Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/sjar
http://dx.doi.org/10.5424/sjar/2013114-4115

Spanish Journal of Agricultural Research 2013 11(4): 997-1003
ISSN: 1695-971-X
eISSN: 2171-9292

# Short communication. Melatonin improves the reproductive performance of seasonal anoestrus goats exposed to buck effect during early post-partum

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## Abstract

The aim of the present study was to determine whether any combination of buck effect and melatonin treatment could improve reproductive performance in Payoya female goats during early postpartum. Forty-four pregnant female goats were used. After birth in spring, they were distributed into two major groups: females submitted to the *buck effect* (BE, N = 22) or not such effect (NBE, N = 22). In turn, the BE animals were subdivided into: 1) no further treatment (CBE, N = 11) and 2) implanted with melatonin (MELBE, N = 11). And the NBE animals were subdivided into: 3) no further treatment (CNBE, N = 12) and 4) implanted with melatonin (MELNBE, N = 10). Melatonin was implanted 10 days after birth. Oestrus activity was tested daily using entire males from day of birth (D0) in the groups NBE and from D55 after birth in the BE groups. Transrectal ultrasonography was performed 10 days after the detection of oestrus and 45 days after mounting to determine pregnancy. Fecundity, fertility and productivity were higher in MELBE animals compared to CNBE animals (fecundity and fertility: 66.7% *vs*. 0.0%, and productivity: 0.73 *vs*. 0.00 kids/female for MELBE and CNBE respectively, $p < 0.05$), and CBE animals (fecundity and fertility: 66.7% *vs*. 14.3%, and productivity: 0.73 *vs*. 0.09 kids/female for MELBE and CBE respectively; $p < 0.05$). No significant differences were recorded between the subgroups of the NBE animals. The present results show that exogenous melatonin improves the reproductive performances of early post-partum Payoya does exposed to male effect during the seasonal anoestrus.

**Additional key words**: goat; postpartum; melatonin; male effect; seasonal anoestrous.

The gestation period of goats only lasts some 150 days; annual reproductive efficiency might therefore be improvable via accelerated breeding programs. However, the seasonal breeding pattern of goats (most goats do not cycle after lambing in the spring) acts as a barrier to such improvements, limiting productivity. Seasonal anoestrous is characterized by the inhibition of luteinizing hormone (LH) secretion by oestradiol (Zarazaga *et al*., 2011a) or, during the postpartum period, by ovarian steroidal and non-steroidal factors involved in the feedback system that reduces LH secretion (Al-Gubory *et al*., 2003). Further, the seasonal inhibition of LH is under photoperiodic control, with long days inhibiting and short days stimulating reproductive activity (Bissonnette, 1941; Zarazaga *et al*., 2011a,b). Bittman & Karsch (1984) suggested this effect of the photoperiod to be mediated by pineal gland's production of melatonin (MEL).

Reactivating reproductive activity in small ruminants when kidding coincides with seasonal anoestrous is very difficult. It is only resumed in the normal breeding season since, when the effect of lactation disappears (Mauleon & Dauzier, 1965), the influence of the photoperiod prevents any earlier return (Mauleon & Dauzier, 1965; Abecia *et al*., 1993). However, when kidding occurs during the breeding season, reproductive activity is resumed soon after (*i.e.,* within the same breeding season), again due to the influence of

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Received: 20-02-13. Accepted: 22-10-13.

Abbreviations used: BE (buck effect group); CBE (females control of the buck effect group); CNBE (females control for the non buck effect group); LH (luteinizing hormone); MELBE (melatonin treated females of the buck effect group); MELNBE (melatonin treated females of the non buck effect group); MEL (melatonin); NBE (non buck effect group).

the photoperiod (Quirke *et al*., 1983; Schirar *et al*., 1989).

Sexual activity can be induced in anovulatory females separated from all male company simply with the reintroduction of males. This phenomenon, known as the male effect, has been extensively studied in ewes and goats (for a review see Delgadillo *et al*., 2009). The introduction of males to anovulatory females induces ovulatory activity in a high percentage of the latter, although strong ovulatory responses depend on the males showing fervent signs of sexual activity (Delgadillo *et al*., 2002). A normal luteal phase may follow the females' first ovulation, but they more commonly develop a short cycle of 5-7 days. Ovulations induced by the male effect are, however, insufficiently synchronised to allow the use of artificial insemination (Restall, 1988).

MEL treatments have been used in dairy goats in deep anoestrus to promote cycling with the intention of using artificial insemination after inducing the male effect (Pellicer-Rubio *et al*., 2007, 2008). Other authors have shown that during seasonal anoestrous, the administration of MEL (preceded or not by a long day photoperiod from November), accompanied or not by the male effect, is able to induce a period of reproductive activity (Zarazaga *et al*., 2009, 2011c, 2012a).

The aim of the present study was to determine whether the combination of buck effect and exogenous melatonin could improve reproductive performance in early postpartum Payoya female goats during seasonal anoestrus.

This experiment was performed in accordance with Spanish Animal Protection Policy RD1201/2005 (BOE, 2005), which conforms to European Union Directive 86/609 regarding the protection of animals used in scientific experiments.

The study was conducted at the experimental farm (37° 20' N 6.91 W) of the University of Huelva, Spain; these installations meet the requirements of the European Community Commission for Scientific Procedure Establishments (OJ, 1986).

The study animals were 44 adult pregnant female Payoya goats. The pregnant females gave birth during the first fortnight of April ($7^{th}$ April ± 1.67). At this moment, females were distributed at random into two major groups (Fig. 1): those that would be submitted to the buck effect (BE, N = 22) and those that would not be submitted to such effect (NBE, N = 22). These major groups were balanced according to the animals' live weight, body condition score (Hervieu *et al*., 1991), age and date of birth.

In turn, the animals in these major groups were distributed into subgroups. The BE animals were subdivided into: 1) females with no further treatment (CBE, N = 11); and 2) females implanted with melatonin (MELBE, N = 11). Before teasing, the females were

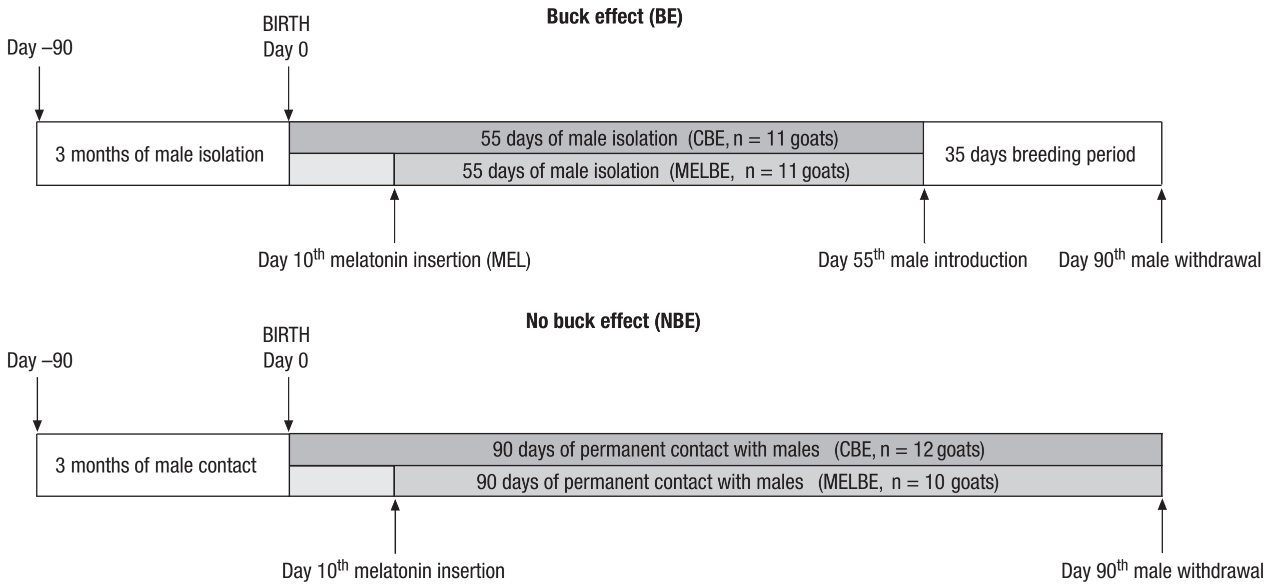


**Figure 1.** Diagram showing the different experimental groups and the number of females involved in each. CBE: females submitted to the buck effect with no further treatment. MELBE: females submitted to the buck effect implanted with melatonin. CNBE: females not submitted to the buck effect with no further treatment. MELNBE: females not submitted to the buck effect implanted with melatonin.

not allowed contact with males for about 55 days, starting on the date of the first birth registered among these females as a whole. The NBE animals were subdivided into: 3) females with no further treatment (CNBE, N = 12); and 4) females implanted with melatonin (MELNBE, N = 10). These females were permanently in contact with bucks, even during the three months before any gave birth.

The MELBE and MELNBE animals received one subcutaneous MEL implant containing 18 mg of the hormone Melovine® (CEVA Salud Animal, Barcelona, Spain) at the base of the left ear, 10 days after giving birth.

All groups were kept in communal yards throughout the experiment, without supplementary light. They were fed daily with lucerne hay and barley straw *ad libitum*, plus a commercial concentrate, according to Institut National de la Recherche Agronomique (INRA, France) standards for maintaining adult body weight and ensuring successful pregnancy and lactation (Morand-Fehr & Sauvant, 1988).

The offspring of all females that gave birth were weaned at 45 days. After weaning, their mothers were milked once daily until the end of the study (6th July ± 1.67).

Eight bucks were used for natural mating. To stimulate their sexual activity during seasonal anoestrous, they received three subcutaneous MEL implants 45 days before the females were expected to give birth. Each implant containing 18 mg of the hormone Melovine® and was inserted at the base of the left ear (Zarazaga *et al*., 2010). The control of oestrous started on the day of birth (D0) in the groups NBE and from the introduction of males (day 55th after birth) in the BE groups. Males were in contact with the females during 90 days and 35 days in the groups NBE and BE groups, respectively. Four groups (one for each female experimental group) of two males were formed at random because teasing was performed separately in each group. The males were fitted with marking harnesses for teasing. Oestrous behaviour was recorded by direct visual observation every day between 09:00 and 12:00 h and between 16:00 and 19:00 h. The immobility of the female at mounting was considered a sign of oestrous behaviour (Mauleon & Dauzier, 1965). The females marked by bucks at other times of day were also considered to be in oestrus.

The occurrence of ovulation and the ovulation rate were assessed by the presence and number of *corpora lutea* observed in each female in transrectal ultrasonography examinations conducted 10 ± 2 days after the detection of oestrus (Simoes *et al*., 2005). The procedure was performed using an Aloka SSD-500 machine connected to a 7.5 MHz linear probe. The presence of *corpora lutea* were also inferred by progesterone levels. For this, daily blood samples were taken from days 3 to 12 and on day 19 after the onset of the mating period of each female.

Blood samples were collected by jugular venipuncture in tubes containing heparin. Plasma was obtained after centrifugation at 3,500 rpm for 30 min and then stored at –20°C until hormone concentrations were measured. Concentrations of plasma progesterone were assayed in duplicate samples of plasma using a commercial enzyme linked immunoassay (ELISA) kit (Ridgeway Science Ltd., Gloucester, UK) in accordance with the manufacturer's instructions, as detailed by Madgwick *et al*. (2005). The mean intra-assay and inter-assay coefficients of variation were 6.0% and 5.4%, respectively. The sensitivity of the assay was 0.1 ng $mL^{-1}$. Females with progesterone concentrations of ≥ 0.5 ng $mL^{-1}$ were considered to have ovulated (Chemineau *et al*., 1984).

The interval between the male introduction and the onset of oestrous behaviour was recorded to the nearest half day. To determine fecundity (percentage of pregnant does/does mounted by the male), transrectal ultrasonography was performed 45 days after exposure to the males (Schrick *et al*., 1993). Fertility (percentage of goats kidding/does mounted by the male), prolificacy (number of kids born per female kidding) and productivity (number of kids born per female in each mating group) were then determined.

Ovulation rates and prolificacies were compared using the Mann-Whitney U test. The proportion of females in each group experiencing ovulation, and their fecundity and fertility results, were compared using the Chi-squared test and the Fisher exact probability test for two-group comparisons. Productivity was compared by factorial ANOVA with the experimental treatments understood as fixed effects. A Duncan test was performed when differences among treatments were observed. The results are reported as means ± standard errors. All analyses were performed using SPSS (2008) software.

Overall, more females showed oestrous activity when the buck effect was induced (overall total 72.7%) than when the males were in permanent contact with females (overall total 27.3%) ($p < 0.01$). The interval between the introduction of the males to teasing was shorter when the male effect was induced (an overall $6.0 \pm 1.4$ *vs*. an overall $16.0 \pm 5.8$ days for the BE and NBE animals respectively; $p < 0.05$). Neither overall fecundity, fertility and prolificacy was modified by the male effect. However, productivity was increased by the male effect (0.41 *vs* 0.09 kids born/female for BE and NBE, respectively, $F_1 = 4.502$, $p < 0.05$).

On the whole, more melatonin treated females showed oestrous and ovulation (52.4% *vs* 13.0% for MEL and C animals, respectively, $p < 0.01$), fecundity and fertility was higher in the melatonin treated females (61.5% *vs* 11.1% for both variables of the MEL and C animals, respectively, $p < 0.05$) and productivity was higher in the melatonin treated females (0.45 *vs* 0.04 goat kids born/female for MEL and C animals, respectively, $F_1 = 7.848$, $p < 0.01$).

A higher percentage of females of the MELBE group showed oestrous than the no buck effect groups (CNBE and MELNBE) ($p < 0.05$). Moreover, a higher percentage of females showing oestrous and ovulation, fertility and fecundity were observed in the MELBE group than the CBE and CNBE. Finally, the productivity of the MELBE group was higher ($F_3 = 5.460$, $p < 0.01$) than that of the other three groups (Table 1).

The buck effect combined with melatonin treatment improved the productivity of the females. However, the reproductive performances of all other animals were poor. This might be explained by the physiological status of the does – those that had given birth were in early postpartum, and the time of the year was that of deep seasonal anoestrous. This might account for the very high incidence of anovulatory oestrus observed, and indicates that, in general, all animals were experiencing strong inhibition of the hypothalamus-pituitary-ovarian axis, with gonadotrophin secretion insufficient to induce ovarian activity.

The results of the present experiment demonstrate that the buck effect combined to melatonin implants can reduce the anoestrous postpartum period in goats at Mediterranean latitudes, even during natural seasonal anoestrous. These are the first such results for goats. In ewes, Kusakari & Ohara (1997) and Abecia *et al*. (2002) observed that the insertion of melatonin implants induced an earlier onset of the ovulatory or oestrous activity and increased reproductive performance (fertility and fecundity) over that of non-treated ewes. These results contrast with those obtained by Kridli & Hallford (1997) and Forcada *et al*. (1995), who observed no resumption of reproductive activity before the normal breeding season in ewes, even in those administered melatonin. In cows, it has even been observed that melatonin treatment causes a significant lengthening of the interval between giving birth and the first postpartum oestrus (Sharpe *et al*., 1986). The reason for these results could be two complementary and non exclusive hypotheses. Firstly, the time between birth and mounting was enough for the uterus regression. This time was similar for all groups so it is not the only possibility. Secondly, the most important reason could be that it has been demonstrated that melatonin enhances the sensitivity of the hypothalamic gonadotropin-releasing hormone (GnRH) pulse gene-

**Table 1.** Reproductive results for the female Payoya goats subjected to different combinations of buck effect and melatonin treatment

| | Buck effect | | No buck effect | |
|---|---|---|---|---|
| | CBE[1] | MELBE[2] | CNBE[3] | MELNBE[4] |
| Females showing oestrous (%) | $63.6^{AB}$ | $81.8^{A}$ | $16.7^{C}$ | $40.0^{BC}$ |
| Interval male introduction to mating (days) | $5.7 \pm 2.2^{A}$ | $6.2 \pm 1.9^{A}$ | $28.5 \pm 14.5^{B}$ | $54.3 \pm 4.2^{C}$ |
| Females showing oestrous and ovulation (%) | $9.1^{B}$ | $63.6^{A}$ | $16.7^{B}$ | $40.0^{AB}$ |
| Ovulation rate of first oestrous (mean ± SE) | $1.0 \pm 0.0^{A}$ | $1.7 \pm 0.2^{A}$ | $1.0 \pm 0.0^{A}$ | $1.5 \pm 0.3^{A}$ |
| Fecundity (%) | $14.3^{B}$ | $66.7^{A}$ | $0.0^{B}$ | $50.0^{AB}$ |
| Fertility (%) | $14.3^{B}$ | $66.7^{A}$ | $0.0^{B}$ | $50.0^{AB}$ |
| Prolificacy[5] (mean ± SE) | $1.0 \pm 0.0^{A}$ | $1.3 \pm 0.2^{A}$ | $0.0 \pm 0.0^{A}$ | $1.0 \pm 0.0^{A}$ |
| Productivity[6] | $0.09^{B}$ | $0.73^{A}$ | $0.00^{B}$ | $0.20^{B}$ |

[1] CBE (n = 11): females submitted to the buck effect with no further treatment. [2] MELBE (n = 11): females submitted to the buck effect implanted with melatonin. [3] CNBE (n = 12): females not submitted to the buck effect with no further treatment. [4] MELNBE (n = 10): females not submitted to the buck effect implanted with melatonin. [5] Prolificacy: number of kids born per female kidding. [6] Productivity: number of kids per female in each mating group. Values with different letters in the same row are significantly different between groups (at least A,B,C: $p < 0.05$).

rator in the presence of the ram (Robinson *et al*., 1991) that allowed a higher response of fertility and fecundity and finally on productivity.

Compared to no treatment, the melatonin alone increased the number of females in oestrus, as well as ovulation, fecundity, fertility and productivity. This agrees with previous results reported for the Murciano-Granadina breed (Zarazaga *et al*., 2012b) and shows that melatonin treatment affords a useful way of increasing reproductive success in Spanish goats probably due to the positive effect of melatonin on the hypothalamus-pituitary axis (Robinson *et al*., 1991). Concerning the time between the time of birth and the day of mating, it is very interesting to indicate that was very similar for the females implanted with melatonin submitted or not to the male effect groups. This result could suggest that when the melatonin treatment is used, the male effect could be not strictly necessary and confirm previous results obtained by our group, indicating that the melatonin treatment by it self is able to resume the reproductive activity in Mediterranean goat breeds (Zarazaga *et al*., 2009). However, the association of the male effect and melatonin remains necessary to increase the percentage of goats showing oestrus and to improve fertility and productivity.

The females that had received no melatonin hormonal treatment (females without melatonin submitted or not to the buck effect) showed the poorest reproductive performances. Using a methodology similar to that described in the present work, Thompson *et al*. (1983) observed that *corpus luteum* function in Nubian dairy goats was absent in postpartum until the beginning of the next breeding season. Even in the non-seasonal Shiba goat, no ovulation occurs during nursing postpartum, suggesting that nursing is a determinant of the resumption of ovulation after having given birth (Takayama *et al*., 2010). The scant cyclicity seen after birth in the females with no melatonin treatment and without buck effect group may be due to the inability of the pituitary to generate sufficiently frequent LH pulses to induce ovulation (Wright *et al*., 1981a,b). It has also been suggested that uterine involution and ovarian insensitivity to gonadotrophins may contribute to postpartum acyclicity. Peters & Lamming (1990) suggested that acyclicity during the postpartum period may be due to the inhibition, at several levels, of the hypothalamus-pituitary-ovarian axis.

On the whole, the male effect itself, irrespective of the melatonin treatment, was unable to modify fecundity or fertility, although it caused a higher percentage of females to enter oestrus and ovulate. Indeed, the exposure of anoestrous does and ewes to bucks and rams, respectively, induced the rapid activation of LH secretion leading to oestrous behaviour and ovulation. Several factors modify oestrous responses and ovulation in females exposed to males, including the time of the year and the intensity of the males' sexual behaviour, etc. (Delgadillo *et al*., 2009; Delgadillo, 2011). The impact of the time of year was recently described by our group when a male effect was induced in February (Celi *et al*., 2013). In the latter work, a larger percentage of females entered oestrous and ovulated, but no differences were observed in terms of fecundity or fertility. While this treatment induced an oestrous response, pregnancy could not occur since the animals probably experienced strong inhibition of the reproductive hypothalamus-pituitary-ovarian axis.

In conclusion, the results of the present experiment show that exogenous melatonin combined to the buck effect on early postpartum Payoya does during seasonal anoestrous overrides the negative effects of this physiological status on reproductive function and leads to significantly better productivity than the other proposed treatments. This suggests such treatment could be of use in goat to improve meat production systems.

## Acknowledgments

This work was funded by the CICYT, Spain, grant PETRI 95-0964.OP. The authors are grateful to CEVA Salud Animal, Barcelona, Spain, for providing the Melovine® implants.

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