Seasonal variation in semen quality of lacaune rams in Brazil

Variação estacional na qualidade do sêmen de carneiros Lacaune no Brasil

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Abstract

Little is known regarding the seasonal semen production of Lacaune rams in southern Brazil. Thus, the aim of this study was to evaluate semen quality and production of Lacaune rams maintained in Rio Grande do Sul (RS). Semen was collected using an artificial vagina, from 12 rams (1 to 4 years of age), kept under intensive conditions. The quantitative and qualitative characteristics of the semen and scrotal circumference of each ram were recorded, from the winter of 2002 to the spring of 2003. Seasonal variations were recorded in almost all the monitored semen characteristics and in scrotal circumference. Sperm concentration was the only semen characteristic evaluated that did not show seasonal variation. Sperm volume varied between 1.1 ± 0.4 mL (winter of 2002) and 1.5 ± 0.4 mL (autumn of 2003). Total number of sperm per ejaculate varied between 4.0 ± 3.3x10^9 (winter of 2002) and 5.7 ± 3.3x10^9 (summer of 2003). For scrotal circumference records, in 2002 significant differences between seasons were observed. Significant differences were also found in accordance with the age of rams ($P < 0.05$) in the winter of 2002. Results showed improvement in motility, concentration and volume of the sperm during summer and autumn when compared to spring and winter. So, in this region of Brazil summer and autumn are the more indicated seasons to maximize the utilization of Lacaune rams for reproductive practices. Further studies must be done to evaluate Lacaune semen freezability in these seasons of the year.

Keywords: Lacaune. Ram. Reproduction. Seasonal semen variation.

Resumo

Existem poucos dados sobre a produção de sêmen da raça Lacaune, no sul do país. Assim, o objetivo deste estudo foi o de avaliar a produção e a qualidade do sêmen de carneiros desta raça, criados no estado do Rio Grande do Sul (RS). Foram utilizados 12 carneiros, de idades entre 1-4 anos, criados em condições intensivas. O sêmen foi colhido por vagina artificial e foram avaliadas as características quantitativas e qualitativas e a circunferência escrotal, do inverno de 2002 até a primavera de 2003. Com exceção da concentração espermática, foram observadas variações estacionais em todas as características estudadas. O volume variou de 1.1 ± 0.4 mL, no inverno de 2002, a 1.5 ± 0.4 mL no outono de 2003. O número total de espermatozóides por ejaculado variou de 4.0 ± 3.3x10^9 (inverno de 2002) a 5.7 ± 3.3x10^9 (verão de 2003). Observou-se variação estacional significativa ($P < 0,05$) na circunferência escrotal em 2002, bem como variação significativa entre as idades dos animais, no inverno do mesmo ano. Os valores médios da produção espermática observados no verão e o outono foram superiores aos obtidos no inverno e primavera. O verão e outono foram considerados as estações mais indicadas para a utilização de machos da raça Lacaune em programas reprodutivos, no RS. Mais estudos devem ser conduzidos, para a verificação de efeitos estacionais sobre o congelamento do sêmen.

Palavras-chave: Lacaune. Carneiro. Reprodução. Variação estacional do sêmen.

Introduction

Several ovine breeds, especially those that originate from temperate regions, have their breeding season modulated mainly by photoperiod and temperature[1]. Regarding sperm production and testicular size in sheep, many authors[2,3,4,5,6] have described seasonal variation. In general, the quality of sperm produc-

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tion in rams is superior during autumn and summer than in spring or winter. This seasonal influence has also been reported on semen freezability[7] and on the fertilizing ability of sperm following cryopreservation[8,9]. The intimate mechanism involved in the seasonal variation of semen quality is a complex combination of endogenous circannual rhythm driven and synchronized by light and melatonin[10], and reflects modifications in sperm production, oxidative defence enzymes and seminal plasma.

In ram-lambs, classical semen parameters were negatively related to sperm DNA fragmentation index and lipid peroxidation. There were indications that sperm oxidative defence enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase, play an important role as a part of the antioxidant system that protects spermatozoa against toxic effect of reactive oxygen species[11] Supplementation of ram spermatozoa with crude seminal plasma, or its protein component (> 10 kDa SPP) before freezing protects them from freeze–thaw damage. The protective effect is greatest when seminal plasma is collected during the breeding season, fractionated with > 10 kDa filters and added directly to the spermatozoa[12].

The dairy milk sheep industry was introduced in Rio Grande do Sul in 1992, mainly on small farms in the hilly areas. The main milk breed of these flocks is the French Lacaune. To date, the knowledge of the reproductive activity of Lacaune rams, in this part of the country, has been previously based on the knowledge of the reproductive activity of wool sheep. Different to wool sheep, dairy sheep industry is associated with intensive reproductive management practices, where ewes need to be exposed to ram out of season, to ensure continuous milk production. Thus, it is important to know how the semen quality of the milk breed rams varies during the different seasons, due to lack of information and studies on this breed under Brazilian climatic characteristics.

The aim of this study was to monitor semen quality and production of Lacaune rams throughout the year. This study is relevant to achieve a more efficient use of the rams and a more effective freezing technique of sheep semen.

Material and Method

This study was conducted on a commercial flock of Lacaune rams in a district of RS, Bento Gonçalves (29° S latitude), in the southernmost state of Brazil. Temperature and rainfall means of the region are presented in figure 1.

Semen was collected with the aid of an artificial vagina from 12 rams (1 to 4 years of age) kept in intensive conditions. The quantitative and qualitative characteristics of the semen of each ram were evaluated twice a season, every 30 - 40 days, from the winter of 2002 to the spring of 2003, except in winter of 2003 (one evaluation). Some of the records (sperm volume, sperm motility and scrotal circumference) were taken just after semen collection. Semen samples were taken to further analysis (sperm concentration, total number of sperms per ejaculate – Nº sperm/ejac – and sperm abnormalities) in the Artificial Insemination Laboratory of Veterinary Faculty of the Federal University of Rio Grande do Sul (UFRGS).

The semen characteristics recorded were: a) volume - directly recorded in the graduated collection glass cup; b) motility - evaluated as a percentage, using a drop of semen diluted in egg yolk sodium citrate solution at 37 ºC, and observed on a prewarmed slide at a 100 x magnification under microscope; c) sperm concentration (conc/mm^3) - semen was diluted (1:400) using a Sahli pipette in a formol citrate solution and measured by counting in a Improved Neubauer chamber at a 400 x magnification under a microscope; d) total number of sperm per ejaculate (N° sperm/ejac) - according the values obtained for volume and sperm concentration and e) sperm abnormalities - semen was diluted (1:10) in a

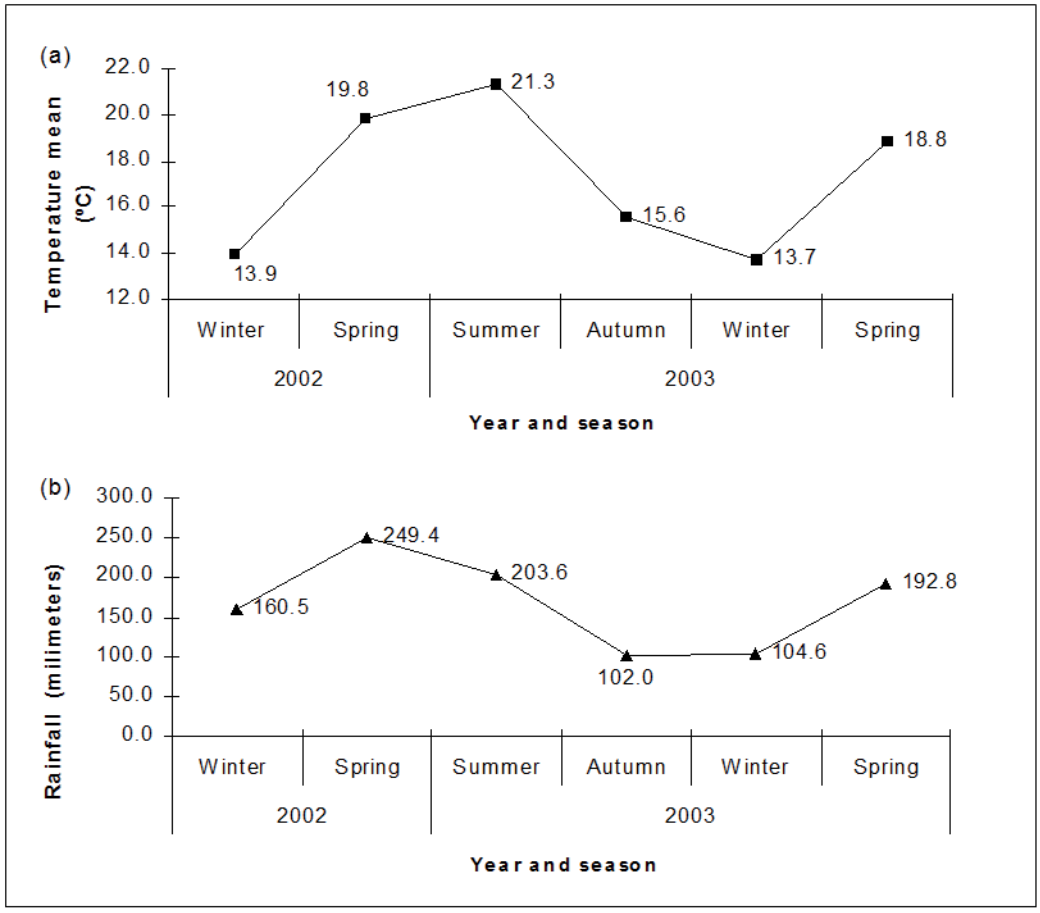

Figure 1 - Climatic conditions in Bento Gonçalves, RS (Brazil) during the experimental period: (a) Mean temperatures ºC, (b) Rainfall (mm)

pre-warmed (37 ºC) formol citrate solution and analyzed by counting 100 cells in stained smears[13], using oil immersion at a 1000 x magnification. The sperm abnormalities were classified in minor and major defects. Total sperm defects were obtained by the sum of minor and major defects[14].

After the semen collection, the measurement for scrotal circumference (in centimeters) was taken at the area of the largest diameter of the scrotum using a measuring tape.

Data were analyzed using mixed models for repeated measures (proc mixed, SAS[15]), considering the rams as the subject and age, season within the year and year as independent variables. The model was fitted with the autoregressive of order 1 (AR(1)) covariance structure. For motility and sperm abnormalities, data were transformed to logarithms before analysis[16].

Results

Table 1 shows the seasonal variation (mean and SD) in the semen production for all rams, as well as values for scrotal circumference, from the winter of 2002 to the spring of 2003.

Seasonal variations were observed in almost all the semen characteristics evaluated and in scrotal circumference. Sperm concentration was the only semen characteristic evaluated that did not show seasonal variation. There were significant differences for scrotal circumference between seasons.

Table 1 - Seasonal variation in semen production and scrotal circumference of 12 Lacaune rams, from the winter of 2002 to the spring of 2003 (mean ± SD)

Semen Characteristics	2002		2003			
	Winter n = 24	Spring n = 24	Summer n = 24	Autumn n = 24	Winter n = 12	Spring n = 24
Volume (mL)	$1.30^{ab} \pm 0.5$	$1.40^{b} \pm 0.7$	$1.40^{b} \pm 0.5$	$1.50^{b} \pm 0.6$	1.1 ± 0.4^{a}	$1.4^{ab} \pm 0.4$
Sperm motility (%)	$86.20^{a} \pm 9.0$	$82.8^{a} \pm 6.9$	$79.8^{ab} \pm 10.20$	$75.8^{b} \pm 12.4$	$79.6^{ab} \pm 11.4$	$67.3^{c} \pm 9.9$
Major Sperm Defects (%)	$3.82^{a} \pm 3.6$	$4.1^{a} \pm 3.2$	$1.1^{b} \pm 1.5$	$4.0^{a} \pm 4.0$	$3.8^{a} \pm 3.25$	$1.54^{b} \pm 1.2$
Minor Sperm Defects (%)	$4.52^{a} \pm 2.9$	$8.1^{ab} \pm 9.8$	$7.8^{abc} \pm 7.0$	$7.9^{bc} \pm 5.4$	$15.5^{d} \pm 13.0$	$13.0^{d} \pm 7.8$
Total Sperm Defects (%)	$8.3^{a} \pm 5.4$	$12.2^{ab} \pm 11.5$	$8.8^{abc} \pm 7.1$	$11.9^{abc} \pm 7.4$	$19.3^{c} \pm 5.3^{c}$	$14.6^{bc} \pm 7.7$
Sperm concentration (10^6)	$3.0^{a} \pm 1.1$	$3.4^{a} \pm 1.5$	$3.8^{b} \pm 1.4$	$3.9^{b} \pm 1.0$	$4.0^{b} \pm 1.3$	$3.2^{b} \pm 1.2$
N° sperm per ejaculate (10^9)	$4.0^{a} \pm 2.4$	$5.2^{ab} \pm 3.0$	$5.7^{b} \pm 3.3$	$5.7^{b} \pm 2.4$	$4.6^{ab} \pm 2.1$	$4.4^{a} \pm 2.0$
Scrotal circumference (cm)	$31.1^{a} \pm 3.4$	$34.1^{b} \pm 2.5$	$33.9^{ab} \pm 2.5$	$32.0^{ab} \pm 2.8$	$33.0^{ab} \pm 2.6$	$33.2^{ab} \pm 2.0$

with different superscript on the same row indicate differences among seasons ($P < 0.05$)

Semen volume increased slightly from winter (1.3 ± 0.5 mL), to autumn (1.5 ± 0.6 mL); decreased in the winter of 2003 (1.10 ± 0.4 mL) ($P < 0.05$) and then increased again (1.4 ± 0.4 mL), in spring of 2003.

Sperm motility varied between 86.2 ± 9.0% (winter of 2002) to 67.3 ± 9.9% (spring of 2003), which was the lowest value and differed ($P < 0.05$) from all the others seasons analyzed.

Total sperm defects varied between 8.3 ± 5.4% (winter of 2002) and 19.3 ± 5.3% (winter of 2003). Means observed in the winter (19.3 ± 5.3) and spring (14.6 ± 7.7%) of 2003 were closely related to a significant ($P < 0.05$) increase occurred in minor defects. Values recorded for major defects were lower than 5% in all seasons. The lowest values recorded for major values were observed in the summer (1.1 ± 1.5%) and spring (1.5 ± 1.2%) of 2003, and had differed significantly ($P < 0.05$) between the other seasons evaluated.

Sperm concentration showed significant differences between 2002 ($3.1 \pm 1.3 \times 10^6/mm^3$) and 2003 ($3.7 \pm 1.2 \times 10^6/mm^3$), with no variation among seasons.

Seasonal differences were observed in the total number of sperm (N° sperm/ejac). The lowest values ($4.0 \pm 2.4 \times 10^9$) were observed in the winter of 2002, followed by a progressively increase until summer and autumn. After that, a slight decrease was detected in winter and spring.

There was a significant seasonal variation in the scrotal circumference. It was also observed a significant variation according to the age of the animal. In 2002, a significant seasonal variation ($P < 0.05$) was recorded between winter (31.1 ± 3.4 cm) and spring values (34.1± 2.5 cm). After that, no significant variations were recorded. Regarding the age of rams, a significant effect on scrotal circumference was observed in the winter of 2002. Values of scrotal circumference of one-year old rams (29.2 ± 2.3 cm) differed ($P < 0.05$) from two (32.3 ± 2.6 cm), three (33.0 ± 2.7 cm) and four year old rams (32.5 ± 2.9 cm).

Discussion

In this study, the mean semen volume recorded for seasons was 1.4 mL, which is similar to values previously reported for French Lacaune rams[17]. Ejaculated volume showed slight variations during the seasons, a decrease being the most evident in winter. Observations suggest that ram semen volume is lower in winter, with higher volumes being recorded in autumn and summer[3,18].

Regarding sperm motility, the lowest values recorded occurred in the spring of 2003. Variations in motility were already detected before spring, but they could not be related to the season. It has been reported that photoperiod has no effect on sperm mo-

tility[19] and that fluctuations in sperm motility occur independently of seasons[20].

No seasonal variations were observed in sperm concentration of the ejaculate, but the values progressively increased from the winter of 2002 to the winter of 2003 followed by a slight decrease in the spring of 2003. The lack of seasonal variations in this semen characteristic has been reported previously[3,21].

Unlike sperm concentration, the total number of sperm showed seasonal variation. Since the total number of spermatozoa is related to volume and to the sperm concentration, the variation was related to these characteristics.

The data presented in table 1 showed that in summer and autumn the total number of sperm showed an increase that probably reflects the sperm concentration, associated with a lack in the variation of semen volume. On the other hand, in winter, despite the lack of variation on sperm concentration, the decrease in semen volume led to a consequent decrease in the total number of sperm. A higher number of sperm per ejaculate in autumn has been reported previously in sheep[21].

In this study, sperm defects variations were recorded among seasons. The higher frequency of variation occurred for minor defects, which was more evident in the winter and spring seasons. Seasonal variation in major defects was also detect, but the mean value recorded for all seasons was less than 5%, and could be considered low and acceptable[4]. Regarding the total sperm defects, the values recorded in this study were lower than those quoted for Corriedale rams in Brazil[22], but higher than reported for rams of the same breed, grazed on natural pastures, in Uruguay[23]. Several reports for other sheep breeds[4,20,21] have detected a reduced number of sperm defects in semen produced in summer and autumn. This was also observed in the current work.

Data on scrotal circumference show variations during the observation period. It was found that the variation on scrotal circumference was more evident in young rams, as the value of one year old animals differed significantly ($P < 0.05$) from those of older animals (winter 2002). This result was as expected and could probably be related to body development of the rams. In winter and spring of 2002, a parallelism was observed between sperm concentration and testicular diameter, similar to that reported previously[24]. Observations on scrotal circumference taken throughout the year (2003) showed only slight variation.

The results of the ejaculated volume, sperm concentration and number of sperm/ejaculate recorded in this work were similar to that based on 771 Lacaune rams, grazed in the Northern hemisphere[17]. Thus, it can be concluded that Lacaune semen quality evaluated in this study is within the standards accepted for reproductive use.

Considering all results related to semen quality and sperm production, findings indicate summer and autumn semen production to be better than in spring and winter, for Lacaune rams. Similar results were reported in semen quality, in different ram breeds[4,8,9,21] That is valuable information to maximize the utilization of Lacaune rams for natural mating in this region of Brazil. Further studies are needed to evaluate the freezability of the Lacaune semen in these seasons of the year as suggested in previous studies[7].

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