Effects of exogenous melatonin treatment on out-of-season ram fertility

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ABSTRACT

Three field experiments were performed to determine the effects of melatonin implants on the fertility of rams. The experiments were performed on three breeds (Rasa Aragonesa, Assaf and Manchega) in three commercial farms in Spain. Melatonin-treated rams (M) and non-implanted control rams (C) mated either ewes oestrous-induced with FGA intravaginal sponges (Rasa Aragonesa and Assaf farms) or melatonin-treated ewes during the anoestrous period (Manchega farm). Scrotal circumference (SC) was measured at melatonin implantation and at ram introduction (nearly 45 d later). Lambing rate, litter size, and fecundity were recorded at parturition. Melatonin implants in rams increased the number of lambs born per ewe in Rasa Aragonesa (19%), Assaf (9%), and Manchega (7%) because of the higher lambing rates exhibited by ewes mated with melatonin-treated rams (Rasa Aragonesa: 80.2% vs 70.5%, P<0.05; Assaf: 74.2% vs 68.0%, NS; Manchega: 90.0% vs 81.5%, P<0.05; for M and C groups, respectively). As a result, the number of extra lambs born per treated ewe was 0.17, 0.10, and 0.10 in the Rasa Aragonesa, Assaf, and Manchega ewes, respectively. In the M and C groups of Rasa Aragonesa rams, mean scrotal circumference increased significantly between the start of the experiments and the onset of mating, but the two groups did not differ significantly at the implantation or at the onset of mating. In the melatonin-treated and control Assaf rams, scrotal circumference increased over the course of the experiments but, at the onset of mating, the melatonin-treated rams had higher SC than did the control rams. Melatonin-treated Manchega rams had scrotal circumferences that were significantly greater than those of the control rams. In the Rasa Aragonesa and Manchega breeds, treating rams using melatonin implants significantly increased the net lamb production of ewes in field conditions. In the Assaf breed, the number of extra lambs born per ewe mated with melatonin treated rams was lower than in the other breeds, even though rams exhibited a significant increase in scrotal circumference. Further research involving the study of semen quality has been planned.

Key words: Melatonin, Rams, Fertility.
RIASSUNTO

EFFETTO DI IMPIANTI DI MELATONINA SULLA FERTILITÀ DEGLI ARIETI

Sono stati realizzati tre esperimenti per determinare l’effetto degli impianti di melatonina sulla fertilità dei maschi. Le prove sono state effettuate in tre aziende agricole spagnole, su tre razze: Aragonese, Assaf e Manchega. Sia i maschi trattati con melatonina, gruppo M, che i maschi non trattati, gruppo C (controllo), hanno montato pecore con estro indotto mediante spugne vaginali di FGA (razze Aragonese e Assaf) e pecore trattate con melatonina durante l’anastre. Nelle tre prove è stato misurata la circonferenza scrotale (CS) sia al momento dell’inserimento degli impianti di melatonina, sia al momento dell’introduzione dei maschi nel gruppo delle pecore (circa 45 giorni più tardi). Ai parti sono state registrate le percentuali di nascite, prolificità e fecondità. I maschi trattati con melatonina hanno fatto incrementare il numero di agnelli nati per parto del 19% nella razza Aragonese, del 9% nella razza Assaf e del 7% nella razza Manchega. Tale risultato è dovuto al maggior tasso di natalità presentato dalle pecore accoppiatesi con maschi trattati con impianto di melatonina (Razza Aragonese: 80,2 vs 70,5%, P<0,05; Assaf: 74,2 vs 68,0%, ns; Manchega: 90,0 vs 81,5%, P<0,05; per i gruppi M e C, rispettivamente). È risultato quindi un incremento dello 0,17, 0,10 e 0,10 agnelli nati per pecora trattata, rispettivamente nelle razze Aragonese, Assaf e Manchega. I maschi di razza Aragonese hanno mostrato un significativo aumento della circonferenza media all’inizio delle monte in entrambi i gruppi, senza differenze significative al momento dell’inserimento degli impianti o all’inizio delle monte. Anche i due gruppi (M e C) della razza Assaf, hanno mostrato un significativo aumento della circonferenza scrotale media durante la prova, anche se i maschi del gruppo M hanno dato valori significativamente maggiori nella seconda misurazione. Anche i maschi di razza Manchega trattati con melatonina hanno mostrato un significativo incremento della circonferenza scrotale, maggiore rispetto al gruppo controllo. In conclusione, il trattamento con melatonina negli arieti aumenta la produzione netta di agnelli in condizioni naturali, in maniera significativa nelle razze Aragonese e Manchega. Nella razza Assaf l’incremento del numero di agnelli per pecora trattata è risultato inferiore, tuttavia nei maschi trattati con melatonina è significativamente aumentata la circonferenza scrotale. Sono stati programmati ulteriori esperimenti per studiare l’effetto della melatonina sulla qualità del seme.

Parole chiave: Melatonina, Ariete, Fertilità.

Introduction

Seasonality, which is regulated by photoperiod, is a limiting factor in sheep reproduction. The circadian secretion of the hormone melatonin by the pineal gland conveys to the neuro-endocrine system information about photoperiod (Bittman et al., 1983). Subcutaneous melatonin implants are used to control oestrus by inducing a short-day-like response (O’Callaghan et al., 1991). Spain (since 2000), Italy (since 2007), and several other countries in the European Union, have sanctioned the commercial use of exogenous melatonin in sheep. Although the treatment was initially intended for use in ewes, attempts have been made to use it to improve the libido semen quality and fertility of rams, and its use in both sexes is recommended at the commercial level. The treatment of rams with melatonin between the middle and the end of spring accelerated the seasonal increase in LH and testis size (Webster et al., 1991) and increased the proportion of ewes that ovulated in response to the introduction of a ram (Rosa et al., 2000).

The objective of this study was to determine whether melatonin implants have a significant effect on ram fertility, as estimated by the reproductive performances of ewes, in the non-breeding season. In addition, the study quantified the effects of melatonin on scrotal circumference. These field trials are preliminary experiments of a more extensive project designed to study...
the effect of melatonin on semen quality and fecundity potential of rams.

**Material and methods**

Three experiments were conducted at three Spanish commercial farms in Huesca (41ºN) (Rasa Aragonesa breed), Zamora (42ºN) (Assaf breed), and Cáceres (39ºN) (Manchega breed).

**Rasa Aragonesa rams**

The Rasa Aragonesa farm, which produces light lambs (weaned at 45 d and slaughtered at 70 d), uses intensive reproductive management. The 5000-ewe flock (LW=55 kg) and 125 rams (LW=70 kg) is divided into 10 groups (500 ewes each). Every 23 d, ewes in one of the groups are oestrous-induced by intravaginal sponges that contain 30 mg (anoestrus period) or 40 mg (breeding season) of fluorogestone acetate (FGA) (Sincropart, CEVA Salud Animal, Barcelona, Spain). Sponges are withdrawn 12 d after insertion, and rams are introduced 48 h later. For the purpose of this out-of-season experiment, on April 26, 25 rams received three subcutaneous implants that each contained 18 mg of melatonin (Melovine, CEVA Salud Animal, Barcelona, Spain). These implants can release melatonin for more than 100 d (Zúñiga et al., 2002). The other 100 rams served as a control group (C). Intravaginal sponges (30 mg FGA) were inserted on May 27. At pessary withdrawal, ewes were injected with 480 I.U. of eCG, and assigned to either the M group (n=100) or the C group (n=400), and mounted by M and C rams, respectively. Rams were introduced into their groups 48 h after pessary withdrawal (on June 10), 45 d after receiving the melatonin implants. To insure that ewes that had a second oestrus were mated, rams and ewes were kept together for 20 d. The 18 ewes (4 M and 14 C) that died or left the farm before lambing were excluded from the analysis.

**Assaf rams**

The dairy sheep farm where this experiment was performed follows a system of 4 lambings in 3 years, and milking periods of 210-240 d. The flock of 500 ewes (LW=70 kg) and 20 rams (LW=100 kg) is divided into three groups. For the purpose of our experiment, the treatment group (M) included nine rams that received three melatonin implants each on May 13. Nine other rams served as the control group (C). On June 12, 200 ewes were oestrous-induced with intravaginal 30-mg FGA sponges and 480 I.U. ecG (Sincropart, CEVA Salud Animal, Barcelona, Spain), assigned to one of two groups (n=100 each), and mated with a melatonin-treated (M) or a non-treated (C) ram. Rams were introduced on June 26, 48 h after pessary withdrawal and 44 d after melatonin implants were inserted. Rams and ewes were kept together until oestrus was eventually repeated. Four ewes (3 M and 1 C) that died before lambing were excluded from the analysis.

**Manchega rams**

At the Manchega farm, the flock of 1800 ewes (LW=65 kg) and 150 rams (LW=80 kg) is managed using the STAR system (5 lambings in 3 years) (Lewis et al., 1996) to produce suckling lambs (slaughtered at 30 d). In this experiment, 50 rams received three melatonin implants each on 10 February (rams M). Another 50 rams served as a control group (C). On the day that rams received melatonin implants, ewes were given a single melatonin implant. About 45 d later, on March 24, 566 ewes were divided into two groups [group M (n=279) and group C (n=287)], and mated with M or C rams. The protocol for the commercial use of melatonin treatment in ewes indicates that a
ram effect should be applied; therefore, at this farm, the rams were completely isolated from the ewes for at least two months before mating.

At the time the melatonin implants were inserted and when the rams were introduced to ewes, scrotal circumference (SC) was measured using a flexible tape. At the Rasa Aragonesa farm, 25 rams in the C group were selected randomly and compared to the rams in the M group. Rams on the three farms and the Rasa Aragonesa and Manchega ewes were fed to provide their live weight maintenance requirements. Assaf ewes were fed to cover their milk production. Water and mineral blocks were available ad libitum.

The Rasa Aragonesa breed, of which there are >2.5 million ewes in Spain, has a short anoestrous period (<100 d) in spring, but a varying proportion (20-50%) of females exhibit ovarian activity throughout the year (Forcada et al., 1992). The Assaf breed is the main dairy sheep in Spain, where there are about 1 million heads. Although there are no published data on its annual ovarian seasonality, Palacios et al. (2002) found that Spanish Assaf ewes are more seasonal than are some other breeds (Merino or Lacaune) reared in Spain at the same latitude. The Manchega sheep, of which there are 1.5 million ewes in Spain, is a Spanish breed that exhibits a long breeding season from June-July until February-March (Gómez-Brunet and López-Sebastián, 1991).

To evaluate the effects of exogenous melatonin treatments to rams on the reproductive performances of ewes, we calculated lambing rate (proportion of ewes that lambed), litter size or prolificacy (number of lambs born per ewe lambed), and fecundity (number of lambs born per ewe treated). To obtain an objective measurement of the effects of the melatonin implants on reproductive performance, we calculated the number of additional lambs born per melatonin-treated ewe (fecundity M - fecundity C) and percentage of increase ((fecundity M - fecundity C)/fecundity M x 100).

Lambing rate, litter size, and fecundity were recorded at parturition. The statistical significance of differences in the lambing rates of groups was assessed using a $\chi^2$ test. To determine whether the three breeds differed in their response to the melatonin treatment, the litter size, fecundity and scrotal circumference of the groups were compared using a factorial analysis of variance based on the following fixed-effects model: $Y=Xb+e$; where $Y$ is the N x 1 vector of records, $b$ denotes the fixed effects in the model (two treatments) with the associated matrix $X$, and $e$ denotes the vector for residual effects. To compare scrotal circumferences at melatonin implantation and at ram introduction, $t$ tests for paired samples were used.

**Results**

Rasa Aragonesa, Assaf, and Manchega ewes mated to rams that received melatonin implants produced 19%, 9% and 7% more lambs born per ewe, respectively, than did ewes mated to rams in the control groups. The effect was particularly strong in the Rasa Aragonesa and Manchega breeds, and ewes mated with melatonin-treated rams had significantly ($P<0.05$) higher lambing rates than did the control ewes. As a result, in those breeds, the average number lambs born per treated ewe was, respectively, 0.17 and 0.10 higher among ewes mated with rams that received melatonin implants than it was among ewes mated with non-treated rams (Table 1). In the Assaf sheep, exogenous melatonin treatments did not have a significant effect on the reproductive performance of ewes.

In the M and C groups of Rasa Aragonesa rams, mean scrotal circumference increased
significantly between the start of the experiments and the onset of mating, but the two groups did not differ significantly at the time the melatonin implants were inserted or at the onset of mating (Table 2). In the melatonin-treated and control Assaf rams, scrotal circumference increased over the course of the experiments but, at the onset of mating, the melatonin-treated rams had higher SC than did the control rams. Among the Manchega rams, the scrotal circumferences of the melatonin-treated rams, but not those of the control rams, increased significantly between the start of the experiments and the onset of mating. Consequently, at the time of ram introduction, melatonin-treated rams had SC that were significantly greater than were those of rams in the control group.

**Discussion**

In our study of three breeds of sheep, the net lamb production by ewes synchronized in oestrus and mated in late spring was greater if they were mated to rams that were treated with exogenous melatonin. Fitzgerald and Stellflug (1991) found that the fertility of melatonin-treated rams in April (86-91%) was similar to that of melatonin-treated rams in autumn (93%), and was significantly higher than that of non-implanted rams (59-62%). In Merino sheep, the fertility of ewes mated with melatonin-treated rams (99%) was significantly higher than the fertility of ewes mated to control males (75%). That increase in the lambing rate after rams were treated with melatonin was greater than the rate observed when only ewes received melatonin implants in the same conditions (synchronized with progesterogen sponges) reported by Forcada et al. (1998) (80% vs 77%), but was lower than the rates observed by Horoz et al. (2003) (95% vs 75%). At the Manchega farm, where all of the ewes were given a melatonin implant, it is remarkable that the implants given to rams increased the lambing rate by 9%, giv-

| Table 1. Lambing rate (percentage of ewes lambed) and mean (±SE) litter size (lambs born per ewe lambed), fecundity (lambs born per ewe treated), and number of extra lambs born per melatonin treated ewe and gain (%), in Rasa Aragonesa, Assaf and Manchega ewes, which were mated by rams implanted (M) or not (C) with melatonin nearly 45 days before ram introduction. |
|---|---|---|---|---|---|---|
| Rasa Aragonesa | Assaf | Manchega |
| | M | C | M | C | M | C |
| N. | 96 | 386 | 97 | 97 | 90 | 81 |
| Lambing rate | 80.2%<sup>a</sup> | 70.5%<sup>b</sup> | 74.20% | 68.0% | 90.0%<sup>a</sup> | 81.5%<sup>b</sup> |
| Litter size | 1.34 ± 0.05 | 1.28 ± 0.15 | 1.61 ± 0.08 | 1.64 ± 0.08 | 1.68 ± 0.03 | 1.72 ± 0.03 |
| Fecundity | 1.07 ± 0.07<sup>a</sup> | 0.90 ± 0.04<sup>b</sup> | 1.18 ± 0.09 | 1.08 ± 0.10 | 1.49 ± 0.04 | 1.39 ± 0.05 |
| Extra lambs<sup>2</sup> | 0.17 | 0.10 | 0.10 | 0.10 | 0.10 |
| Gain<sup>3</sup> | 19% | 9% | 7% | 7% | 7% |

Within rows (for each breed), means with different superscripts are significantly different (P<0.05).

<sup>1</sup>Melatonin implantation to rams: April 26, May 13 and February 10; ram introduction: June 10, June 26 and March 24, respectively.

<sup>2</sup>Fecundity M – Fecundity C;

<sup>3</sup>[(Fecundity M – fecundity C)/fecundity M] x 100.
en that the ewes mated with control rams exhibited a high lambing rate (81.5%).

The experimental protocols were adapted to the mating dates at the three farms. Consequently, rams received melatonin implants from February to May. Given the breeding seasons of each breed in Spain (Manchega, Gómez-Brunet and López-Sebastián, 1991; Rasa Aragonesa and Forcada et al., 1992; Assaf, Palacios et al., 2002), in our study, the treatments were given in the anoestrous period. Nevertheless, the timing of the treatment might have had an effect on a ram’s fertility, particularly because we have observed in another study an effect of farm and month of treatment on the effects of melatonin treatment in ewes (Abecia et al., 2007).

Melatonin implants administered in the non-breeding season can improve progressive motility and morphologically normal sperm rates (Kaya et al., 2000). Moreover, in the non-breeding season, exogenous melatonin implants can increase intact acrosome rates and reduce aspartate aminotransferase activity post-thaw in the off-season ejaculates (Kaya et al., 2001). Further evidence of the positive effect of exogenous melatonin on semen quality has been described by Kokolis et al. (2000), who observed an increase in total acrosin activity between days 35-56 (autumn) and days 49-70 (spring) after melatonin implantation. In cases where acrosin activity is low, melatonin might improve fertilization rates in sheep during the non-breeding season, after induced oestrus. Melatonin treatments in heat-stressed rams in summer significantly improved rectal and scrotal-skin temperatures, libido, semen quality, and fertility (El-Darawany, 1999).

In our study, the mean SC of Rasa Aragonesa rams increased significantly between the onset of the treatment (late spring) and ram introduction (early summer), regardless of whether the ram received exogenous melatonin implants. In Assaf rams, the temporal increase was significantly greater in the treated rams than in the non-treated rams, whereas among the Manchega control rams SC did not increase significantly over the course of the experiment. In Mediterranean breeds of sheep, SC increases from May to August, when maximum sizes are achieved (Santiago-Moreno et al., 2005). In two Greek breeds of sheep, testicular volume follows a clear seasonal trend, with maximum and minimum volumes in July-August and February-April, respectively (Avdi et al., 2004). Melatonin implants can have positive effects on sperm quality and increase the scrotal circumference and testes volume of normospermic and pathospermic rams in anoestrous seasons (Coyan et al., 1998). Melatonin implants can have positive effects on sperm quality and increase the scrotal circumference and testes volume of normospermic and pathospermic rams in anoestrous seasons (Coyan et al., 1998).

| Table 2. Mean (±SE) scrotal circumference (cm) of Rasa Aragonesa, Assaf and Manchega rams, at implantation (three implants containing 18 mg melatonin) (SC1) (April 26, May 13 and February 10, respectively), and at ram introduction (SC2), nearly 45 days later (June 10, June 26 and March 24, respectively). |
|---------------------------------------------------------------|
| **Rasa Aragonesa** | **Assaf** | **Manchega** |
| **M (n = 25)** | **C (n= 25)** | **M (n = 9)** | **C (n = 9)** | **M (n = 50)** | **C (n = 50)** |
| SC1                | 34.24 ± 0.58<sup>a</sup> | 34.61 ± 0.69<sup>a</sup> | 28.55 ± 1.40<sup>a</sup> | 26.00 ± 1.40<sup>a</sup> | 34.39 ± 0.46<sup>a</sup> | 35.02 ± 0.42 |
| SC2                | 36.37 ± 0.63<sup>b</sup> | 36.17 ± 0.61<sup>b</sup> | 36.44 ± 1.21<sup>bx</sup> | 33.33 ± 0.69<sup>by</sup> | 37.14 ± 0.37<sup>bx</sup> | 35.49 ± 0.47<sup>y</sup> |

*Within columns, “a,b” means P<0.05; within rows (for each breed), “x,y” means P<0.05.*
Melatonin treatments during the non-breeding period can increase testicular growth (Fitzgerald and Stellflug, 1991).

Conclusions

Exogenous melatonin implants in rams improved the net lamb production of ewes under field conditions, and significantly so in the Rasa Aragonesa breed. In the Assaf and Manchega breeds, the number of extra lambs born per ewe mated with rams that received melatonin implants was lower than in the other two breeds, but treated rams exhibited a significantly greater increase in SC. Further research involving the study of semen quality has been planned to confirm such results and to investigate the physiological mechanisms involved in these observations, which might differ among breeds.

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