

**EFFECT OF MELATONIN DURING THE SEASONAL ANOESTROUS ON  
THE REACTIVATION OF THE SEXUAL ACTIVITY AND SEMEN  
PRODUCTION AT THE NORMAL BREEDING SEASON ON  
MEDITERRANEAN BUCKS**

**[EFECTO DE LA MELATONINA DURANTE EL ANESTRO ESTACIONAL  
EN LA REACTIVACIÓN DE LA ACTIVIDAD SEXUAL Y PRODUCCIÓN  
SEMINAL DURANTE LA ESTACIÓN REPRODUCTIVA DE MACHOS  
CABRIOS DEL MEDITERRANEO]**

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**SUMMARY**

One experiment was conducted to determine if the onset of the reproductive activity and semen production could be modified by a previous treatment with exogenous melatonin, used to enhance reproductive activity during the seasonal anoestrous in Mediterranean bucks. Two balanced groups of bucks were used. The 18<sup>th</sup> March 2005, one group (M group) received 3 s.c. implants of melatonin (N=7) and other group (N=4) was used like control (C group). From June to September, body weight and testosterone was measured weekly and testicular weight every 15 days. The reproductive activity of each buck was assessed using characteristics of the testosterone profile. During the first 8 days of each month, volume of ejaculate and sperm concentration was assessed. Each of these 8-d periods was divided into 3-d periods of daily sperm collection separated by 2 d of rest. The semen was collected using an artificial vagina. The effect of treatment and month was studied on each variable. An interaction, month-treatment was observed on ejaculate volume, July was the month with higher volume ( $0.62 \pm 0.06$  mL vs  $1.07 \pm 0.15$  mL for M and C group respectively,  $P < 0.05$ ). Sperm concentration was influenced by treatment ( $6.13 \times 10^9 \pm 2.49 \times 10^8$  vs  $4.26 \times 10^9 \pm 3.12 \times 10^8$  sperm/mL, for M and C, respectively,  $P < 0.05$ ). The onset of the reproductive activity, after the study of the testosterone concentrations was similar for both groups (31st August  $\pm 7.89$  days and 4th September  $\pm 25.66$  days for M and C group, respectively). Results demonstrate that melatonin treatment during the seasonal anoestrous does not influence the onset of the normal breeding season or ejaculate volume but it seems that could increase the sperm concentration at the normal breeding season in Mediterranean goat males.

**Keywords:** Buck, Melatonin, Seasonality, Semen.

**INTRODUCTION**

The influence of photoperiod on sexual activity is greatest at high latitudes, decreasing gradually towards the equator (Hafez, 1952; Chemineau et al., 1995). Daylight length influences the secretion of the pineal gland, being responsible for transducing the photoperiodic cue into a hormonal signal (melatonin) (Hansen, 1985). Photoperiod information is conveyed to the neuro-endocrine system by the circadian secretion of melatonin from the pineal gland (Bittman et al., 1983). The profile of melatonin secretion is therefore an endocrine signal that relays photoperiodic information to the reproductive axis. As short days are characterized by a longer duration of melatonin secretion than long days, different attempts have been made to mimic the effect of short days by artificially prolonging the duration of high levels of circulating melatonin. Two mechanisms can be put forward to explain that action of melatonin implants: either they provide a short-day signal (very long duration) or they remove photoperiodic information (which is inhibitory at that time) because of the absence of a detectable rhythm. Short-day treatment can therefore be replaced by melatonin treatments so that the maintenance of constantly high levels of melatonin mimics a short day effect. Studies do not exist about the effect of exogenous melatonin used during the seasonal anoestrous on the onset of the normal breeding season in Mediterranean bucks.

**MATERIAL AND METHODS**

The study was conducted at the experimental farm of the University of Huelva (latitude 37° 15' N). The males were distributed at random into two groups balanced according to their live weight and testicular

weight. Group treated with exogenous melatonin: M group (n=7, received 3 s. i. the 18<sup>th</sup> March containing 18 mg of the hormone (Melovine®, CEVA Salud Animal, Barcelona, Spain) and a control group (C group) without melatonin (n=4). We studied testosterone concentrations (TC) weekly, testicular weight (TW) every 15 days, volume and sperm concentrations monthly from 1<sup>st</sup> June to 30<sup>th</sup> September after a period of reproductive activity induced by the previous treatment (Gatica et al., 2006). The reproductive state was assessed using characteristics of the testosterone profile. Firstly, baseline level was defined as the averaged of the at least lowest plasma samples obtained during the experiment normally sampled during the seasonal anoestrous. I think than the authors must re-write this part because there is no average in lowest sample.... Testicular activity was defined when two or more consecutive plasma samples had testosterone concentrations differing from the highest value obtained from this baseline by more than 3 SD. To study volume and sperm concentration, semen collection was assessed monthly during the first 8 days of each month (2 periods of 3days of daily sperm collection separated by 2 day of rest). The ejaculated semen volume was recorded immediately after collection from a graduated collection vial. Concentration was analyzed using a computer-aided sperm analyzer (Sperm Class Analyzer, Microptic, Barcelona, Spain). The effect of treatment and time was studied on each variable.

## RESULTS

No effect of treatment was observed on TS ( $278.39 \pm 8.85$  gr and  $292.39 \pm 12.81$  gr for M and C group, respectively). This line is related to TW and I did not find in he materials and method the techniques to evaluate this variable. The onset of the testicular activity, in order to the testosterone concentrations analysis, was not modified by the previous treatment with melatonin (31<sup>st</sup> August  $\pm 7.89$  days and 4<sup>th</sup> September  $\pm 25.66$  days for M and C group, respectively). Figure 1 shows the monthly mean of the ejaculate volume. A significant interaction time-treatment ( $P < 0.05$ ) was observed with higher ejaculate volume for the C group in July ( $P < 0.01$ ) ( $0.62 \pm 0.07$  mL vs  $0.94 \pm 0.05$  mL for M and C group), but no effect of treatment was observed ( $1.05 \pm 0.06$  mL vs  $0.95 \pm 0.15$  mL for M and C group). Figure 2 shows the monthly sperm concentration; a clear effect of treatment was observed on this parameter ( $6.13 \times 10^9 \pm 2.49 \times 10^8$  vs  $4.26 \times 10^9 \pm 3.12 \times 10^8$  sperm/mL, for M and C, respectively,  $P < 0.05$ ) and differences between groups were observed in June, July and September, with higher values for the M group (at least  $P < 0.05$ ).

## DISCUSSION

There were no difference between groups on the reactivation of the testicular activity. The testosterone concentration response was later for both groups, compared with data described by Delgadillo et al. (2001) in Creole males treated with artificial long days and melatonin in January. In this trial the control group started reproductive activity in July (testosterone concentrations  $>5$  ng/mL) and group treated with artificial long days and melatonin started reproductive activity in June. Similarly, Todini et al. (2007), working with four Mediterranean goat breeds they determined testosterone concentrations throughout 1 year in blood samples collected weekly, maintained without interactions with does. In this experiment the bucks of all the four breeds displayed a clear seasonality of plasma testosterone, with very low levels from January to May and high levels from July to November; hormone levels and pattern of seasonality were affected by breed and not by climate. Moreover, Zarazaga et al. (unpublished data) for the Payoya bucks in natural conditions that described the onset of the reproductive activity on the first fortnight of August. These differences could be originated by the different criteria used to define the onset of the reproductive activity. The results obtained for ejaculate volume, were similar to that obtained by Gatica et al. (2006) in males treated with exogenous melatonin during the anoestrous with the same animals but lower than described by Zarazaga et al. (unpublished data) for the Payoya bucks during autumn season that was  $1.44 \pm 0.04$  mL. In the present experiment, the M group showed a very high sperm concentration, higher than described in bucks treated with melatonin during anoestrous (Gatica et al., 2006), or by Zarazaga et al. (unpublished data) during the breeding season on Payoya bucks using similar methodology and similar computer-aided sperm analyzer. However control group showed similar results than in those experiments, indicating that the origin of these differences could be the very high individual variability observed for this parameter.

## CONCLUSION

Results demonstrate that melatonin treatment used during the seasonal anoestrous and that induced a clear reproductive activity during the seasonal anoestrous, does not influence the onset of the normal breeding season or ejaculate volume but it seems that could increase the sperm concentration at the normal breeding season in Mediterranean goat males.

## ACKNOWLEDGMENTS

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Figure 1: Mean ( $\pm$ S.E.M.) for ejaculate volume (mL) of Mediterranean goat bucks treated with melatonin (M group,  $\blacksquare$ ) or without melatonin treatment (C group,  $\square$ ). (\*\*:  $P < 0.01$ ).

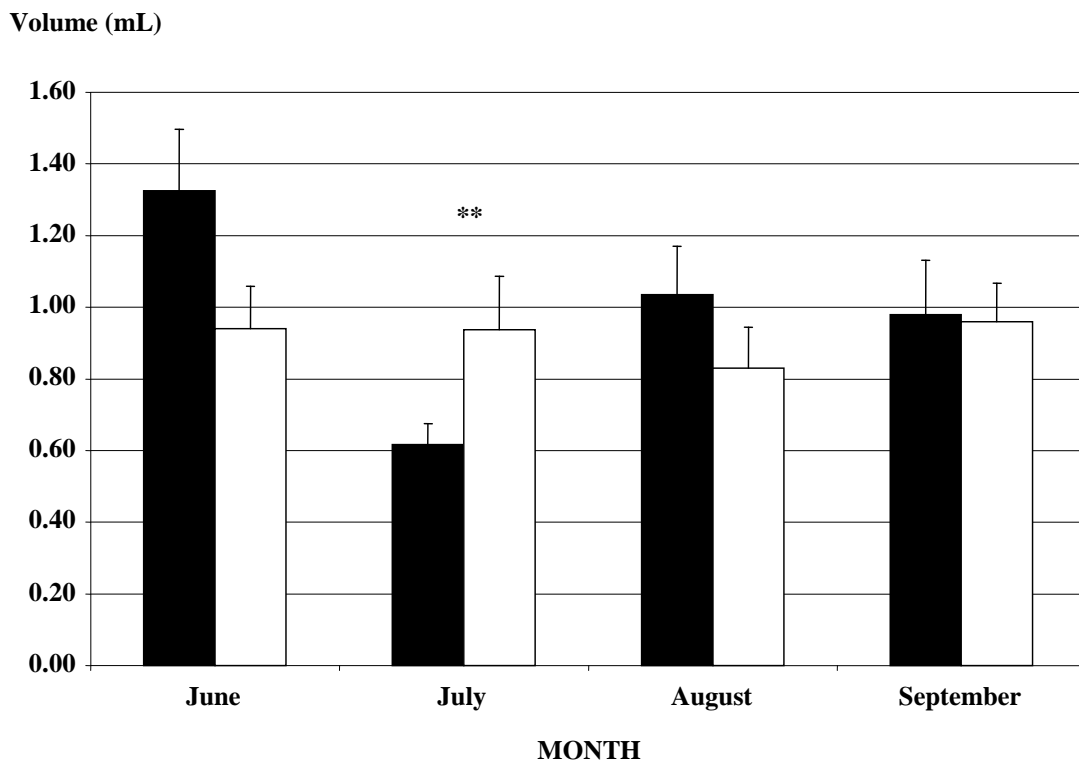
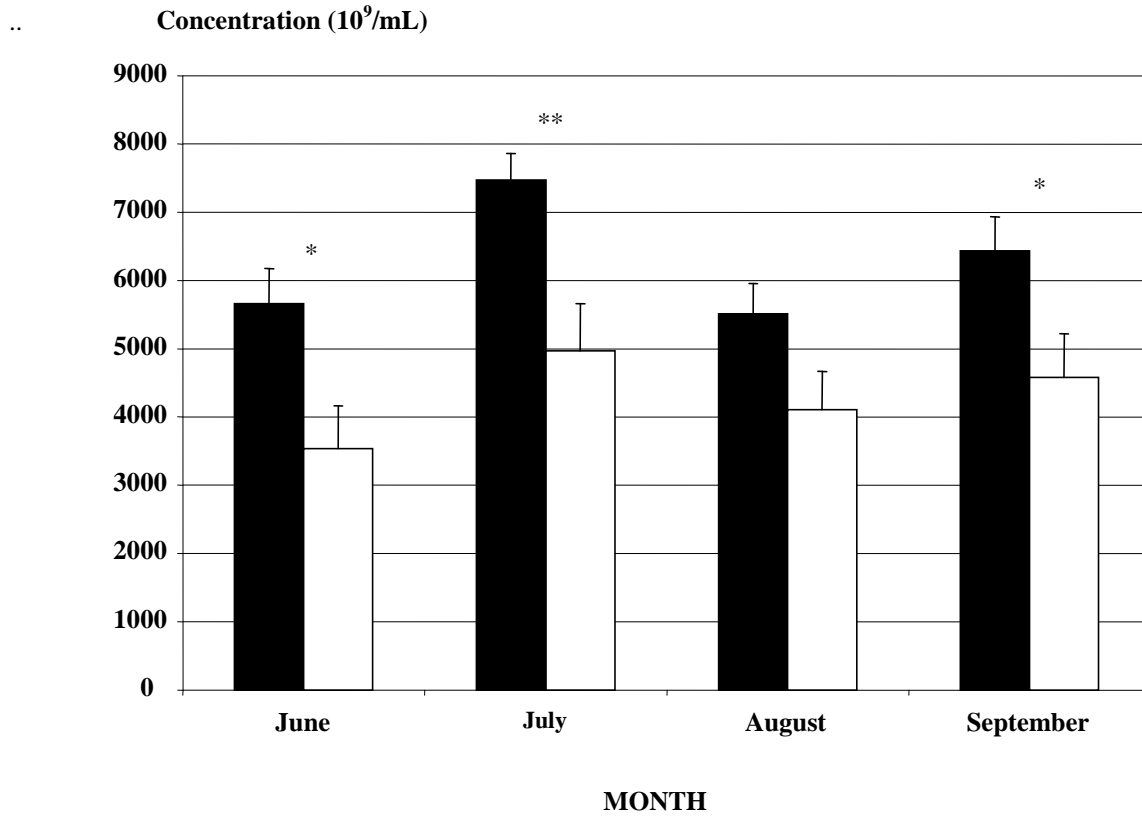


Figure 2: Mean ( $\pm$ S.E.M.) for sperm concentration (mL) of Mediterranean goat bucks treated with melatonin (M group,  $\blacksquare$ ) or without melatonin treatment (C group,  $\square$ ). (\*:  $P < 0.05$  and \*\*:  $P < 0.01$ ).



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