility and head morphometry variables

Whereas there was no body weight effect on values for sperm variables, seasonal variation affected semen kinematic motility except for the percentage of rapidly progressive motile spermatozoa (rapid spermatozoa; Table 5, Fig. 3). There was the greatest percentage of motile spermatozoa (total motility, TM) in the autumn ($P < 0.05$) in comparison with mean percentages in the spring and summer. The least percentage of sperm moving progressively forward (progressive motility, PM) was recorded in the winter ($P < 0.05$) in comparison with mean percentage values in the spring, summer and autumn. There was a greater value for sperm velocities, curvilinear velocity (VCL), straight line velocity (VSL) and average path velocity (VAP) during the autumn, followed by lesser values ($P < 0.05$ and $P < 0.001$, respectively for VCL and VAP and VSL) during the winter and spring.

Values for sperm head morphometric CASA measurements are also depicted in Table 5 and Fig. 4. Season influenced head morphometric dimensions ($P < 0.001$). There was an increase in head length, width, area and perimeter during the autumn.

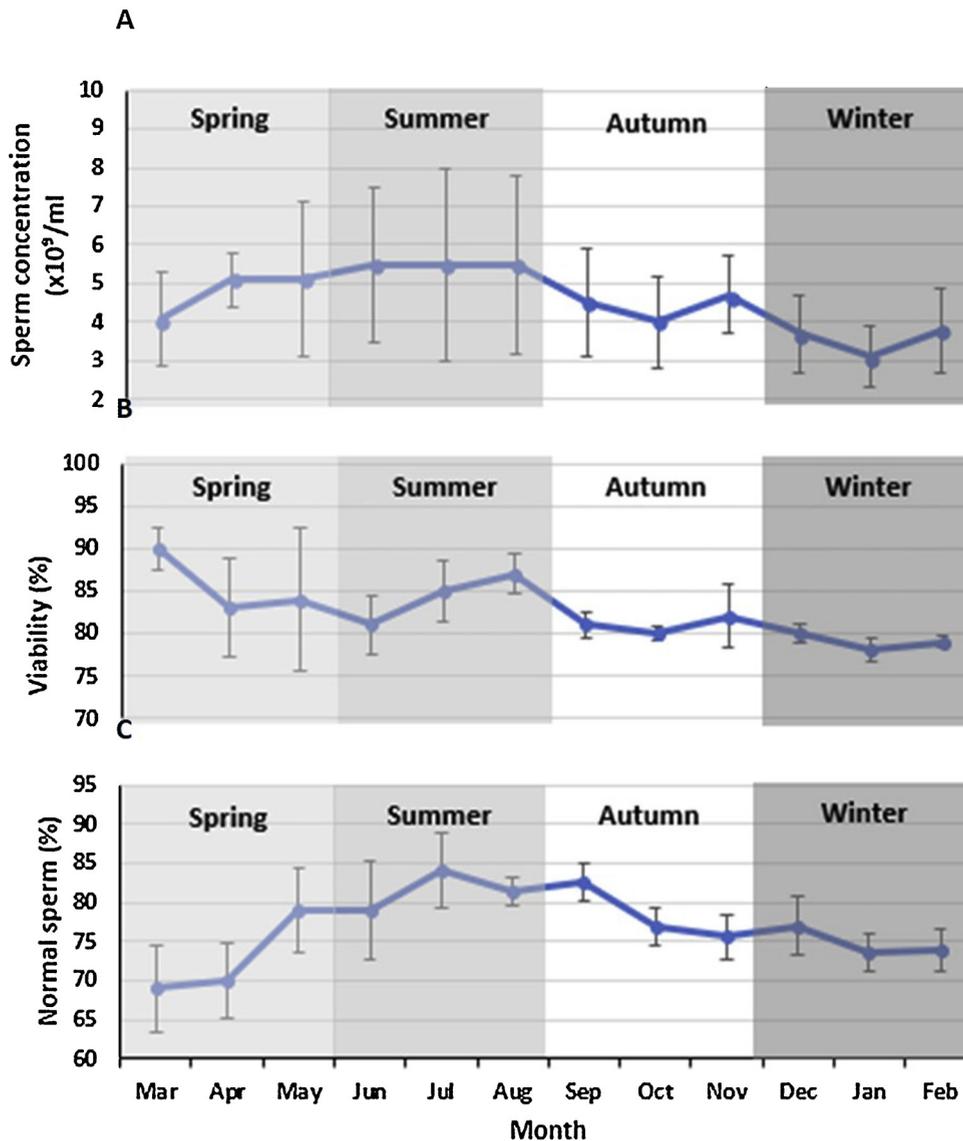


Fig. 2. Monthly variations in sperm concentration (A), viability (B) and normal sperm (C) in Beni Arouss bucks; Data are shown as mean \pm SD ($P < 0.05$).

3.4. Correlation between testicular measurements, general seminal characteristics and sperm motility and head morphometry variables

Correlation coefficients of physical measurements with conventional seminal characteristics and CASA of motility and morphometry are shown in Table 6. The values for a majority of conventional seminal characteristics were positively correlated ($P < 0.05$) with values for physical measurements but the relationship was rather minimal ($r < 0.5$). Most of the values for variables assessed with CASA were not correlated with values for testicular measurements. Similarly, the values for conventional and CASA variables were not correlated. Some of these values were moderately correlated ($P < 0.05$; $r < 0.5$). Among physical measurement variables, values for the SC and TD had the largest coefficients of correlation and with TL ($r = 0.7 - 0.8$; $P < 0.05$). As expected, there was a positive correlation between values for semen volume and sperm concentration ($r = 0.3$; $P < 0.05$). Testicular size was also positively correlated with values for semen volume ($r > 0.3$; $P < 0.05$) and sperm concentration ($r > 0.2$; $P < 0.05$). For CASA variables, values for TM, PM and rapid spermatozoa were correlated ($P < 0.05$; $r = 0.7 - 0.9$). Likewise, values for velocities (VCL, VSL and VAP) were positively correlated ($r = 0.7 - 0.9$; $P < 0.05$). Values for morphometric variables were correlated ($r = 0.6 - 0.9$; $P < 0.05$) except for length and width ($r < 0.5$). Correlation between the values for conventional and CASA motility indicated there was a positive correlation between values for sperm viability and PM ($r = 0.4$; $P < 0.05$) and between values for the percentage of normal sperm and VSL and VAP ($r = 0.4$; $P < 0.05$).

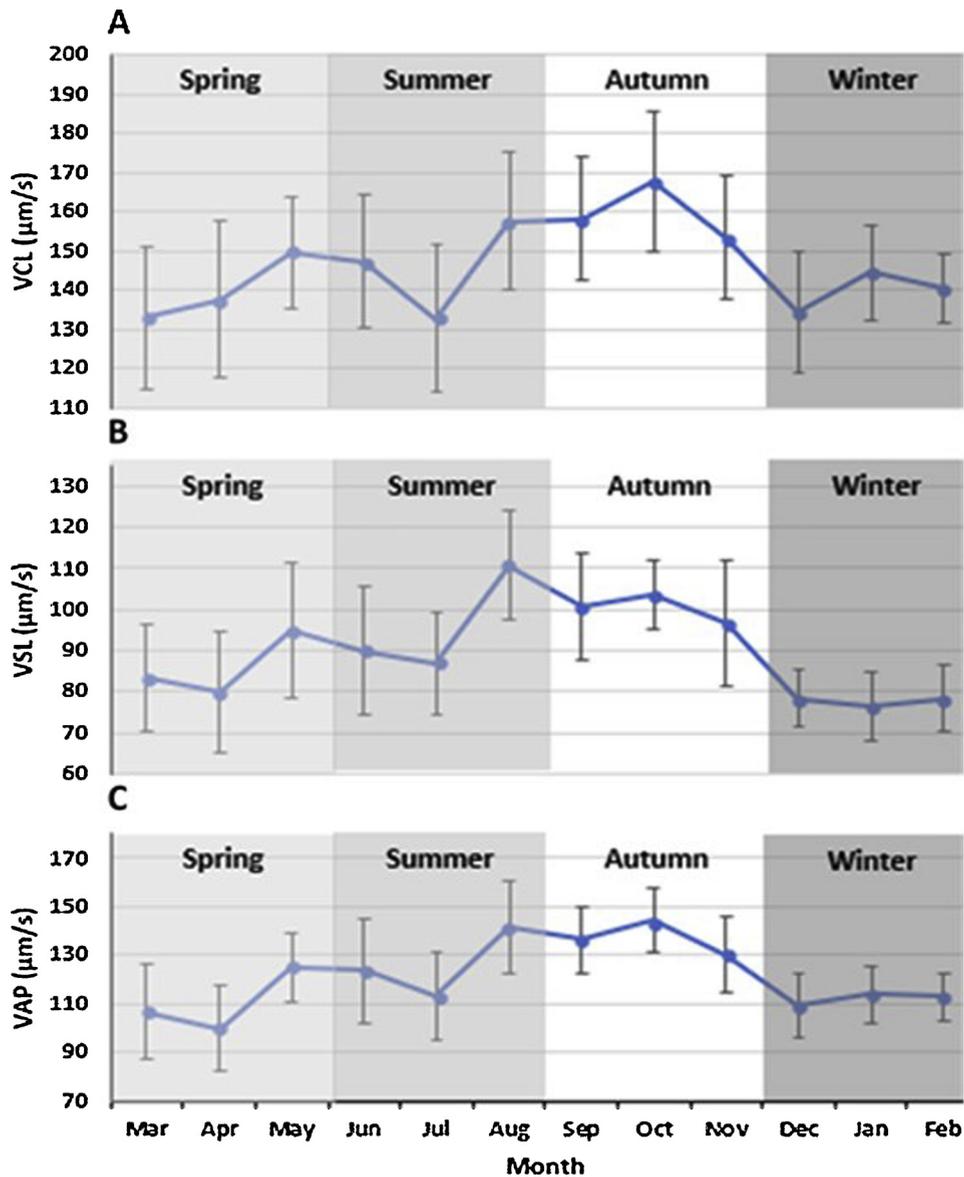


Fig. 3. Monthly variations in values for sperm motility variables: (A) curvilinear velocity (VCL, $\mu\text{m/s}$), (B) straight line velocity (VSL, $\mu\text{m/s}$) and (C) average path velocity (VAP, $\mu\text{m/s}$) in Beni Arouss bucks; Data are shown as mean \pm SD ($P < 0.05$).

3.5. Global reproduction performance score

Variations of the global reproductive performance score with season and for each buck are shown in Fig. 5. In all bucks, the maximum reproductive performance score was observed in the summer and/or autumn.

4. Discussion

This study is the first in which there was evaluation of seasonal changes in reproductive physiology in Beni Arouss males by measuring testes size, reproductive behavior and seminal characteristics using conventional methods and CASA. Because the Beni Arouss goat population which is indigenous to the North of Morocco has been recently considered to be classified as a local breed (official Journal of Kingdom of Morocco; No. 6430; 01/2016), actions are being taken to ensure selection of this status. In the present study, reproductive characteristics of seven sexually mature bucks were investigated. Animals were not selected for study inclusion based on weight and age but rather based on being sexually mature which resulted in some of the individual differences in results among animals. Body weight was taken into consideration in the statistical model and affected values for testicle size as well as semen volume and concentration results, whilst the values for other variables were not affected by body weight. Ahmad and Noakes (1996)

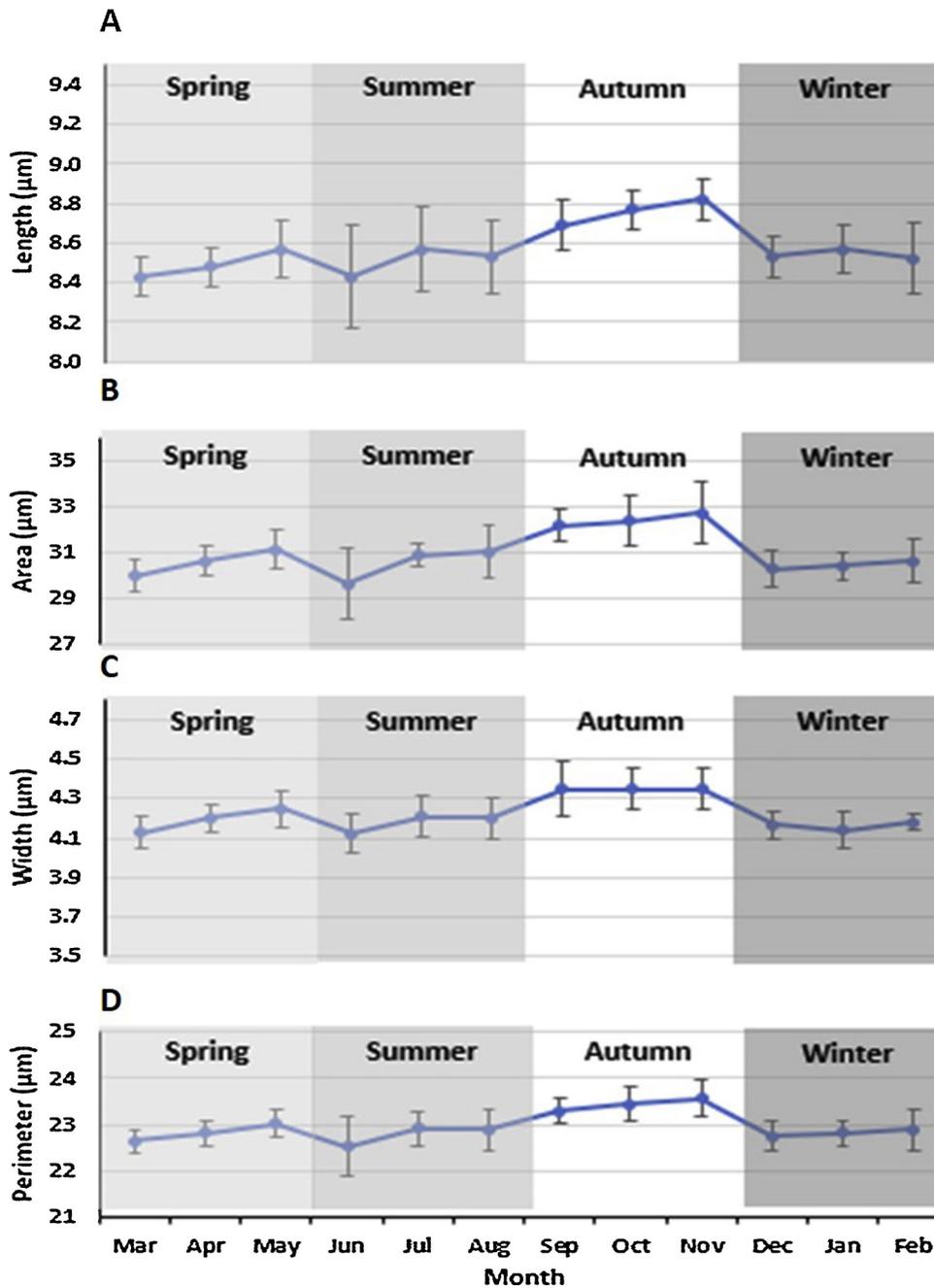


Fig. 4. Monthly variations in values for sperm morphometry variables: (A) length (μm), (B) area (μm), (C) width (μm) and (D) perimeter (μm) in Beni Arous bucks; Data are shown as mean \pm SD ($P < 0.05$).

and Delgadillo et al. (1991) reported body weight and age among factors affecting semen characteristics, but seasonality had greater effect on these characteristics in the present study. In Mediterranean regions, reproductive characteristics of bucks are also affected by feed quality and availability (Perez and Mateas, 1996; Zarazaga et al., 2009). Throughout the present study, the same feeding program was used, thereby minimizing nutrition-related changes on reproduction performance.

The results of the present study indicate reproductive activity of Beni Arous bucks was maintained throughout the year in natural photoperiod conditions (35°N 5°O), however, bucks have seasonal variations in reproductive activity. In the Mediterranean area, from previous studies it has been reported that seasonal variations in reproductive characteristics of bucks occurred as a result of photoperiod and especially at some latitudes (Perez and Mateas, 1996; Zarazaga et al., 2009; Arrebola et al., 2010; Gallego-Calvo et al., 2015; Arrebola and Abecia, 2017). Bucks in latitudes between 30° and 40°N in the Mediterranean areas (e.g., Murciano-Granadina, Verata, Payoya and Blanca Andaluza breeds) had a moderate seasonal increase in values for many reproductive variables

Table 6
Correlation coefficients between values for buck reproductive variables; First ejaculate of each monthly collection was considered for seven bucks.

SC	TD	TL	Semen volume	Sperm concentration	Viability	Normal sperm	TM	PM	Rapid sperm-atozoa	VCL	VSL	VAP	Length	Width	Area	Perimeter
SC	-	0.84	0.37	0.30	NS	0.32	NS	0.26	NS	NS	NS	0.22	NS	0.28	NS	NS
TD	-	0.67	0.36	0.25	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
TL	-	-	0.35	0.21	NS	0.22	NS	0.31	NS	NS	0.26	NS	NS	0.29	NS	NS
Semen volume	-	-	-	0.35	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sperm concentration	-	-	-	-	NS	NS	NS	NS	NS	NS	NS	NS	-0.34	NS	-0.23	-0.31
Viability	-	-	-	-	-	NS	NS	0.40	NS	NS	NS	NS	NS	NS	NS	NS
Normal sperm	-	-	-	-	-	-	NS	0.28	NS	0.28	0.42	0.42	0.24	0.24	0.25	0.23
TM	-	-	-	-	-	-	-	0.67	0.96	0.36	NS	0.23	NS	NS	NS	NS
PM	-	-	-	-	-	-	-	-	0.75	0.27	0.44	0.32	NS	NS	NS	NS
Rapid spermatozoa	-	-	-	-	-	-	-	-	-	0.44	NS	0.31	NS	NS	NS	NS
VCL	-	-	-	-	-	-	-	-	-	-	0.75	0.91	0.42	NS	0.33	0.41
VSL	-	-	-	-	-	-	-	-	-	-	-	0.91	0.23	0.30	0.29	0.27
VAP	-	-	-	-	-	-	-	-	-	-	-	-	0.38	0.27	0.36	0.39
Length	-	-	-	-	-	-	-	-	-	-	-	-	-	0.47	0.82	0.94
Width	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.86	0.63
Area	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.93
Perimeter	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

SC, scrotal circumference; TD, testicular diameter; TL, testicular length; TM, total motility; PM, progressive motility; VCL, curvilinear velocity; VSL, straight line velocity; VAP, average path velocity; Correlation coefficients are indicated if $P < 0.05$; NS: non-significant correlation.

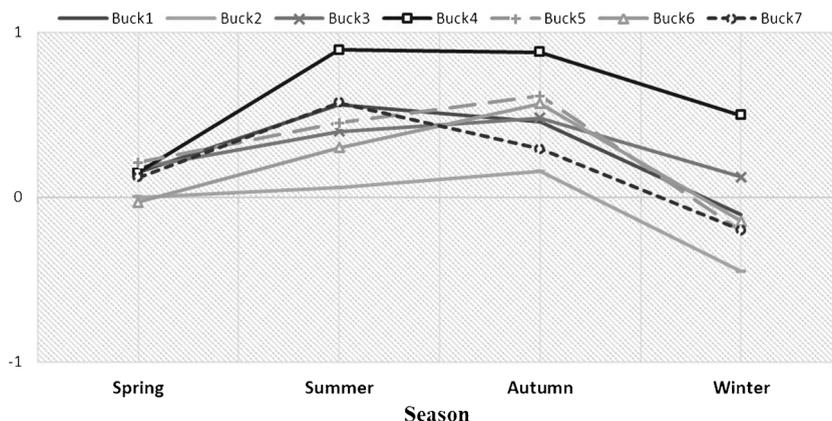


Fig. 5. Seasonal variations of the global reproduction performance score in Beni Arouss bucks.

during the summer and autumn (Roca et al., 1992; Perez and Mateas, 1996; Zarazaga et al., 2009; Arrebola et al., 2010; Gallego-Calvo et al., 2015; Arrebola and Abecia, 2017). Male goats of several breeds that were located in the higher latitudes (e.g. Alpine and Saanen breeds raised in latitudes above 40 °N), had marked seasonal variations in semen production and sexual activity that were maximal during days when there was a decreasing photoperiod (Delgadillo and Chemineau, 1992; Chemineau et al., 1999). In latitudinal zones of less than 30 °N, the seasonality of reproduction is quasi absent in these breeds (Chemineau, 1986).

Similar to animals of other breeds that are located in lower latitudes (30–40 °N) in the Mediterranean and the Middle East regions such as the Blanca Andaluza (Gallego-Calvo et al., 2015), Payoya (Zarazaga et al., 2009), Murciano-Granadina (Arrebola et al., 2010), Verata (Perez and Mateas, 1996), Zaraibi (Barkawi et al., 2006) and Markhoz (Talebi et al., 2009) the greatest values for most of the variables for animals of the Beni Arouss breed were observed during summer and autumn and the least values were in winter. Apparently North Moroccan bucks respond to seasonal changes in similar ways as Murciano-Granadina bucks in Spain (Arrebola et al., 2010) and Zaraibi bucks in Iran (Barkawi et al., 2006) by not being affected by the relatively greater temperatures during the summer.

In the current study, values for testicular measurements were recorded monthly and all the data indicate values were at a maximum in September. When the data were grouped by season there were the greatest values for SC, TD and TL in the summer and autumn with the least values being in the winter. These findings are comparable to those of bucks of the Murciano-Granadina (Roca et al., 1992), Atlas Moroccan (Douk, 1996), Verata (Perez and Mateas, 1996), Saanen and Nubian (Babiker Abdelhai, 2003), Damascus (Al-Ghalban et al., 2004), Rayini (Zamiri and Heidari, 2006) and North Moroccan (Chentouf et al., 2011) breeds or populations. There were fewer mounts before the first ejaculation during the summer and early autumn (September) suggesting that libido was greatest during this season of the year. This trend is comparable to that reported by Barkawi et al. (2006) during the summer where there were fewer mounts and a lesser reaction time per first and second ejaculation.

There were greater sperm concentrations in the spring and summer than winter with a maximum between June and August. Findings in the present study are consistent with those of Arrebola et al. (2010); Chentouf et al. (2011); Wang et al. (2015) and Arrebola and Abecia (2017). In Creole bucks in Mexico, there was the greatest sperm concentration from May to September and the least in February and March (Delgadillo et al., 1999), which is consistent with results of the present study. Also in the present study, there were no seasonal effects on semen volume. Similarly, volume did not differ between seasons in bucks of the Malaguena (Perez and Mateas, 1996), Korean Native (Choe et al., 2006), Murciano-Granadina (Arrebola et al., 2010; Arrebola and Abecia, 2017) and Payoya (Arrebola and Abecia, 2017) breeds. Semen viability ranged from 89% to 76% and was relatively consistent throughout the year. The values, however, were greatest in the spring and summer and maximum in August. These results are consistent with those of Al-Ghalban et al. (2004). In British bucks, however, the values were greatest for live sperm during autumn and winter and least during the spring and summer (Ahmad and Noakes, 1996). In Korean Native and North Moroccan indigenous bucks, there were no seasonal variations for sperm viability (Choe et al., 2006; Chentouf et al., 2011). The percentage of normal sperm was greater during the summer (maximum between July and September, being about 83%), while dead sperm values were greater during the winter and spring (26%). This finding is consistent with that of Perez and Mateas (1996) in Verata bucks and similar to those reported for bucks of different breeds (Karagiannidis et al., 2000; Barkawi et al., 2006; Talebi et al., 2009). In these reports, there were the least values for normal sperm in the winter and spring, while there was the greatest proportion of normal spermatozoa in the autumn. There was less normal sperm morphology in the winter in Murciano-Granadina bucks (Roca et al., 1992). In Damascus and Rayini bucks, however, there were greater values for normal sperm in the spring and summer, while the least values were in the autumn (Al-Ghalban et al., 2004; Zamiri and Heidari, 2006).

Sperm quality was also assessed using the CASA system for the sperm motility and head morphometry characteristic variables. Sperm motility and especially progressive sperm motility and sperm velocities are essential for sperm migration efficiency, oocyte penetration, capacitation and, therefore, for potentially predicting fertilizing capacity (Cox et al., 2006). In the current research, results indicate that values for most of motility variables (PM, VSL and VAP) were greater in the summer and autumn than those in the winter. In semen from Xinong-Saanen bucks, Wang et al. (2015) also reported that values for some of the CASA motility variables

were greater in the autumn than those observed in other seasons. Zarazaga et al. (2009) and Gallego-Calvo et al. (2015), working with Payoya and Blanca Andaluza bucks, reported that there were lesser values for sperm motility variables during the winter compared with the summer, with no differences being detected among the other seasons.

Sperm head morphometry has also been analyzed in relation to fertility in many species. The variation in sperm head dimensions can be considered as an indicator of fertility potential in goats (Hidalgo and Dorado, 2009). In stallions, the sperm morphometry analysis of head area and perimeter were less in stallions with greater fertility (Waheed et al., 2015). Accordingly, dimensions of the sperm head appear to be useful for differentiation between males of relatively greater as compared with those with relatively lesser fertility. Similarly, there was a negative correlation between spermatozoa with a short and wide head and fertility when there was use of fresh ram semen (Martinez-Rodriguez et al., 2015). Inconsistent with this finding, Bravo et al. (2014) reported there was a greater spermatozoa head length and size in rams during the breeding season. For goats, to our knowledge, there have been no previous studies of the effect of season on buck sperm head morphometry. Although the physiological significance of these variables seems unclear and not correlated with values related to semen viability, there was an increase of length, width, surface and perimeter of spermatozoa heads in the autumn.

Regarding correlation analyses, there were correlations between values for physical measurements and conventional seminal characteristics but these did not appear to be a predictor of semen quality. Correlation analysis between values for conventional semen characteristics and CASA variables indicated that viability of sperm was of a similar pattern of change as that for increased progressive motility. Also, normal spermatozoa swim faster and values for this variable are correlated with normal morphology, VSL and VAP. This finding is consistent with that of several studies with human sperm (Katz et al., 1982; Morales et al., 1988; Sivanarayana et al., 2012).

To assess seasonality of reproduction performance of individual animals while considering values for all reproductive variables, a global reproduction score was calculated in the present study. This score takes into account the time-related modifications of values for each variable which in turn is weighted based on relative importance of the variable to fertility capacity (see Table 1 for details). Accordingly, this score reflects the magnitude of seasonal changes for each buck rather than an absolute evaluation of reproduction capacity. There is general agreement of the primary effects of sperm motility, concentration, viability and spermatozoa morphology (Gomendio et al., 2007; Gillan et al., 2008; Love, 2011), thus, the relative weight of these semen characteristics and sperm motility was greatest, followed by sperm morphometry, sexual behavior and testicular measurements in calculation of the global reproduction score. In developing this scoring system, the previous detailed results where there was assessment of reproductive activity of North Moroccan bucks was particularly considered with these results indicating there was greater reproductive function when photoperiod starts decreasing (at summer solstice) and this trend for changes in reproductive function continues into the autumn period. This scoring system further indicates that individual bucks do not undergo seasonal changes of similar magnitude and that some animals have a greater increase in the score in the summer or autumn whereas others have a pronounced decrease in the winter. These individual variations are not assessed by considering mean results but are known to be important in individual animals (Bravo et al., 2011; Vicente-Fiel et al., 2013).

5. Conclusion

Results of the present study indicate that reproduction of North Moroccan bucks have a distinct seasonal pattern for reproductive capacity. There were the greatest values for semen variables during the summer and autumn which suggests that ejaculates collected during the summer and autumn would be more suitable for cryopreservation than those collected during the other seasons of the year. Even though there was a significant seasonal effect, there were values for reproductive characteristic and sperm quality variables that were sustained throughout the year that were greater than the minimal limits for use of semen in AI in goat and sheep centers. The reproductive performance score indicated that seasonal changes of reproduction capacity differ between individuals.

Conflict of interest

The authors declare that there is no conflict of interest that would prejudice the impartiality in conducting the experiment and publishing the manuscript.

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