Positive Effects of Melatonin Treatment on the Reproductive Performance of Young Border Leicester Rams Mated to Merino Ewes in Spring: Preliminary Observations

DO Kleemann1, JM Kelly1, LJ Arney2, IL Farley3, AJ Tilbrook3 and SK Walker3

1Turrerfield Research Centre, South Australian Research and Development Institute, Rosedale, SA, Australia; 2Inverbrackie, Strathalbyn, SA, Australia; 3Marmon Hill, Jabuk, SA, Australia; 4South Australian Research and Development Institute, Roseworthy, SA, Australia

Contents

Poor reproductive performance of Merino ewe flocks when mated to Border Leicester rams during spring may be due to seasonality of the Border Leicester breed. Two approaches were taken to test this assumption. Six young (12 months old) or six mixed-age (12, 24 and ≥36 months old) Border Leicester rams were either treated or not treated with melatonin implants (2 × 2 design) 6 weeks before the four groups of rams were each put with approximately 300 Merino ewes for an 8-week mating period. Implants were inserted in early September (experiment 1). The second approach was to yard or not yard ewes and mixed-age rams on several occasions during the first 3 weeks of the mating period (experiment 2). Pregnancy rate and twinning percentage were assessed by ultrasonography. In experiment 1, melatonin treatment in young rams increased (p < 0.001) pregnancy rate from 5.0% to 92.6%, but mixed-age rams did not respond (90.7% vs 89.5% for melatonin and non-melatonin treatments, respectively). Twinning rate was similar (p > 0.05) for ewes mated to either melatonin or non-melatonin-treated young rams (36.8% vs 40.0%, respectively), whereas melatonin significantly improved (p < 0.05) twinning rate in those ewes mated to mixed-age rams (49.1% vs 36.1%). After 6 weeks of melatonin treatment, scrotal circumference was greater (p < 0.05) in both young and mixed-aged rams than in untreated counterparts. In experiment 2, yarding of ewes and rams overnight on several occasions early in the mating period reduced (p < 0.001) pregnancy rate compared with non-yarded counterparts (89.5% vs 65.5%). Twinning rate was not affected (37.7% vs 36.1%, respectively). In summary, melatonin treatment of Border Leicester rams significantly improved flock reproductive performance in spring due to improved pregnancy rates with young rams and improved litter size with mixed-age rams.

Introduction

The Border Leicester breed, originating from England, forms an integral part of an efficient global lamb-meat industry. Generally, Border Leicester rams are used to produce first-cross ewe progeny, particularly in Australia, that are valued for their fertility and mothering ability (Fogarty et al. 1992; Stephens et al. 2006). The mating of these ewes to terminal meat sires such as the Dorset or Suffolk produces rapidly growing lambs with preferred carcase characteristics.

Reproduction in the Border Leicester breed is, however, highly seasonal, particularly at northern latitudes where pronounced changes in day length restrict breeding to the autumn period (Hafez 1952). In Australia, with less obvious changes in day length, the breeding season generally occurs between February and early April (Smith 1967; Williams et al. 1974; Hall and Killeen 1989). Sexual activity of Border Leicester rams is less constrained by the effects of season compared with the ewe, but activity is still reduced in the spring–summer period (Lindsay and Ellsmore 1968; D’Occhio and Brooks 1983). On the other hand, the Merino ewe which is used to produce Border Leicester x Merino females for terminal matings is generally able to cycle spontaneously or be induced to cycle throughout the year (Martin et al. 1986). The Border Leicester lamb-meat industry attempts to capitalize on the lack of seasonality in the Merino, but matings that occur during the October to December period have highly variable success rates particularly when young rams are used. This variability, which can account for 10–20% fewer lambs, on average, being weaned compared with Merino rams (L Arney and I Farley; personal communication) is thought to be primarily due to poor libido.

This study examined two approaches to overcome this problem, namely treatment with melatonin implants in spring and exposing rams to additional sexual stimuli early in the mating period. Treatment of rams with melatonin, the endocrine signal through which day length impacts the reproductive axis (Bittman et al. 1983), enhances testicular size, sexual behaviour and semen quality (Lincoln and Ebling 1985; Fitzgerald and Stellflug 1991; Hanif and Williams 1991; Rosa et al. 2000a; Casao et al. 2010). Furthermore, in pre-pubertal rams, melatonin treatment advances puberty in association with increases in testicular weight and sperm production per ejaculate (Chemineau et al. 1992). While these studies included some highly seasonal breeds (e.g. Soay, Texel and Suffolk; Hafez 1952), comparable studies appear not to have been conducted in the Border Leicester. On the other hand, several factors are known to influence sexual motivation in rams including sexual rearing pre- and post-puberty (e.g. Hulet et al. 1964; Katz et al. 1988; Tilbrook and Cameron 1990), competition between rams (Lindsay and Robinson 1961a; Lindsay and Ellsmore 1968), watching another ram’s performance (see Tilbrook and Cameron 1990) and exposure to oestrous ewes (Knight and Gibb 1990; Rosa et al. 2000b). Of particular interest is the ability to trigger sexual interest in young rams by brief exposure to oestrous ewes (Price et al. 1991; Rosa and Bryant 1998). The strategy adopted in the current study, namely yarding rams with ewes overnight early in the mating period, is supported by the findings of Lindsay and Robinson (1961b) where mating in small pens markedly increased the percentage of ewes served compared with paddock-mated ewes.

The current on-farm study determined pregnancy rate and litter size in Merino ewes mated in spring to either...
young or mixed-aged Border Leicester rams. In experiment 1, rams were treated with melatonin for 6 weeks prior to mating, and their reproductive performance compared with that of untreated rams. In experiment 2, mixed-age rams were yarded overnight with ewes on several occasions early in the mating period, and their reproductive performance compared with rams that remained in the paddock.

Materials and Methods

The Border Leicester rams were born and reared at Strathalbyn, South Australia (latitude 35.3°S, longitude 138.9°E), while all rams were managed and mated to 1500 Merino ewes at Jabuk, South Australia (latitude 35.4°S, longitude 140.1°E). The two locations have a Mediterranean-like climate with wet winters and dry summers predominating. Corresponding annual mean rainfall (mm) and annual mean maximum and minimum temperatures (°C) for the two locations are 491, 21.3, 9.5 and 310, 23.4, 9.1.

The practices and procedures of the experiments were approved by the Animal Ethics Committee of Primary Industries and Regions, South Australia (PIRSA).

Experiment 1

The experiment was designed as a 2 × 2 factorial with ram age (12 months old vs a mixture of 12, 24, 36 and 48 months old) and melatonin treatment (melatonin, no melatonin) forming the main effects. Standard farm practice is to mate a syndicate of mixed-age rams. Rams were allocated at random to treatments after stratification on liveweight. Six 12-month-old and six mixed-age Border Leicester (two 12 months old and four mature) rams were each treated with synthetic melatonin implants in early September, 2013. Three melatonin implants (18 mg per implant; Regulin®, Ceva Animal Health Pty Ltd, Glenorie, New South Wales, Australia) were inserted subcutaneously at the base of the ear. The skin and needle were cleaned with 70% ethanol prior to implantation. Six 12-month-old and six mixed-age Border Leicester (2 12 months old and four mature) untreated rams were included in the study. Each group of treated and untreated rams were put to approximately 300 mixed-age Merino ewes (mid-October) with the 8-week mating period commencing 6 weeks after the start of melatonin treatment. Ewes were allocated at random to the four treatment groups on the basis of age (2.5, 3.5 and 4.5-year old). Scrotal circumference (Foster et al. 1989), live weight and body condition score (Russel et al. 1969) were recorded on the day of implant insertion, and 6 weeks later on, the day that rams were put to ewes. The same operator measured scrotal circumference and body condition during the experiments. A sample of 40 Merino ewes from each age group was condition-scored at the start of mating, and a further 40 ewes from within each treatment group were scored 8 weeks later at the end of the mating period. Ewes were scanned for pregnancy and litter size by an experienced commercial operator when foetal age ranged from approximately 44 to 100 days.

Experiment 2

This experiment was conducted on mixed-age rams. Treatment consisted of yarding rams overnight with ewes on six occasions during the first 3 weeks of the mating period. Control rams remained with ewes under paddock conditions. The yarded group of six rams and 300 ewes were contained within a 0.2-ha area with access to water. Allocation of rams and ewes, measurements and timing of events were the same as described in experiment 1.

Statistical analysis

In experiment 1, data were analysed as a 2 × 2 factorial with melatonin and age of ram forming the main effects and interaction term. When appropriate, age of ewe was included as a fixed effect with all interactions (melatonin, age of ram, age of ewe) included in the model. Data from experiment 2 were analysed using treatments (yarding, no yarding) and when appropriate, age of ewe and the first order interaction term in the model. Continuous and non-continuous data in both experiments were analysed using the GLM and CATMOD procedures in SAS, respectively.

Results

Experiment 1

Ram liveweight, body condition and scrotal circumference

Scrotal size was larger (p < 0.01) for mixed age than young rams at the start of melatonin treatment and tended to be greater at the start of mating (p = 0.08, Table 1). After 6 weeks of melatonin treatment, scrotal circumference (Table 1) and gain in scrotal circumference (Fig. 1) were greater (p < 0.05) in both young and mixed-aged rams than in untreated counterparts. Live-weight and body condition score were not influenced (p > 0.05) by melatonin treatment (Table 1).

Pregnancy rate, twinning rate and ewe body condition

Melatonin treatment in young rams increased (p < 0.001) the pregnancy rate from 5.0% to 92.6%, but a significant response was not obtained with mixed-age rams (90.7% vs 89.5% for melatonin and non-melatonin treatments, respectively; Fig. 2). Twinning rate was similar (p > 0.05) for ewes mated to either melatonin- or non-melatonin-treated young rams (36.8% vs 40.0%, respectively), whereas melatonin significantly improved (p < 0.05) twinning rate in ewes mated to mixed-age rams (49.1% vs 36.1%; Fig. 3). Overall, body condition score of ewes at the start of mating was 3.56 ± 0.02. Body condition score of ewes 8 weeks later did not vary (p > 0.05) between the four treatment groups (range 3.11 ± 0.03 to 3.18 ± 0.03).

Experiment 2

Ram liveweight, body condition and scrotal circumference

There were no significant differences (p > 0.05) between yarded and non-yarded treatment groups for ram liveweight, body condition score and scrotal
circumference at the start of mating. Corresponding values were 98.6 ± 6.8, 4.44 ± 0.15, 34.0 ± 0.8 and 100.7 ± 6.8, 4.33 ± 0.15, 32.8 ± 0.8. Yarded and non-yarded groups did not vary (p > 0.05) in body condition at the end of the mating period (3.90 ± 0.20 and 4.13 ± 0.18, respectively).

**Pregnancy rate, twinning rate and ewe body condition**

Yarding of ewes and rams overnight on six occasions during the first 3 weeks of the mating period reduced pregnancy rate, twinning rate and ewe body condition (p < 0.05; 37.7% vs 36.1%, respectively). Body condition score of yarded and non-yarded groups did not vary (p > 0.05) in body condition at the end of the mating period (3.90 ± 0.20 and 4.13 ± 0.18, respectively).

**Discussion**

This study has shown for the first time that poor reproductive performance of young Border Leicester rams, when first exposed to Merino ewes during spring (October–November), can be overcome by administering melatonin 6 weeks beforehand. The substantial increase in pregnancy rate in this study (from 5% to 93%) is indicative of a major shift in sexual ability of rams, presumably associated with changes in both libido and spermatogenesis as has previously been demonstrated (Williams et al. 1990; Hanif and Williams 1991).

While these studies did not use ewe fertility as the ultimate test of reproductive performance, they showed that melatonin treatment was responsible for increases in testicular diameter, sexual behaviour and semen quality in 12-month-old rams of the seasonal Suffolk breed. The current study also indicated that testicular size, as measured by scrotal circumference, was enhanced by melatonin treatment.

Unlike young rams, treatment of mixed-age rams with melatonin did not improve pregnancy rate. In contrast, other comparable studies with mature or mixed-aged rams (Fitzgerald and Stellflug 1991; Palacin et al. 2008) have reported increases in pregnancy rate of 10–29% following melatonin treatment. These experiments were conducted at higher latitudes (43.5°N and 39°–42°N) compared with the current study (35°S) and with breeds (Booroola Merino and Polypay, Fitzgerald and Stellflug 1991; Rasa Aragonesa, Spanish Assaf and Manchega, Palacin et al. 2008) that are reputedly less seasonal than the Border Leicester (Hafez 1952; Palacin et al. 2008). One or both of these factors may be implicated in the differences obtained between studies. Despite melatonin treatment of mixed-age rams having no effect on pregnancy rate in the current experiment, it was associated with an increase in litter size. This response could have resulted from improved semen quality (Casao et al. 2010, 2013; Rosa et al. 2012) with associated increases in fertilization rate and decreases in both embryo mortality and partial failure of multiple ovulations. The latter is known to be a significant source

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**Fig. 1.** Least-squares means (±SEM) for daily gain in scrotal circumference over 6 weeks for young or mixed-age Border Leicester rams either treated or not treated with melatonin implants. Different letters indicate significant differences (p < 0.05) between the four treatments

**Fig. 2.** Pregnancy rate (%) of mature age Merino ewes mated to young or mixed-age Border Leicester rams either treated or not treated with melatonin implants. Different letters indicate significant differences (p < 0.05) between the four treatments
The differences between ages in pregnancy rate might be both physiological and behavioural. Despite melatonin increasing the testicular size in both ages, there was only an increase in pregnancy rates from the young rams. The lack of effect in mixed-age rams may have been due to their testes being sufficiently large prior to treatment to produce adequate viable sperm to fertilize most ewes (Cameron et al. 1987). Another possibility is that sexual experience and motivation in the young rams were increased with treatment, meaning that they mated more ewes more times compared with the more experienced mixed-age rams where sexual behaviour was less limiting (Tilbrook and Cameron 1990). Further research would be required to establish the extent to which these factors impacted pregnancy rate.

Most graziers in Australia put a syndicate of rams to the ewe flock. Syndicates usually consist of either mature (>1.5 year old), young (≤1.5 year old) or mixed-age groups (Kleemann and Walker 1992), the latter two being used in experiment 1. The main reason for using young Border Leicester rams is to maximize the rate of genetic progress, but their use (e.g. in spring) before they attain full sexual maturity can compromise flock reproductive performance. In Australia, there is also the temptation to use Border Leicester rams in spring to capitalize on the lack of seasonality in the Merino ewe. The results of this study clearly offer breeders a solution to the dilemma of mating young Border Leicester rams in spring, namely a previous 6-week treatment period with melatonin. Similar studies need to determine how relevant this strategy is to other seasonal breeds (e.g. Suffolk). However, such treatment, across ram breeds, would only be relevant in those environments where ewe breeds with worthwhile levels of cyclicity in spring are available.

In conclusion, administration of melatonin to young Border Leicester rams 6 weeks before the mating period in spring dramatically increased pregnancy rate in mature age Merino ewes. Melatonin given to mixed-age Border Leicester rams did not enhance pregnancy rate but did increase twinning rate. Yarding of mixed-age rams with ewes overnight was not successful and, in fact, was detrimental to flock fertility. Yet, the performance of the control group (90% of ewes pregnant) indicates that the sexual activity of the mixed-age ram syndicate was satisfactory, and at this level of performance, the likelihood of improvement through manipulation of any kind is unlikely. Similar studies that focus on the performance of young rams may be worthwhile. Why pregnancy rate was substantially reduced from 90% to 66% with overnight yarding is unknown, but it could be related to suppression of mating activity of subordinate rams as well as dominant rams themselves spending less time engaged in mating activity (Lindsay 1966; Lindsay et al. 1976). Another possibility is that the yarding induced stress responses that suppressed sexual behaviour. The activity associated with yarding is known to induce acute stress responses (Turner et al. 2010), and these responses can inhibit aspects of sexual behaviour in ewes that are essential for mating (Pierce et al. 2008).

In conclusion, administration of melatonin to young Border Leicester rams 6 weeks before the mating period in spring dramatically increased pregnancy rate in mature age Merino ewes. Melatonin given to mixed-age Border Leicester rams did not enhance pregnancy rate but did increase twinning rate. Yarding of mixed-age rams with ewes overnight was not successful and, in fact, was detrimental to flock fertility. Further field and pen studies are required to elucidate why melatonin treatment is beneficial to the reproductive performance of the Border Leicester ram, particularly the young ram when mated in spring.

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Conflict of interest

The authors have no conflict of interest to declare.

Author contributions

Drs Kleemann, Kelly, Tilbrook and Walker designed the research, drafted and revised the paper. Messrs Arney, Farley and Dr

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Kleemann finalised the design, implanted rams with melatonin, allocated rams and ewes to treatments and collected data. Messrs Arney and Farley revised the paper, provided the melatonin implants and supplied rams, ewes and farm facilities for conducting the research.

References


Cameron AWN, Tilbrook AJ, Lindsay DR, Fairnie IJ, Keogh EJ, 1987: The number of spermatozoa required by naturally mated ewes and the ability of rams to meet these requirements. Anim Reprod Sci 13, 91–104.


Lindsay DR, 1966: Modification of behavioural oestrus in the ewe by social and hormonal factors. Anim Behav 14, 73–83.


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Author’s address (for correspondence): Dr DO Kleemann, Turrefield Research Centre, South Australian Research and Development Institute, Holland Road, Roseadle, SA 5550, Australia.

E-mail: dave.kleemann@sa.gov.au

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