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Reproductive response and LH secretion in ewes treated with melatonin implants and induced to ovulate with the ram effect

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Abstract

Two experiments were conducted to examine the effects of treating seasonally anoestrous ewes with melatonin before ram introduction on reproductive response, and on LH secretion in anoestrous ewes induced to ovulate by rams.

In Experiment 1, a total of 667 ewes from three flocks involving Merino (Flock 1, $N=149$), Merino entrefino (Flock 2, $N=325$) and Rasa Aragonesa (Flock 3, $N=203$) breeds were used. Within each flock, ewes isolated from rams since the previous lambing were assigned at random to receive melatonin implants of Regulin (75, 175 and 105 in Merino, Merino entrefino and Rasa Aragonesa flocks, respectively) or to serve as untreated controls (74 in Merino, 150 in Merino entrefino and 98 in Rasa Aragonesa flocks). Fertile rams were introduced into all flocks 5 weeks after implantation in March (Flocks 1 and 2) or April (Flock 3), and remained with the ewes for a 50 day mating period. Percentage of ewes with luteal activity at ram introduction did not differ between melatonin treated and control ewes in any flock. There were no significant differences in either the mean interval from ram introduction to lambing or the distribution of lambing. Implantation with melatonin resulted in an improvement of prolificacy in all three flocks, although this only reached statistical significance in the Merino flock (1.15 vs. 1.03 in treated and control ewes, respectively, $P<0.05$). Fertility was increased significantly ($P<0.05$) in the Merino entrefino flock (64.5% in treated vs. 51.3% in control ewes).

In Experiment 2, two trials were undertaken utilizing a total of 63 ewes. Trial 1 involved 24 mature Manchega ewes and Trial 2 involved 39 Merino ewe lambs. Half of the animals in each trial received a Regulin implant on 28 February (Trial 1) or 12 March (Trial 2) and the remaining half acted as controls. Rams were introduced 5 weeks after implantation and remained with the ewes for a 25 day period. In both trials, anoestrous ewes at ram introduction were bled at 20 min intervals for 3 h before

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and 5 h after ram introduction and then at 3 h intervals over the next 24 h for assessment of plasma concentrations of LH. Secretion of LH before or following introduction of rams was not affected by melatonin. Both treated and control anoestrous ewes in each trial responded to introduction of rams with an increase in the frequency of the LH pulses ($P < 0.05$), but no significant changes were detected in pulse amplitude or mean levels of LH. A preovulatory surge of LH was detected between 8 and 26 h after ram introduction, but neither mean interval from ram introduction to the peak of LH surge, nor the magnitude of the LH peak, was influenced by melatonin treatment.

Results from this study show that: (1) melatonin implants administered during early seasonal anoestrus have the potential to improve reproductive performance in Spanish breeds of sheep, but the response is conditioned by breed, management system and environmental factors; (2) melatonin did not modify the secretion of LH in anoestrous ewes induced to ovulate by the ram effect under our experimental conditions.

Keywords: Melatonin implants; Ram effect; LH secretion; Ewes

1. Introduction

From recognition that photoperiodic control of reproductive seasonality in sheep is achieved via the patterns of secretion of melatonin from the pineal gland (Karsch et al., 1984), treatment of anoestrous ewes with melatonin has been proven to be effective in advancing the onset of the breeding season and increasing reproductive performance in sheep, goats and deer (Adam et al., 1986; Stellflug et al., 1988; Kouimtzis et al., 1989; Durotoye et al., 1991; Sharon et al., 1992; Staples et al., 1992).

Recently, new strategies involving the use of melatonin implants combined with the ram effect have been shown to be more advantageous in terms of obtaining an earlier mean conception date, more synchronization in either mating or lambing patterns and also higher lambing percentages (Williams et al., 1992). In Spanish Flocks, the reproductive response of ewes treated with melatonin implants before the introduction of rams during the anoestrous season is variable in relation to breed, month of treatment, management system and reproductive history (López Sebastian and Inskeep, 1991), and therefore more studies are necessary to optimise practical methods for the use of melatonin to improve reproductive performance in Spanish sheep breeds.

There is experimental evidence that exposure to melatonin stimulates progesterone production by the bovine corpus luteum in vitro (Webley and Luck, 1986). Other studies (Wallace et al., 1989) have showed that treatment of ewes with melatonin increases plasma concentrations of progesterone during the luteal phase of the oestrous cycle, leading to the suggestion that the improvement in fecundity of ewes treated with melatonin could be attributable, at least in part, to the luteotrophic effect of this hormone (Durutoye et al., 1991), althought no definitive conclusions have been made.

With regard to gonadotrophin secretion, it is well established that the onset of ovarian activity during the non-breeding season in both naturally and induced ovulating ewes is characterized by an increase in the frequency of LH pulses in the peripheral circulation (Martin, 1984). However equivocal results have been reported in relation to the effects of melatonin on LH secretion. Some investigators (Robinson et al., 1991; Webster et al., 1991) have stated that pulsatile secretion of LH is influenced by melatonin treatment, whereas others (Kennaway et al., 1982; Poulton et al., 1987) have indicated that it has no effect. This discrepancy between studies has led to the question of whether melatonin has an influence on the endogenous secretion of LH.

This work was undertaken to obtain more information on the effectiveness of the subcutaneous implants of melatonin given before introduction of rams during seasonal anoestrous in three Spanish sheep breeds. The other objective was to evaluate the effects of melatonin treatment on LH secretion in both mature ewes and ewe lambs induced to ovulate with the ram effect during the non-breeding season.

2. Materials and methods

2.1. Experiment 1

The objective of this experiment was to determine the effects on the reproductive response of treatment during the non-breeding season with melatonin implants before introduction of rams in three breeds of sheep in extensive production systems. The study was carried out on a total of 667 ewes from three flocks involving Merino (Flock 1, $N=149$), Merino entrefino (Flock 2, $N=325$) and Rasa Aragonesa (Flock 3, $N=203$) breeds of sheep located in Badajoz, Caceres and Huesca, respectively (latitude 39–41° N).

Within each flock, the ewes were randomly divided into two groups (Table 1). One group (M) received a subcutaneous implant containing 18 mg of melatonin (Regulin, Hoechst) and the other group (C) were not treated and served as controls. Throughout the experiment, M and C ewes in each flock were run together as a single group. Dates of previous lambing, weaning, implantation, ram introduction and number of animals in each treatment group for the three Flocks are presented in Table 1.

Intact rams were introduced at a ratio of 1:15, about 5 weeks after implantation with melatonin, and remained with the ewes for a minimum mating period of 50 days. The date of lambing and the numbers of lambs born for individual ewes were recorded from 148 to 198 days after ram introduction. The approximate date of fertile mating was retrospectively calculated from the date of lambing by assuming a gestation length of 148 days.

Table 1
Dates of previous lambing, weaning, implantation, ram introduction and number of ewes in the treatment groups (M = melatonin; C = control) for each flock in experiment 1

Flock	Breed	Treatment group	No. of ewes	Date of previous lambing	Date of weaning	Date of implantation	Date of ram introduction
1	Merino	M	75	December	14 April	12 March	17 April
		C	74	December	14 April	12 March	17 April
2	Merino	M	175	January/February	17 April	7 March	17 April
	Entrefino	C	150	January/February	17 April	7 March	17 April
3	Rasa	M	105	February/March	15 April	5 April	9 May
	Aragonesa	C	98	February/March	15 April	5 April	9 May

In the three flocks the proportion of ewes with luteal activity on the day of introduction of rams was evaluated by determining the concentration of progesterone in a single blood sample collected at ram introduction. Progesterone concentration greater than 0.5 ng ml^{-1} was considered evidence of luteal activity.

2.2. Experiment 2

This experiment was primarily designed to examine changes in plasma concentrations of LH in mature ewes and ewe lambs treated with melatonin and induced to ovulate with the ram effect during anoestrous season. Reproductive response was also evaluated.

Two trials were conducted utilizing a total of 63 ewes from an experimental flock located in Madrid at a latitude of 40° N. Trial 1 involved 24 mature Manchega ewes that had lambed 4–5 months before the start of experiment and Trial 2 involved 39 ewe lambs of Merino breed. Within each trial, the animals were assigned at random to receive treatment with melatonin implants (Regulin) or served as controls, and the two treatment groups remained together throughout the experimental period.

2.2.1. Trial 1

Twelve ewes out of a total of 24 mature Manchega ewes used in this study received one Regulin implant by subcutaneous application on 28 February and the remaining 12 acted as untreated controls. Five weeks after melatonin was implanted, two intact rams were introduced with the ewes for a mating period of 25 days. Ewes had been previously isolated from rams since the previous lambing. The date of lambing and the number of lambs born to each ewe was recorded at lambing.

A blood sample was collected from each ewe 7 days before and on the day of ram introduction, and assayed for progesterone in order to determine the number of ewes with ovarian activity the time of introduction of the rams.

Based on the progesterone concentrations, five treated and six control ewes identified as being in anoestrous at ram introduction were bled every 20 min for 3 h before and 5 h after ram introduction and then at 3 h intervals over the next 24 h for assessment of plasma concentrations of LH.

2.2.2. Trial 2

On 12 March, 20 out of 39 Merino ewe lambs received one melatonin implant and 19 were not treated. Five weeks after melatonin treatment, two vasectomized rams with marking harnesses were introduced to the ewes and remained with them for a 25 day period. Occurrence of oestrus as indicated by crayon marks from the rams was recorded three times a week.

As described in Trial 1, anoestrous ewe lambs at ram introduction were identified by measuring plasma concentrations of progesterone in two blood samples taken on the day of ram introduction and 7 days before.

Blood samples for assessment of LH secretion were obtained from seven treated and seven control anoestrous ewe lambs at the time of ram introduction. The number and the frequency of blood samples obtained to determine plasma concentrations of LH was similar to that described for Trial 1 in anoestrous ewes.

For all 39 ewe lambs, blood samples were taken on Days 42 and 50 after ram introduction and analysed for progesterone in order to identify ewes which remain cycling through this period.

2.3. Blood sample collections and hormone assays

All blood samples were obtained from the jugular vein into heparinized vacutainer tubes (5 ml) and centrifuged inmediately at 3000 r.p.m. for 15 min. Plasma was stored at -20°C until it was assayed for progesterone or LH.

Concentrations of progesterone were measured in duplicate in 200 μl of plasma by radioimmunoassay as described by López Sebastian et al. (1984). The minimal detectable concentration was 16 pg per tube, efficiency of extraction was 86% and intra- and interassay coefficients of variation were 10.4% and 13.6%, respectively.

Concentrations of LH were analysed in duplicate plasma samples of 100 μl by the method described by Mondain-Monval et al. (1984) modified by Gómez Brunet et al. (1992) to assay ovine plasma by an homologous radioinmunoassay using a specific antibody raised in the rabbit (INRA, Nouzilly, France), NIAMDD-oLH-22 as standard and LER-oLH-1734 as tracer. Sensitivity of the assay was 0.15 ng ml^{-1} of plasma and intra- and interassay coefficients of variation were 9.5% and 14.8%, respectively.

2.4. Data analysis

Data for LH concentrations are presented as means and standard errors of the means. Concentrations of LH from samples taken every 20 min were examined for secretory pulses by the method described by Martin et al. (1983). In brief, a pulse of LH was defined: (1) both the increase and the subsequent decrease in concentration had to exceed the sum of the assay errors appropriate for the concentration at the onset and the peak of the pulse, and (2) the increase had to occupy no more than two sampling intervals and the decline had to begin within two sampling intervals of attainment of the peak. Pulse amplitude was calculated by subtracting the concentration at the onset of a pulse (nadir) from the peak concentration. Comparisons of LH concentrations between treatment groups were carried out by performing an analysis of variance. Data on reproductive response were analysed by analysis of variance for differences between means: prolificacy (number of lambs born/ewes lambed) and intervals from ram introduction to lambing or date of first oestrous (Experiment 2, Trial 2). The χ-square method was used for comparisons of proportions: fertility (number of ewes lambing/ewes treated and percentage of ewes with luteal activity or showing oestrous (Experiment 2, Trial 2).

3. Results

3.1. Experiment 1

Percentages of ewes with luteal activity at ram introduction did not differ between melatonin treated and control groups in any flock (Table 2), although in Flock 1, this

Table 2
Percentage of ewes with luteal activity at ram introduction, interval from introduction of rams to lambing, fertility and prolificacy in melatonin treated (M) and control ewes (C) in the three flocks of experiment 1

	Merino flock		Merino entrefino flock		Rasa Aragonesa flock	
	M	C	M	C	M	C
No. of ewes	75	74	175	150	105	98
Ewes with luteal activity at ram introduction (%)	14.6	9.4	7.4	5.3	12.3	11.2
Interval from introduction of rams to lambing (days ± SEM)	166.2 ± 1.36	165 ± 1.16	179.6 ± 0.79	181.9 ± 1.09	180.8 ± 1.75	183 ± 1.82
Fertility (%)	78.6	81.1	64.5	51.3	36.2	26.5
Prolificacy	1.15	1.03	1.11	1.07	1.18	1.07

percentage was slightly greater in ewes treated with melatonin compared with controls.

Within each flock, no differences were found in the intervals from introduction of rams to lambing between treated and control ewes (Table 2). Irrespective of melatonin treatment, the interval from ram introduction to lambing for those ewes that did lamb in each flock was shorter ($P<0.05$) in Flock 1 (165.9 ± 1.26 days) than in the other two flocks (180.7 ± 0.94 and 181.9 ± 1.73 days in Flocks 2 and 3, respectively).

Detailed lambing data showed two peaks of lambing for ewes in the two groups in Flock 1, one occurring between 150 and 158 days after ram introduction, and the other occurring between 164 and 173 days, indicating that in this flock fertile mating took place primarily during the first 10 days and from Days 16 to 25 after ram introduction. By contrast, in Flocks 2 and 3 both treated and control ewes lambed over the period from 167 to 198 days after ram introduction with most ewes lambing after Day 173, so breeding in these flocks occurred for the majority of ewes in the second half of the mating period.

Fertility was increased by melatonin in Flock 2 only, where the proportion of ewes lambing by the end of the 50 day mating period was higher in melatonin treated compared with control ewes ($P<0.05$). No significant differences were observed in Flocks 1 and 3 (Table 2) although in Flock 3, fertility tended to be greater in melatonin treated than in control ewes.

An increase in prolificay resulting from treatment with melatonin was apparent in all three flocks (Table 2), but only in Merino ewes (Flock 1) did this difference reach statistical significance ($P<0.05$).

3.2. Experiment 2

3.2.1. Trial 1

The percentage of ewes which were cycling at the time of ram introduction was high for both melatonin treated (58.3%) and control ewes (50%) with no significant differences between them.

Melatonin treatment had no effect on either the distribution or mean interval from ram introduction to lambing. In both treated and control ewes, lambing commenced 149 days after ram introduction and continued to Day 174, resulting in mean lambing dates of 159.8 ± 0.69 days and 163.1 ± 0.64 days, respectively, for melatonin treated and control ewes.

Fertility was similar in melatonin treated (91.6%) and control ewes (100%). Prolificacy was not significantly affected by melatonin treatment (1.45 vs. 1.33 in melatonin and control ewes, respectively).

Results obtained in the study of the secretory patterns of LH in five treated and six control anoestrous ewes at ram introduction, showed that treatment with melatonin had no effect on any of the parameters of LH secretion measured before or after introduction of rams (Table 3).

In both melatonin treated and control anoestrous ewes, there was an increase ($P < 0.01$) in the frequency of pulses of LH after rams were introduced (Table 3). No significant changes were detected in pulse amplitude or mean levels of LH relative to introduction of rams.

All anoestrous ewes from each group, showed a preovulatory surge of LH in the period 8–23 h after rams were introduced, but neither the time nor the magnitude of the peak of the preovulatory surge of LH appeared to be influenced by melatonin treatment.

3.2.2. Trial 2

The proportion of ewe lambs that were showing ovarian activity at the time of ram introduction did not differ between melatonin treated and control ewe lambs (40% and 37%, respectively).

Treatment with melatonin had no significant effect on the percentage of ewe lambs showing oestrus during the 25 day period after ram introduction (60% in melatonin treated,

Table 3
LH pulse, frequency amplitudes, and mean concentrations in anoestrous Manchega ewes receiving melatonin treatment (M) or untreated controls (C), induced to ovulate by the ram effect (mean values ± SEM: experiment 2, trial 1)

	Group M	Group C
No. of ewes	5	6
Before ram introduction		
Pulse frequency (no. pulses per 3 h)	0.60 ± 0.24	0.50 ± 0.22
Amplitude (ng ml^{-1})	1.47 ± 0.39	2.28 ± 0.68
Mean LH (ng ml^{-1})	0.26 ± 0.07	0.33 ± 0.10
After ram introduction		
Pulse frequency (no. pulses per 5 h)	2.40 ± 0.24	2.66 ± 0.33
Amplitude (ng ml^{-1})	1.47 ± 0.11	1.98 ± 0.40
Mean LH (ng ml^{-1})	1.19 ± 0.11	0.84 ± 0.10
Preovulatory surge of LH (ng ml^{-1})	33.7 ± 8.27	26.2 ± 3.75
Mean interval from ram introduction to LH surge (h)	16.4 ± 2.40	20.0 ± 2.68

Table 4
LH pulse, frequency amplitudes, and mean concentrations in anoestrous Merino ewe lambs receiving melatonin treatment (M) or untreated controls (C), induced to ovulate by the ram effect (mean values ± SEM: experiment 2, trial 2)

	Group M	Group C
No. of ewe lambs	7	7
Before ram introduction		
Pulse frequency (no. pulses per 3 h)	0.57 ± 0.28	0.83 ± 0.15
Amplitude (ng/ml)	1.41 ± 0.29	1.52 ± 0.52
Mean LH (ng/ml)	0.23 ± 0.10	0.24 ± 0.08
After ram introduction		
Pulse frequency (no. pulses per 5 h)	1.71 ± 0.28	2.00 ± 0.23
Amplitude (ng ml^{-1})	1.02 ± 0.28	1.13 ± 0.22
Mean LH (ng ml^{-1})	0.20 ± 0.04	0.41 ± 0.23
Preovulatory surge of LH (ng ml^{-1})	64.5 ± 15.00	74.0 ± 8.69
Mean interval from ram introduction to LH surge (h)	16.5 ± 1.78	14.8 ± 0.50

and 53% in control ewes), or on the mean interval from ram introduction to first detected oestrous (10.0 ± 0.63 days and 10.2 ± 0.85 days for melatonin treated and control ewe lambs, respectively) or the distribution pattern of oestrus.

Concentrations of plasma progesterone in the blood samples taken on Days 42 and 50 after ram introduction indicated that with the exception of one melatonin treated and two control ewe lambs, all ewes remained cycling at 1.5 months after introduction of rams.

As in the mature ewes (Trial 1), melatonin treatment had no effect on the secretion of LH, either before or after ram introduction. In the two treatment groups, the frequency of pulses of LH increased ($P<0.05$) after ram introduction (Table 4), but there was no significant variation in pulse amplitude or mean plasma concentrations in the period inmediately before or after introduction of the rams (Table 4).

All seven anoestrous ewe lambs from each group responded to ram introduction with a preovulatory surge of LH, occurring between 11 h and 26 h after their introduction, with a similar mean time between introduction of rams and peak of LH surge for control and melatonin treated ewe lambs (Table 4). No significant differences were found in the mean concentration of the LH at the peak surge between melatonin treated and control ewe lambs.

4. Discussion

Data from Experiment 1 show a considerable variation among the flocks in several aspects of their reproductive response as result of implantation with melatonin. In fact, treatment with Regulin implants 5 weeks before introduction of rams during seasonal anoestrus improved fertility in the Merino entrefino breed (Flock 2) and increased prolificacy in the Merino breed (Flock 1). However, there was no evidence for an effect of melatonin on any of the reproductive parameters in the Rasa Aragonesa flock (Flock 3). These results confirm the findings of previous studies carried out with different breeds of sheep in extensive production systems (Haresign et al., 1990; López Sebastian and Inskeep, 1991; Staples et al., 1992) revealing that continous administration of melatonin can be be used to improve reproductive performance in ewes treated during the non-breeding season, but a high degree of variation related to breed, management system, and environmental and physiological factors is to be expected.

On the basis of progesterone levels at ram introduction and the lambing dates recorded in Experiment 1, it is suggested that most ewes in each flock were anoestrous before ram introduction, and that oestrus and ovulation were stimulated by the introduction of rams. The lack of differences between melatonin treated and control groups in the mean interval from ram introduction to lambing and the distribution pattern of lambing supports the fact that within each flock, ewes in the two groups were mated at a similar time after ram introduction, with no differences in mean date or patterns of mating. This finding disagrees with reports by Haresign (1992) in which melatonin treatment before introduction of rams was shown to be effective in advancing and condensing the mating patterns in treated ewes compared with controls. In the present study, unlike that conducted by Haresign (1992), treated and control ewes had been kept as one group from time of implantation. It is probable, as has been postulated by Haresign et al. (1990), that there was a social facilitation effect between treated and control animals running together which contributed to the tightening of the mating period for both groups. It is not clear, however, whether the facilitation effect is because of a ewe–ewe interaction or a ewe–ram–ewe interaction.

In evaluating the individual flock lambing dates in Experiment 1, irrespective of melatonin treatment, it should be noted that the mean interval from ram introduction to lambing was approximately 15 days earlier in the Merino flock (Flock 1) than in Merino entrefino and Rasa Aragonesa flocks (Flocks 2 and 3, respectively). This finding could be a function of the proportion of ewes already cycling at the time of ram introduction as well as the interval from ram introduction to first induced oestrus in response to the ram effect.

The lack of differences in fertility between melatonin treated and control ewes in Rasa Aragonesa flock disagrees with observations on the same breed reported by López Sebastian and Inskeep (1991), who found significant increases in fertility in melatonin treated ewes. In other studies (Folch et al., 1991), fertility was also enhanced in melatonin treated Rasa Aragonesa ewes, although the magnitude of the response was variable from one farm to another. A large portion of the differences in response between studies may be attributable to differences in the time of implantation relative to previous lambing, date of melatonin treatment or ram introduction relative to the onset of the breeding season of this breed or could be associated with different management systems. Failure to observe any major difference in the reproductive response between melatonin treated and control ewes in both trials of Experiment 2 may have been a result of the high proportion of ewes showing ovarian activity at ram introduction, as well as the low number of animals involved in the study.

Melatonin treatment has been shown to be effective in increasing litter size in several breeds of sheep in the UK (Haresign, 1992), Australia (Staples et al., 1992), Greece (Kouimtzis et al., 1989) and Spain (Folch et al., 1991; López Sebastian and Inskeep, 1991). In the current study, only treated ewes of the Merino breed showed a significant increase in the number of lambs born per ewe lambing, but there was a tendency in all breeds for higher prolificacy in ewes treated with melatonin implants compared with control (Table 2). Although the design of these experiments does not permit us to know whether this general increase in prolificacy in treated ewes was a result of an increase in ovulation rate or a reduction in embryo mortality, studies in British (Haresign, 1992) and Spanish ewes (Folch et al., 1991) have indicated a consistent effect of melatonin on ovulation rate. This could explain the greater incidence of twin births in treated ewes in this study.

The main objective in Experiment 2 was to investigate the effects of melatonin treatment on LH secretion in mature ewes (Trial 1) and ewe lambs (Trial 2) induced to ovulate by the ram effect. It is recognized that a low plasma progesterone concentration from a single sample taken at ram introduction does not guarantee that a ewe is anoestrous, and so only ewes judged to be anoestrous on the basis of progesterone concentrations measured in two blood samples collected 7 days before and at ram introduction were eligible to respond to ram effect and therefore suitable for examining the LH response.

Previous experiments have investigated the effect of melatonin on LH secretion during seasonal anoestrous, and produced equivocal results. Robinson et al. (1991) provided evidence that in the ewe, melatonin treatment initiates breeding activity by an abrupt increase in LH pulse frequency over the 3–4 days before the first ovulation, by acting directly on the Gn-RH pulse generator. In contrast, Kennaway et al.(1982) and Poulton et al. (1987) did not find differences between melatonin treated and control ewes in the frequency of LH pulses any times before the onset of oestrus cycles. In our study, there was no effect of melatonin on any of the LH parameters measured. However, these results are not comparable with the above described because in our study ovulation was induced with the ram effect, and measurement of pulsatile secretion of LH was only performed during the periods inmediately before and after introduction of rams.

In the present study both groups of Manchega ewes and Merino ewe lambs had identical responses to the introduction of rams in terms of LH secretion (Tables 3 and 4), irrespective of melatonin treatment. However, it is possible that under our experimental conditions the full effects of melatonin were masked by the good response to the ram effect of both melatonin treated and control ewes.

It can be concluded from these experiments that: (1) melatonin implants administered during early seasonal anoestrous have the ability to be used to improve the reproductive performance of Spanish breeds of sheep, but the response is conditioned by breed, management system and environmental factors; (2) melatonin did not modify the secretion of LH in anoestrous ewes induced to ovulate by the ram effect under our experimental conditions.

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A clinical and ultrasonographic study of induced testicular and epididymal lesions in goats and a ram

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Abstract

Sequential ultrasonographic changes in the testis and the epididymis, semen quality and plasma testosterone concentrations were monitored after unilateral intra-testicular and intra-epididymal injections of chlorhexidine gluconate solution in three goats and a ram; the untreated, normal testis and epididymis were imaged as controls. Sixteen weeks after treatment unilateral vasectomy, ipsilateral to the normal testis and epididymis, was performed in two goats and a ram. Gross and histological changes postmortem were also recorded.

Ejaculates collected after injection showed a decrease in mass and individual motility, sperm concentration and total sperm per ejaculate with an increase in the percentages of dead and abnormal spermatozoa. Those collected after vasectomy on the normal side had less than 20% motile sperm, most of which were dead (77–100%) and abnormal (62–100%). When observed ultrasonographically, within 24 h, the testis showed hypoechoic areas corresponding to the sites of injection. Later, the lesions decreased in echogenicity and showed acoustic shadowing, and were surrounded by a distinctly hyperechoic border. The epididymal tail showed an increased echogenicity, and had anechoic lesions with ill-defined borders within 24 h after injection. A hyperechoic border was subsequently observed around the lesions. Gross and histological examination of the testis revealed areas of long-standing tubular necrosis surrounded by mineralised tissue at the sites of injection. The epididymal tail showed fibrosis and sperm granuloma formation. Ultrasound imaging appears to be useful in diagnosing the presence of focal lesions of the testis or the epididymis.

Keywords: Ultrasound; Testicular lesions; Goats; Rams

1. Introduction

Intra-epididymal injection of chlorhexidine gluconate has been used as a substitute for vasectomy in male cats (Pineda and Dooley, 1984), dogs (Pineda et al., 1977; Pineda,

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