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 therefore be 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). 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 (Rougé, 1974; Muduuli et al., 1979; Corteele, 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 & Corteele, 1992; Muhuyi et al., 1992; Pintado et al., 1992; Tuli & Holtz, 1995) and the fertilizing capacity of frozen-thawed sperm (Corteele et al., 1978) to be better in sperm collected during the breeding season. However, other authors (Peskovatskov et al., 1974; Sommermatter & 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 20’N, 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 -20°C until analysis for testosterone using a commercial enzyme-linked immunoassay (ELISA) kit (Demeditec Diagnostics, Kiel-Welself, 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 (35°C), 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 processing, chilling and sperm freezing
Selected semen samples, i.e., those with a sperm concentration of >3500·10⁶ 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, and distilled water to 100 mL) was added at room temperature (20°C), resulting in a final sperm concentration of 800·10⁶ spermatozoa/mL, 6% egg yolk and 4% glycerol. Lecithin in the egg yolk was inactivated by subjecting the latter to 56°C for 30 min before its use in the extenders. Both extenders were prepared in the laboratory using reagent-grade chemicals purchased from Panreac Quimica S.A. (Barcelona, Spain) and the Sigma Chemical Co. (St. Louis, MO, USA). The sperm was then chilled from room temperature to 5°C 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 37°C 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 $\text{Season} \times WWS$ 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...
Seasonality of Blanca Andaluza bucks

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

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

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.

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

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.
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).
terone 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 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.

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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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References


