SPERM CHARACTERISTICS OF MANCHEGO RAM LAMBS TREATED BY MELATONIN IMPLANTS

CARACTERÍSTICAS SEMINALES DE CORDEROS DE RAZA MANCHEGA TRATADOS CON IMPLANTES DE MELATONINA

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Palabras clave adicionales

Variaciones estacionales. Fotoperiodo. Semen.

SUMMARY

An experiment was carried out to investigate if the administration of melatonin implants could improve sperm parameters of Manchego ram lambs. Two groups of Manchego ram lambs (n=6 in each group) were used in this study. One group received a melatonin implant and the other one served as control. The experiment began May 17 when rams were 137.9±2.1 days of age and ended Oct. 1 during which time all rams were mantained as one group under natural lighting. Semen collection (2 ejaculates/ ram/week) began Aug. 1 and during the whole experimental period the total number of spermatozoa per ejaculate, sperm motility (percentage) and percentage of intact acrosomes (PIA) were calculated for each ram. Acrosome status was measured by phase contrast microscopy. Data of sperm characteristics were analyzed by analysis of variance test. Mean values of total number of spermatozoa per ejaculate were higher ($p<0.05$) for animals of melatonin group ($4.6\pm0.20X10^9$ spermatozoa) than those obtained for control group ($4.0\pm0.11X10^9$ spermatozoa). Variations in motility were not found between the two animal groups in any study period. By contrast, the melatonin treatment produced an improvement ($p<0.05$) of the PIA in the melatonin group between week 4 and 8 of semen collection period. These data indicate that melatonin implants have no effect on sperm motility when the implant is administered May 17, although the implants improved the total number of spermatozoa per ejaculate and the PIA in the semen of young rams. This work encourages further studies for the effects of the melatonin implants on fertility of young ram semen.

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RESUMEN

El presente estudio se ha desarrollado para investigar si la aplicación de implantes de melatonina podría mejorar las características espermáticas de los moruecos jóvenes de raza Manchega. Para ello, se han empleado dos grupos de corderos de raza

Manchega (n=6 animales en cada grupo). Uno de estos lotes recibió un implante de melatonina y el otro sirvió como control. El experimento se inició el 17 de mayo, momento en el cual los animales tenían 137,9±2,1 días de edad, y finalizó el 1 de octubre. Durante todo el periodo de estudio los machos fueron mantenidos en un único lote bajo las condiciones naturales del fotoperiodo. La recogida seminal (2 eyaculados/morueco/semana) se inició el 1 de agosto evaluándose durante todo el período experimental: el número total de espermatozoides por eyaculado, la motilidad espermática (p.100) y el porcentaje de espermatozoides con acrosoma intacto (PIA) para cada morueco. El estado del acrosoma fue evaluado por microscopía de contraste de fases. Los datos seminales obtenidos fueron analizados por medio de análisis de varianza multifactoriales. Los valores globales para el número total de espermatozoides por eyaculado fueron superiores ($p<0,05$) en el grupo de moruecos tratado con los implantes de melatonina ($4,6\pm0,20x10^9$) que en el lote control ($4,0\pm0,11x10^9$). Por otro lado, no se encontraron variaciones en cuanto a los porcentajes de motilidad individual entre los dos grupos dentro del periodo de tiempo estudiado. Sin embargo, el tratamiento con melatonina produce un incremento ($p<0,05$) en la tasa de espermatozoides que presentan su acrosoma intacto durante las semanas 4ª a 8ª del periodo estudiado. Nuestros datos indican que la aplicación de implantes de melatonina el día 17 de mayo no mejora las tasas de motilidad espermática, sin embargo el empleo de dicho tratamiento, sí incrementa el número total de espermatozoides por eyaculado y el PIA en el semen de corderos de raza manchega.

INTRODUCTION

Large seasonal variations in quali- and quantitative semen production were reported in ram (Pelletier *et al.*, 1988). These effects are mainly mediated by the photoperiod, which acts on the central nervous system by the modification of the duration of night-time melatonin secretion (Folch, 1984). Recently, it has been shown that melatonin implants were able to prevent these seasonal variations in adult rams (Chemineau *et al.*, 1988); however, little is known about the effects of melatonin implants on sperm characteristics of ram lambs. Thus, the purpose of the present study was to investigate if the administration of melatonin implants could improve quali- and quantitative sperm parameters of Manchego ram lambs.

MATERIALS AND METHODS

The study was carried out between may 17 and october 1, 1991, at the Artificial Insemination Research Center at Valdepeñas, Spain, at 38° 46' N, 3° 23' W and an altitude of 705 m.

ANIMALS

Two groups of Manchego ram lambs (n=6 in each group) were used in this study. They were 137.9±2.1 days of age when the experiment commenced. The animals were randomly alloted to one of two treatments to investigate the effect of melatonin on seminal characteristics of Manchego ram lambs. One group received a melatonin implant (Regulin^R, 18mg) (MG) and the other one served as control (CG). The experiment began may 17 and ended october 1, during which time all the lambs were mantained as one group under natural lighting, allowed to walk freely and given mineral blocks and water *ad libitum*. They were also fed with a 18 p.100 protein supplement each morning.

SEMEN COLLECTION

Semen collection began august 1, when animals were adapted to ejaculate

in an Artificial Vagina. Semen was collected twice a week. In each collection session, one ejaculate per ram was obtained. An ovariectomized ewe was used as a mount animal for semen collection. The tubes containing freshly collected semen were transferred to the laboratory and inmersed in a water bath at 37°C.

SEMEN EVALUATION

Total number of spermatozoa, sperm motility and percentage of intact acrosomes were calculated weekly for each ram during the whole experimental period. The total number of spermatozoa (TS) per ejaculate was calculated by measuring the volume and the sperm concentration of the ejaculate. The ejaculated volume was read immediately after collection from a graduated test tube. Sperm concentration was measured by a spectrophotometer after diluting a sample of semen with 0.05 p.100 glutaraldehyde solution (1:500 dilution rate) (Vijil, 1986). The number of spermatozoa per ejaculate was then calculated.

Sperm quality was estimated by evaluating the percentage of motile sperm (PM) and the percentage of intact acrosomes (PIA) which was assessed through visual stimation of semen fixed in buffered 2 p.100 glutaraldehyde solution and counting a total of 200 cells under a phase-contrast microscope (Pursel and Johnson, 1974). The percentage of spermatozoa displaying forward progressive motility was estimated by diluting a drop of semen with 0.1 M sodium citrate, transferring it to a warm slide, mounting it with a coverslip and examining it under the microscope (40xobjective).

Data of seminal characteristics were compared by Analysis of Variance test (ANOVA) using a weekly mean for each ram and testing the effects of group, week and interaction treatment-week. All statistical analysis were performed using the General Linear Models procedures of the Statistical Analysis Systems Institute.

Table I. Mean values for ejaculate characteristics of Manchego ram lambs from melatonin (MG) or control group (CG).* (Valores medios de las características seminales de corderos de raza manchega tratados (MG) o no (CG), con implantes de melatonina)*.

	Parameters**		
Treatment	TS ($X10^9$)	PM (p.100)	PIA (p.100)
MG	4.6±0.20[a]	80.0±2.37	84.3±0.35[A]
CG	4.0±0.11[b]	80.1±1.06	79.1±0.23[B]

*Means, for each parameter in the same column with different superscripts differ: [a,b] ($p<0.05$) and [A,B] ($p<0.01$).

**Seminal parameters: TS: total number of spermatozoa; PM: Percentage of sperm motility and PIA: Percentage of intact acrosomes.

RESULTS

Data showing the mean values for ejaculate characteristics of Manchego ram lambs used in this study are presented in **table I**. These means were significantly different between the two groups of animals for the total number of spermatozoa per ejaculate ($p<0.05$) and for the percentage of intact acrosomes ($p<0.01$). These two means were higher

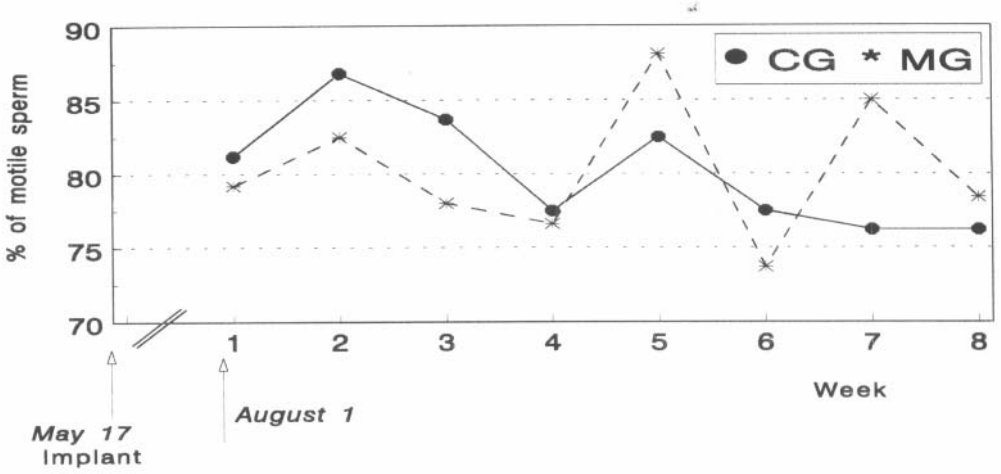

Figure 1. *Percentage of motile spermatozoa from ejaculates of Manchego ram lambs subjected to melatonin treatment. Weekly mean. MG: melatonin group. CG: control group.* (Porcentaje de espermatozoides móviles en el semen de corderos manchegos tratados con implantes de melatonina. Medias semanales. MG: Lote tratado. CG: Lote control).

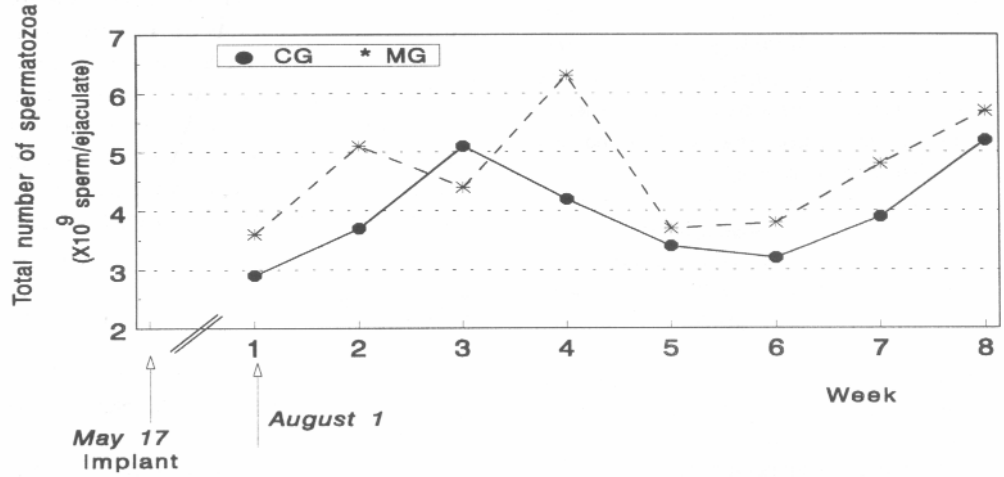

Figure 2. *Total number of spermatozoa per ejaculate of Manchego ram lambs subjected to melatonin treatment. Weekly mean. MG: melatonin group. CG: control group.* (Número total de espermatozoides por eyaculado en el semen de corderos manchegos tratados con implantes de melatonina. Medias semanales. MG: Lote tratado. CG: Lote control).

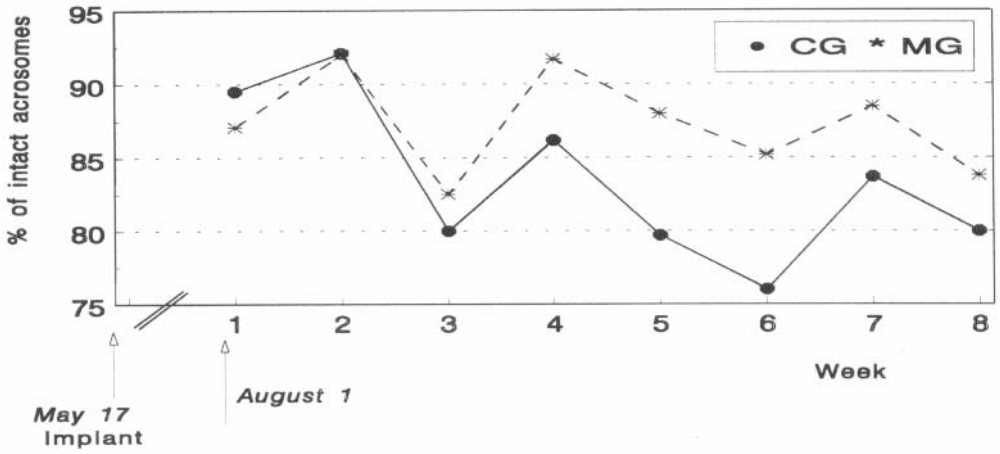

Figure 3. *Percentage of intact acrosomes from ejaculates of Manchego ram lambs subjected to melatonin treatment. Weekly mean. MG: melatonin group. CG: control group.* (Porcentaje de espermatozoides con acrosomas intactos en el semen de corderos manchegos tratados con implantes de melatonina. Medias semanales. MG: Lote tratado. CG: Lote control).

for MG than CG rams. On the other hand, the percentage means of sperm motility did not vary ($p>0.05$) with treatment. **Figures 1** to **3** show the evolution through the experimental period of the studied parameters. Significant variations in the sperm motility were not found between the 2 groups at any study period. Interaction between group and week was detected for this parameter ($p<0.001$), indicating that sperm motility varied between the two groups differently with time **(figure1)**. Evolution of the total number of spermatozoa per ejaculate in relation with time is showed in **figure 2**. As can be seen, the means of TS were generally higher in ejaculates from MG than those of CG animals. A significant ($p<0.05$) treatment-week interaction was detected in this parameter, indicating that TS also varied between the two groups differently with time.

Finally, percentages of intact acrosomes of spermatozoa from Manchego ram lambs of two groups subjected to melatonin treatment are ilustrated in **figure 3**. Melatonin implants generated a significant improvement ($p<0.05$) of the PIA in the experimental group between the weeks 4 and 8 of the semen collection period. Interaction between group and week was not detected for this seminal characteristics; this suggests that PIA did not vary between groups differently with time.

DISCUSSION

Large seasonal variations in quali- and quantitative semen production were reported in the ram (Pelletier *et al.*, 1988). These effects are mainly mediated by the photoperiod, which acts on the central

nervous system by the modification of the duration of night-time melatonin secretion (Folch, 1984). The use of exogenous melatonin to manipulate reproduction in various mammals has lately been studied extensively. Recently, it has been shown that melatonin implants were able to prevent these seasonal variations in adult rams (Chemineau *et al.*, 1988); however, little is known about the effects of melatonin implants on sperm characteristics of ram lambs. Therefore, the purpose of the present study was to investigate if the administration of melatonin implants could improve quali- and quantitative sperm parameters of Manchego ram lambs. Our results indicated that exogenous administration of melatonin implants improve the mean values for the total number of spermatozoa per ejaculate and for the percentage of intact acrosomes on Manchego ram lambs. This allowed to produce more Artificial Insemination doses from melatonin group ejaculates than from the control group. Motility means of spermatozoa produced by the two groups of rams were no different. By contrast, Hudgens and Diekman (1988), reported that an oral daily dose of 2 mg of melatonin had little effect on reproductive characteristics of ram lambs.

Weekly evolution in seminal parameters studied indicates that melatonin implants generated a significant improvement ($p<0.05$) of the PIA between the weeks 4 and 8 of the semen evaluation period. In the same way, the means of TS were generally higher in ejaculates from MG rams. On the other hand, significant variations in sperm motility were not found between the two groups at any study period. The present observations suggest that sperm motility is less sensitive to the influence of melatonin in the ram ejaculates than TS and/or than PIA. The different values of TS and PIA found in the two groups of rams suggest that melatonin implants may give rise to good testicular function, mediating a high spermatogenic activity and stimulating the differenciation of A_0 into A_1 spermatogonia (Lanford *et al.*, 1987). It may be attributed to an increased LH sensitivity of the Leydig cells to melatonin. By contrast, the fact that significant variations in sperm motility were not found between the two groups at any study period, is probably due to a time lag epididymal function in the ram lambs. These data indicate that melatonin implants have kind effects on seminal parameters in Manchego ram lambs when the implant is administered may 17. However, this work encourages further studies for the effects of the melatonin implants on the fertility of young ram semen.

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