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RESEARCH ARTICLE

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Seasonal changes in reproductive activity, sperm variables and sperm freezability in Blanca Andaluza bucks

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Abstract

Interest in the preservation of endangered breeds such as the Blanca Andaluza goat, has increased and some steps should be therefore taken to ensure it. The study was designed to determine the seasonal reproductive pattern of Blanca Andaluza bucks, and whether this affects the quality of their semen and its freezability over the year. Seven bucks were used and their body weight, testicular weight, plasma testosterone concentration and fresh sperm quality determined every week. The collected sperm was cryopreserved and stored; it was then thawed and the same sperm quality variables measured every fortnight. High plasma testosterone concentrations were recorded during the summer and autumn, and low concentrations were recorded during winter and spring ($p<0.001$). No differences were seen between seasons in terms of the percentage of bucks ejaculating, the percentage of active bucks, or ejaculate volume. However, the sperm concentration, the total number of sperm per ejaculate, and the values for most fresh sperm variables were lower during the winter period (at least $p<0.05$). After freezing-thawing, the quality of winter-collected sperm was better, in some respects, than that of summer-collected sperm (at least $p<0.05$). These results reveal that Blanca Andaluza bucks show seasonal reproductive activity in terms of their plasma testosterone concentration, but no clear change in their sexual behaviour between seasons was observed. The values of fresh sperm variables also vary over the year, reaching their lowest during winter. However, after freezing-thawing, winter-collected sperm is of overall better quality than sperm collected during the summer.

Additional key words: endangered goat breed; testosterone; seasonality; fresh semen; cooled semen; semen cryopreservation.

Abbreviations used: BW (body weight); LH (luteinizing hormone); LIN (linearity coefficient); STR (straightness coefficient); TW (testicular weight); VAP (average velocity); VCL (curvilinear velocity); VSL (straight-line velocity); WOB (Wobble coefficient); WWS (week within the season).

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Introduction

The Blanca Andaluza breed of goat, which is native to Spain and adapted to Mediterranean environmental conditions, is endangered according to the Official Catalogue of Spanish Livestock Breeds (RD 2129/2008; BOE, 2009). Steps should therefore be taken to ensure its preservation.

In addition to its use in the genetic improvement of livestock animals, semen cryopreservation is essential in the preservation of endangered genetic resources. It is not sure whether male Blanca Andaluza goats show reproductive seasonality. Understanding how this might affect semen quality and freezability over the year could throw light on how to improve the quality of cryopreserved sperm.

The photoperiod has been suggested the main factor influencing seasonality in buck reproductive activity (Delgadillo *et al*., 1993). Short days and decreasing day-length stimulate the secretion of luteinizing hormone (LH), which in turn, induces testicular growth and the release of testosterone, resulting in quantitative and qualitative improvements in semen production plus increased sexual behaviour. In contrast, long days and in-

creasing daylength reduce LH secretion and testicular growth, leading to a fall in the plasma testosterone concentration, reduced sperm quality, and diminished sexual behaviour (Rouger, 1974; Muduuli *et al.*, 1979; Corteel, 1981; Thimonier *et al*., 1986; Pelletier *et al*., 1988; Delgadillo & Chemineau, 1992; Zarazaga *et al*., 2009).

The information in the literature regarding the freezability and fertilizing capacity of buck sperm collected during the breeding and non-breeding season is contradictory. Some authors report sperm freezability, frozen-thawed sperm variables (Nunez *et al*., 1982; Boue & Corteel, 1992; Muhuyi *et al*., 1992; Pintado *et al*., 1992; Tuli & Holtz, 1995) and the fertilizing capacity of frozen-thawed sperm (Corteel *et al*., 1978) to be better in sperm collected during the breeding season. However, other authors (Peskovatskov *et al*., 1974; Summermatter & Flukiger, 1982) report no seasonal differences.

The aims of the present work were to: 1) determine whether Blanca Andaluza bucks show a seasonal pattern of reproductive activity; and 2) examine the quality of frozen-thawed sperm collected at different times of the year. The results obtained could be of use in programmes designed to preserve this breed of goat.

Material and methods

General

All procedures were performed by trained personnel in strict accordance with Spanish guidelines for the protection of experimental animals (RD 53/2013; BOE, 2013), and in agreement with European Union Directive 86/609. The study was conducted at the University of Huelva experimental farm (37º 20ºN, 6º 54' W), which meets the requirements of the European Community Commission for Scientific Procedure Establishments (2010/63; OJEU, 2010).

The animals examined were seven Blanca Andaluza bucks (8 months old at the start of the experiment), previously trained to mount a teaser doe and to ejaculate into an artificial vagina. These animals were fed daily with barley straw (*ad libitum*), lucerne hay and a commercial concentrate, according to their body weight and in agreement with INRA standards (Morand-Fehr & Sauvant, 1988). All animals had free access to water and mineral blocks containing trace elements and vitamins.

Experimental design

Data collection began on 14th November 2012 and ended on 9th July 2014. However, since the bucks were young at the start of the experiment, only data from the last year (July 2013 to July 2014) were used in analyses. The experiment was designed to determine the effect of season on reproductive status and sperm variables. Summer, autumn, winter and spring were defined as the periods between June 21st and September 22nd, September 23rd and December 20th, December 21st and March 20th, March 21st and June 20th, respectively.

Body weight, testicular weight, and plasma testosterone concentrations

Body weight (BW), testicular weight (TW) and plasma testosterone concentrations were recorded weekly throughout the study. Testicular weight was assessed by comparative palpation using an orchidometer; the same operator always performed this task (Oldham *et al*., 1978). Blood for determining the plasma testosterone concentration was obtained by jugular venipuncture, employing vacuum tubes containing heparin. This was performed once per week at 09:00 h over the entire experimental period. Plasma was obtained by centrifuging the collected blood at 3000 *g* for 30 min. It was then stored at -20ºC until analysis for testosterone using a commercial enzyme-linked immunoassay (ELISA) kit (Demeditec Diagnostics, Kiel-Wellsee, Germany). All plasma samples were analysed at the same time at the end of the experiment. The intra-assay coefficient of variation, estimated from plasma standards, was 8.9% for samples containing 0.5 ng/mL testosterone, 5.1% for samples with 2 ng/mL, and 7.3% for a samples containing 16 ng/mL.

Semen collection and sexual behaviour

Semen was collected weekly. On each occasion, the sexual behaviour of each buck was assessed by presenting it with an intact oestrus-induced doe, allowing 5 min for the male to ejaculate. Oestrus was induced in teaser does via a subcutaneous injection of 2 mg of oestradiol cypionate (Sigma-Aldrich Química, S.A., Spain) (Delgadillo *et al*., 1999; Zarazaga *et al*., 2009). The ejaculation latency, the percentage of bucks that ejaculated, and the percentage of active males (bucks that attempted to ejaculate at least twice, but did not achieve ejaculation within 5 min), were recorded. Animals were always tested in the same order and by the same handlers.

Sperm evaluation

A total of 324 ejaculates were evaluated. The volume of ejaculated semen was recorded immediately after

collection in a graduated collection vial. Overall motility was immediately assessed by transferring a drop of undiluted semen to a warm slide (35ºC), placing a cover slip on it, and observing it under a microscope at 40×. Results were recorded on an arbitrary scale of 0 to 5 (0 = no motility, 5 = 100% motility) (Baril *et al*., 1993).

Sperm concentration and kinematic motility variables were measured using a CASA system (ISAS, Proiser SL, Valencia, Spain). Sperm concentration was measured employing a Bürker chamber after diluting an aliquot of semen with a 0.05% formaldehyde saline solution (1:400) and observing at 400× magnification. The total number of spermatozoa per ejaculate was calculated from the ejaculate volume and sperm concentration. The kinematic motility variables recorded by the CASA system were: percentage of static, motile and progressive motile spermatozoa, curvilinear velocity (VCL, µm/s, *i.e*., the velocity of the actual trajectory of the sperm), straight-line velocity (VSL, µm/s, *i.e*., the velocity calculated using the straight-line distance between the beginning and end of the sperm track), average velocity (VAP, µm/s, *i.e*., the velocity over the smooth calculated path), and the linearity coefficient (LIN), straightness coefficient (STR) and wobble coefficient (WOB). Sperm were classified as being of medium velocity when their VCL was between 45 and 75 µm/s and rapid when their VCL was >75 µm/s. Sperm were deemed progressively motile when their trajectory was straight for at least 80% of the path taken, as suggested by the manufacturer of the CASA system.

Sperm processing, chilling and sperm freezing

Selected semen samples, *i.e*., those with a sperm concentration of $>3500 \cdot 10^6$ spermatozoa/mL, and an overall motility of ≥4 according to the above criteria, were frozen every fortnight. These criteria determined that a total of 128 ejaculates were processed. After checking that these criteria were met, the chosen samples were diluted in washing solution at 1:10 (v:v) (250 mM Tris, 28 mM glucose, 104 mM citric acid, 0.05% streptomycin, 500 UI penicillin/mL) at 37ºC, and then centrifuged once (700 *g* for 15 min) to eliminate the seminal plasma. The supernatant was then removed (now at room temperature [20ºC]) and the pellet diluted in a Tris-yolk extender (250 mM Tris, 28 mM glucose, 104 mM citric acid, 12% egg yolk, 0.05% streptomycin, 500 UI penicillin/mL and distilled water to 100 mL). After 5 min, a similar volume of a second extender (250 mM Tris, 28 mM glucose, 104 mM citric acid, 12% egg yolk, 8% glycerol, 0.05% streptomycin, 500 UI penicillin/mL, and distilled water to 100 mL) was added at room temperature (20ºC), resulting in a final sperm concentration of $800 \cdot 10^6$ spermatozoa/mL, 6% egg yolk and 4% glycerol. Lecithin in the egg yolk was inactivated by subjecting the latter to 56ºC for 30 min before its use in the extenders. Both extenders were prepared in the laboratory using reagent-grade chemicals purchased from Panreac Química S.A. (Barcelona, Spain) and the Sigma Chemical Co. (St. Louis, MO, USA). The sperm was then chilled from room temperature to 5ºC in a cooler over a period of least 3 h, before being packed in 0.25 or 0.50 mL plastic straws. Finally, the straws were placed horizontally on a rack situated 4 cm above the surface of a liquid nitrogen bath for 15 min before being plunged into the same bath. The frozen straws were then stored in liquid nitrogen.

Post-chilling and post-thawing assessment of sperm variables

Sperm quality was evaluated after the 3 h chilling period and again after freezing-thawing; thawing was performed no later than one day after freezing. The frozen straws were thawed in a water bath at 37ºC for 30 s. Both the chilled and frozen-thawed sperm was transferred to a warm slide and the kinematic motility variables measured as described above.

Statistical analyses

The effect of season (spring, summer, autumn and winter) and week within the season (WWS, with 13 weeks per season) on BW, TW and plasma testosterone concentration was analysed using the repeated measures option of the General Linear Model procedure provided by the SPSS package for Windows (2008). In this model, the different seasons and WWS were taken as intra-subject factors. Significant differences in the influence of WWS were analysed using the Bonferroni test.

ANOVA including the male as fixed factor was used to examine the effect of season on all the studied variables in fresh, chilled and frozen-thawed sperm. Variables expressed as percentages (sperm motility, bucks that ejaculated, and percentage of active males), and the values for overall motility were arcsine-transformed before analysis. When differences between seasons were detected, they were examined using the Tukey t-test. ANOVA was also used to compare the effect of season on the motility variables of the rapid and medium velocity fresh, chilled and frozen-thawed sperm. Significance was set at $p<0.05$.

Results

Body weight, testicular weight and plasma testosterone concentration

Repeated measures analysis showed season to have a significant effect on BW and plasma testosterone concentration ($p<0.01$). The BW was higher during spring than any other season, and plasma testosterone was higher during summer and autumn than during spring or winter (Table 1). Moreover, the interaction *Season* × *WWS* had a significant ($p<0.01$) effect on plasma testosterone, with concentrations increasing over the weeks of summer, and decreasing over the weeks of autumn. During spring and winter, testosterone concentrations remained at low levels, during summer testosterone concentrations increased, and during autumn they decreased (Fig. 1).

Sexual behaviour and fresh sperm variables

No differences were seen (Table 2) between seasons in terms of the percentage of bucks that ejaculated

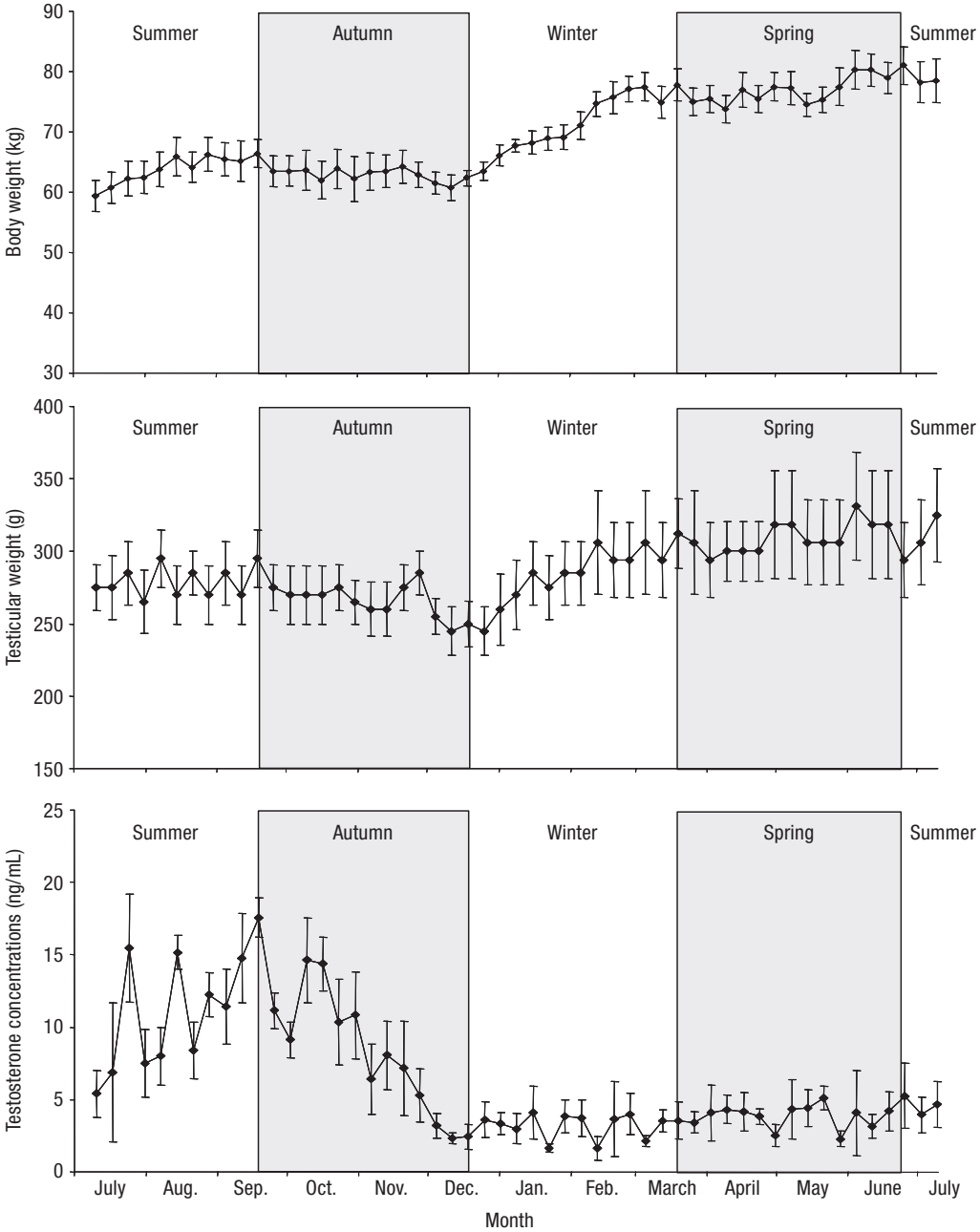

Figure 1. Weekly means (±SEM) over one year for body weight (kg, top), testicular weight (g, middle) and plasma testosterone concentration (bottom, ng/mL) for seven Blanca Andaluza bucks maintained under natural photoperiod conditions.

Table 1. Live weight, testicular weight and plasma testosterone concentration over the four seasons of the year. Values for each season are means ± SEM.

	Summer	Autumn	Winter	Spring
Live weight (kg)	67.1 ± 1.1b	62.9 ± 0.7c	71.7 ± 0.8d	76.8 ± 0.7a
Testicular weight (g)	284.3 ± 5.5	265.8 ± 4.5	283.9 ± 6.6	309.6 ± 7.7
Testosterone concentration (ng/mL)	10.00 ± 0.81a	8.11 ± 0.73a	3.24 ± 0.33b	3.84 ± 0.36b

Different letters in the same row indicate significant differences between groups (p<0.05).

Table 2. Sexual behaviour and values for fresh sperm variables over the four seasons of the year. Values for each season are means ± SEM.

	Summer	Autumn	Winter	Spring
Bucks that ejaculate (%)	82.1	87.7	83.1	90.4
Active males (%)	89.6	93.8	93.2	96.2
Ejaculation latency (s)	51.8 ± 6.0	43.5 ± 6.4	55.7 ± 5.1	36.3 ± 3.6
Ejaculate volume (mL)	0.87 ± 0.04	0.91 ± 0.04	0.93 ± 0.09	0.95 ± 0.05
Semen concentration (10^6 sperm/mL)	7776.8 ± 757.6a	5346.2 ± 395.1b	3336.1 ± 449.4c	4141.0 ± 448.6bc
Total sperm per ejaculate (10^6 sperm/ejaculate)	6606.4 ± 663.0a	5011.4 ± 478.6ab	3291.7 ± 635.2b	4382.6 ± 617.6b
Global motility	4.52 ± 0.15a	4.21 ± 0.15a	3.38 ± 0.28b	4.00 ± 0.24ab

Different letters in the same row indicate significant differences between groups (p <0.05).

(85.6%), the percentage of bucks considered active (93.0%), ejaculation latency (47.0 ± 2.8 s), or ejaculate volume (0.91 ± 0.03 mL). However, differences were seen between seasons in terms of sperm concentration and the number of sperm per ejaculate, with values lower during winter and higher in summer (at least, p<0.01; Table 2). Finally, overall motility varied between seasons (p<0.01; Table 2), with the lowest values recorded during winter.

Fresh sperm kinematic motility variables

The VSL, LIN, STR and WOB values, and the percentages of motile, rapid and progressive spermatozoa, varied between season, with the lowest values recorded during winter (at least p<0.05; Fig. 2). For the rapid spermatozoa, only the values of VSL and LIN varied between seasons (Table 3), with the lowest values recorded in winter and the highest in summer. For the medium velocity spermatozoa, the values of all the kinematic motility variables varied between seasons, again with the lowest values recorded in winter and the highest in summer (Table 4).

Chilled sperm variables

Only the values of VCL, LIN, STR and WOB varied between seasons (at least p<0.05; Fig. 3), with the highest VCL and lowest LIN, STR and WOB values recorded in autumn. For the rapid spermatozoa, VSL, LIN, STR and WOB all varied between seasons, with the highest values recorded in spring (Table 3). For the medium velocity spermatozoa, the values of all the kinematic motility variables (except for VCL) varied between seasons, with the lowest recorded in autumn (Table 4).

Frozen-thawed sperm variables

The VCL and VAP results differed between seasons, with higher values recorded in winter than in summer. Very large differences were also observed in terms of the percentage of motile, rapid and progressive spermatozoa, with the best results recorded in winter (Fig. 4).

When examined separately, neither the rapid nor medium velocity spermatozoa differed between seasons in terms of any kinematic motility variable (Tables 3 and 4).

Discussion

The present results show that, when maintained under natural photoperiod conditions, Blanca Andaluza bucks show marked seasonal variation in their reproductive activity, as measured by the plasma testosterone concentration and fresh sperm quality and freezability. When plasma testosterone concentrations were high the bucks showed their lowest body weight and vice versa. In general the lowest values for fresh semen variables were recorded in winter. However, winter-collected sperm returned better quality results after freezing-thawing than summer-collected sperm.

Table 3. Values for kinematic motility variables for rapid velocity spermatozoa in fresh, chilled and freeze-thawed sperm over the four seasons of the year. Values for each group are means ± SEM.

	Variable	Summer	Autumn	Winter	Spring
Fresh semen	VCL	108.3 ± 1.3	107.2 ± 2.5	100.0 ± 4.3	98.7 ± 6.0
	VSL	86.7 ± 1.8a	78.3 ± 2.4ab	70.1 ± 4.4b	79.36 ± 5.0ab
	VAP	100.6 ± 1.6	94.2 ± 2.7	86.8 ± 4.3	92.4 ± 5.7
	LIN	79.9 ± 1.2a	71.4 ± 2.0ab	65.8 ± 3.8b	71.8 ± 4.4ab
	STR	86.0 ± 0.8	81.4 ± 1.8	75.5 ± 3.7	76.6 ± 4.6
	WOB	92.8 ± 0.6	85.9 ± 2.2	82.0 ± 3.6	83.3 ± 5.0
Chilled semen	VCL	100.7 ± 1.4	101.4 ± 1.7	96.2 ± 1.0	99.1 ± 1.5
	VSL	54.6 ± 2.4ab	51.4 ± 2.3b	55.7 ± 2.1ab	64.3 ± 3.3a
	VAP	77.7 ± 2.2	77.7 ± 2.2	77.9 ± 1.6	85.6 ± 2.2
	LIN	54.2 ± 2.4b	50.7 ± 2.0b	57.9 ± 2.1ab	64.8 ± 2.9a
	STR	69.7 ± 1.6ab	65.8 ± 1.5b	71.2 ± 1.5ab	74.7 ± 2.1a
	WOB	77.1 ± 1.9b	76.5 ± 1.6b	81.0 ± 1.4ab	86.3 ± 1.6a
Frozen-thawed semen	VCL	85.1 ± 4.5	89.3 ± 0.6	92.6 ± 0.9	93.6 ± 1.0
	VSL	56.2 ± 3.4	53.7 ± 1.0	59.5 ± 2.2	61.2 ± 2.1
	VAP	69.7 ± 3.9	71.3 ± 1.2	77.1 ± 1.6	79.9 ± 1.3
	LIN	62.7 ± 3.8	60.1 ± 1.0	64.3 ± 2.4	65.4 ± 2.3
	STR	76.4 ± 4.2	75.3 ± 0.6	77.0 ± 1.9	76.4 ± 1.9
	WOB	77.8 ± 4.3	79.8 ± 1.0	83.3 ± 1.4	85.4 ± 1.1

VCL, curvilinear velocity, µm/s; VSL, straight-line velocity, µm/s; VAP, average path velocity, µm/s; LIN, linearity coefficient, %; STR, straightness coefficient, %; WOB, Wobble coefficient, %. Different letters in the same row indicate significant differences between groups (p<0.05).

Table 4. Values for kinematic motility variables for medium velocity spermatozoa in fresh, chilled and freeze-thawed sperm over the four seasons of the year. Values for each group are means ± SEM.

	Variable	Summer	Autumn	Winter	Spring
Fresh semen	VCL	63.0 ± 1.2a	60.1 ± 1.6a	50.7 ± 3.9b	57.9 ± 2.8ab
	VSL	50.4 ± 1.2a	44.1 ± 1.8ab	35.8 ± 3.1b	46.6 ± 2.8a
	VAP	57.9 ± 1.2a	51.9 ± 1.8a	42.6 ± 3.5b	52.8 ± 2.9a
	LIN	80.3 ± 1.2a	72.4 ± 2.1a	59.8 ± 4.8b	74.6 ± 4.1a
	STR	87.1 ± 0.8a	84.5 ± 1.2a	70.4 ± 5.1b	81.8 ± 4.0ab
	WOB	92.0 ± 0.7a	85.4 ± 1.8a	71.0 ± 5.3b	84.9 ± 4.0a
Chilled semen	VCL	62.6 ± 0.3	62.5 ± 0.4	65.1 ± 2.6	61.8 ± 0.8
	VSL	36.0 ± 1.5ab	32.0 ± 1.4b	38.6 ± 1.7a	38.4 ± 1.2a
	VAP	48.5 ± 1.2ab	47.2 ± 0.8b	52.4 ± 2.3a	50.2 ± 1.1ab
	LIN	57.4 ± 2.3ab	51.1 ± 2.0b	59.4 ± 1.8ab	62.1 ± 1.9a
	STR	73.6 ± 1.6ab	67.3 ± 2.0b	73.8 ± 1.4ab	76.2 ± 1.1a
	WOB	77.4 ± 1.6ab	75.5 ± 1.0b	80.3 ± 1.1ab	81.3 ± 1.4a
Frozen-thawed semen	VCL	59.6 ± 0.6	60.9 ± 0.4	61.7 ± 0.6	60.8 ± 0.7
	VSL	39.8 ± 1.8	38.6 ± 0.6	40.9 ± 1.3	39.9 ± 0.7
	VAP	49.3 ± 1.4	49.7 ± 0.5	51.4 ± 1.0	50.8 ± 0.6
	LIN	66.3 ± 2.7	63.5 ± 1.1	66.3 ± 1.9	65.7 ± 1.3
	STR	79.6 ± 2.1	77.7 ± 0.8	79.4 ± 1.3	78.6 ± 1.0
	WOB	82.5 ± 1.8	81.7 ± 0.7	83.4 ± 1.3	83.4 ± 0.7

VCL: curvilinear velocity, µm/s; VSL: straight-line velocity, µm/s; VAP: average path velocity, µm/s; LIN: linearity coefficient, %; STR: straightness coefficient, %; WOB: Wobble coefficient, %. Different letters in the same row indicate significant differences between groups (p<0.05).

The plasma testosterone concentrations recorded were clearly associated with the natural photoperiod; high testosterone concentrations were recorded during summer and autumn (decreasing daylength), and low concentrations were recorded during winter and spring (increasing daylength). These results reveal the existence of a well-defined breeding season characterised by high testosterone production in these animals. The seasonal changes in testosterone secretion seen were very similar to those reported for Payoya bucks living

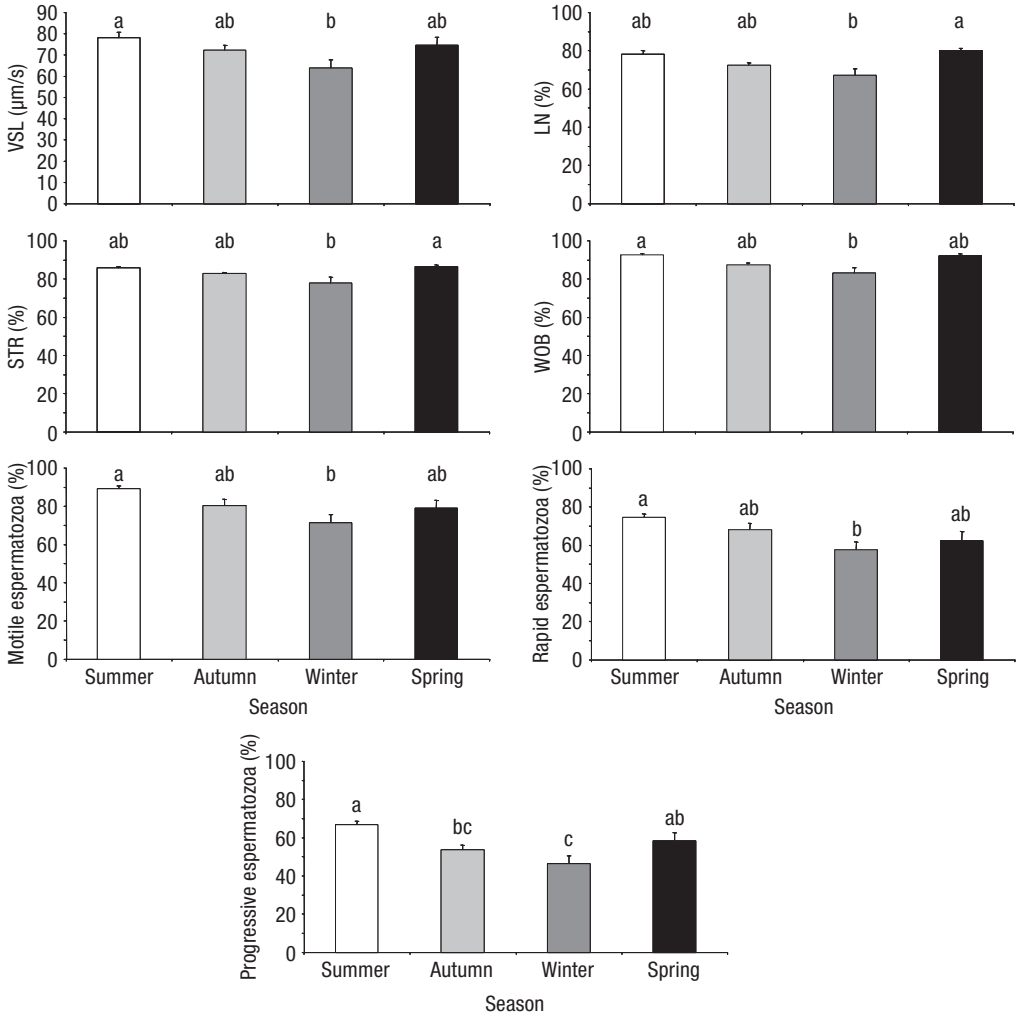

Figure 2. Mean values (±SEM) for straight-line velocity (VSL, µm/s), linearity coefficient (LIN, %), straightness coefficient (STR, %), Wobble coefficient (WOB, %), motile spermatozoa (%), rapid spermatozoa (%) and progressive spermatozoa (%) in fresh semen over the four seasons of the year (Summer: n=86; Autumn: n=80; Winter: n=76; Spring: n=82). Different letters above the histogram bars, indicate significant differences between groups (p<0.05).

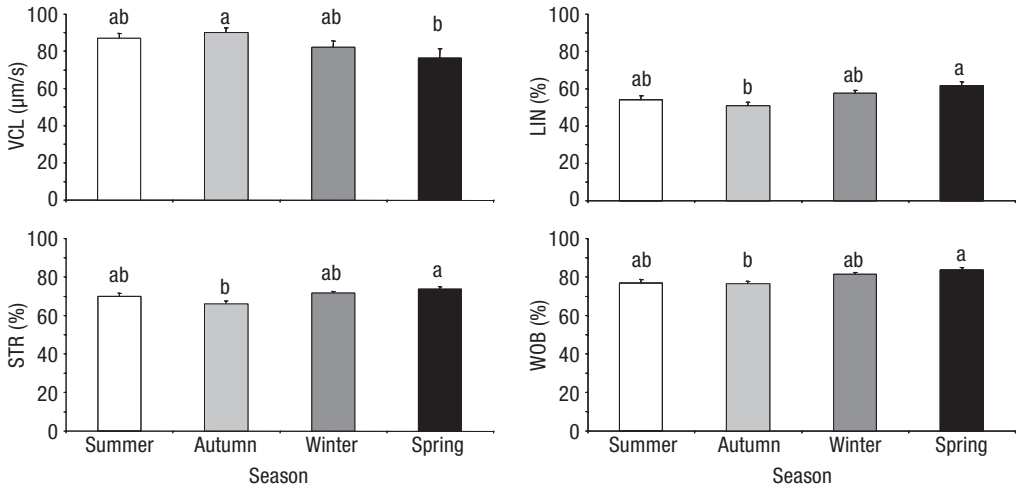

Figure 3. Mean values (±SEM) for curvilinear velocity (VCL, µm/s), linearity coefficient (LIN, %), straightness coefficient (STR, %), Wobble coefficient (WOB, %) in chilled sperm over the four seasons of the year (Summer: n=40; Autumn: n=40; Winter: n=24; Spring: n=24). Different letters above the histogram bars, indicate significant differences between groups (p<0.05).

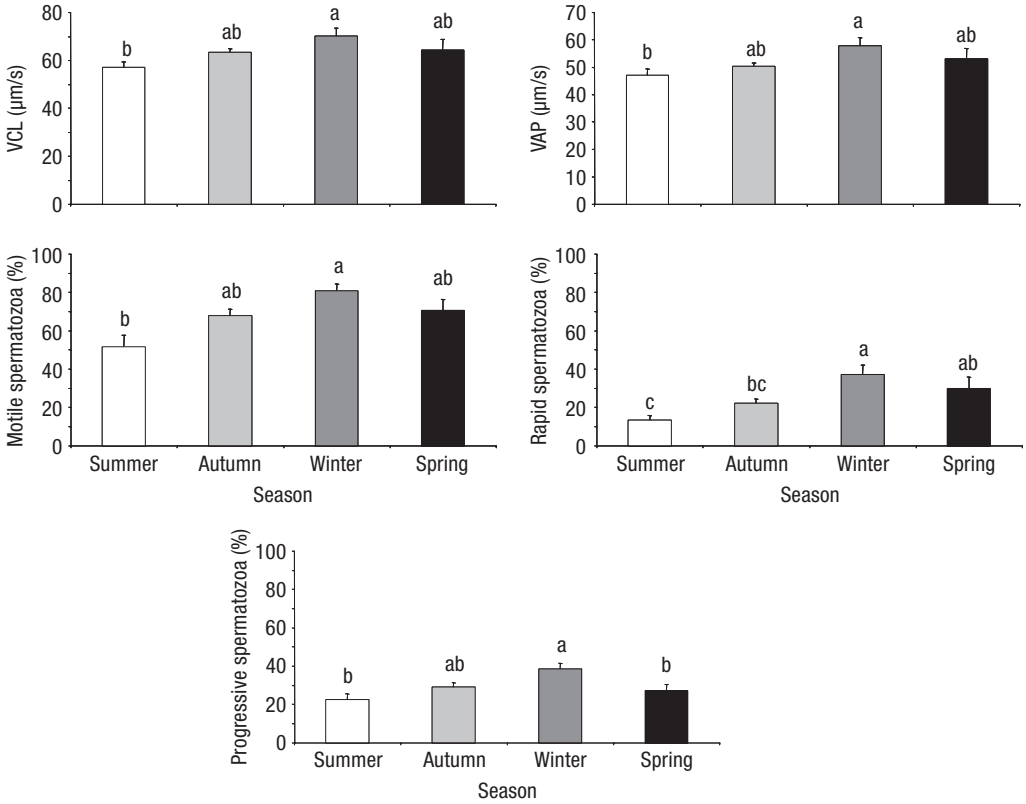

Figure 4. Mean values (±SEM) for curvilinear velocity (VCL, µm/s), average path velocity (VAP, µm/s), motile spermatozoa (%), rapid spermatozoa (%) and progressive spermatozoa (%) in frozen-thawed sperm over the four seasons of the year (Summer: n=40; Autumn: n=40; Winter: n=24; Spring: n=24). Different letters above the histogram bar, indicate significant differences between groups (p<0.05).

at the same latitude (Zarazaga *et al*., 2009), for Creole bucks in Mexico (Delgadillo *et al*., 1999; 2004), for Blanca Andaluza bucks under artificial photoperiod (Gallego-Calvo *et al*., 2015) and bucks of other Mediterranean goat breeds (Todini *et al*., 2007). However, in Alpine bucks living at 46ºN, increases in plasma testosterone are delayed until late August–September (Delgadillo & Chemineau, 1992). This is probably due to a longer lag time between the perception of the photoperiodic signal and the expression of physiological responses in these animals (Delgadillo *et al*., 2004).

The changes seen in plasma testosterone were inversely associated with changes in BW. Such BW changes have previously been reported in both sexes of this species (Delgadillo *et al*., 1991; Walkden-Brown *et al*., 1994a; Delgadillo *et al*., 1999, 2004; Zarazaga *et al*., 2009). It has been suggested that differences in food intake might explain them (Walkden-Brown *et al*., 1994b; Argo *et al*., 1999). It may be that, as the males show more breeding activity due to their higher testosterone concentrations (even homosexual behaviour), their browsing time is reduced.

Lower sperm concentrations, smaller total numbers of spermatozoa per ejaculate, and lower overall motility were also seen during winter when plasma testosterone was low. These findings are similar to those obtained by Karagiannidis *et al*. (2000) who worked with Alpine, Saanen and Damascus goats, and that reported by Roca *et al*. (1992), Pérez & Mateos (1996), Zarazaga *et al*. (2009) and Dorado *et al*. (2010) for other Spanish goat breeds. However, the seasonal changes in plasma testosterone were not associated with any variation in TW, the percentage of ejaculating bucks or active bucks, ejaculation latency, or ejaculate volume. This contrasts with results obtained by our group for Payoya bucks (Zarazaga *et al*., 2009), and suggests that Blanca Andaluza bucks are less seasonal than other Spanish goat breeds – at least in terms of the above variables. Recently, Gallego-Calvo *et al*. (2014), working with females of this breed, reported that around 10% of does showed ovarian activity throughout the year; this has not been described for other Spanish goat breeds.

For the fresh sperm, the values for the kinematic motility variables and the percentages of motile, rapid and progressive sperms were lower during winter compared to summer, with no differences seen between the other seasons. This, along with the lower sperm concentration, suggests winter to be the worst period during which to collect Blanca Andaluza semen. This

agrees with previous results obtained by our group on Payoya bucks (Zarazaga *et al*., 2009). In that earlier experiment, the values for VCL, VSL and VAP were at their lowest in December. It was surprising to see, therefore, that winter-collected sperm showed better post-thaw quality than summer-collected sperm. It may be that the larger number of sperms in the summer ejaculates impeded the removal of the seminal plasma by the washing solution (the same volume was used in both seasons), leading to its poorer cryopreservation. Some enzymes produced in the bulbourethral gland are responsible for the degradation of egg yolk and skimmed milk products, producing compounds toxic to sperm (Roy; 1957; Iritani & Nishikawa, 1961).

The values for the kinematic motility variables and the percentage of motile spermatozoa decreased progressively from fresh to chilled to frozen thawed-sperm. This might be expected since this kind of processing can be harmful to sperm ultrastructure, biochemistry and function (Watson, 2000), resulting in reduced motility, membrane integrity, and fertilizing capacity (Purdy, 2006).

In conclusion, the results of this work support the idea that Blanca Andaluza bucks are subjected to marked seasonality in terms of their plasma testosterone concentration, with more intense secretion occurring during summer and autumn (decreasing daylength). However, no clear changes in their sexual behaviour are seen over the year. The lowest fresh semen quality was obtained in winter, a period with very low testosterone concentrations. However, after freezing-thawing, winter-collected sperm returned better sperm quality results than summer-collected sperm.

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