Optimum use of subcutaneous melatonin implants to enhance the reproductive performance of seasonal and non-seasonal sheep joined in spring and early summer

A.H. Williams\textsuperscript{a}, S.R. McPhee\textsuperscript{b}, J.L. Reeve\textsuperscript{c} and L.D. Staples\textsuperscript{b}

\textsuperscript{a}Victorian Institute of Animal Science, Werribee, Vic. 3030, Australia
\textsuperscript{b}Applied Biotechnologies Pty Ltd, PO Box 59, East Kew, Vic. 3102, Australia
\textsuperscript{c}Rutherglen Research Institute, Department of Agriculture, Rutherglen, Vic. 3685, Australia

ABSTRACT


This paper reviews data from five research trials and 108 clinical trials conducted in three countries to validate the optimum use of melatonin to advance seasonal breeding patterns of a variety of breeds of sheep.

In order to define the optimum time for treatment in breeding flocks, ewes of three different breeds were treated with controlled-release 18-mg melatonin implants (Regulin\textsuperscript{a}), with treatments commencing at various times ranging from 9 to 3 weeks prior to joining with fertile rams. Mating and conception patterns were recorded by regular observation for raddle marks and fecundity was assessed by mid-pregnancy ultrasonography. These research trials were conducted at a latitude of 38\textdegree S in Merino ewes of slight seasonality, in Merino-based cross-breeds of intermediate seasonality and in highly seasonal British breed Romney Marsh ewes. A single implant treatment, commencing at 30–40 days prior to spring or early summer joining, resulted in coincidence in timing of the peak of the inductive phase of the ovarian response to melatonin with the peak of oestrus and mating which arose from the pheromonal influence of the rams. Thus, this treatment strategy maximises the potential advantages to be obtained from both the melatonin treatment and the rams.

Subsequent clinical trial programmes in Australia, the UK and New Zealand confirmed this treatment strategy and tested the available periods of the year during which effective responses could be obtained under farm conditions. It was shown that, whereas Merino and Merino cross-breeds were responsive to melatonin treatment over a wide part of spring and early summer, the British breed ewes responded only during a relatively short period of the season around the time of the summer solstice. Treatments which commenced too early in British breeds were ineffective or gave negative results, possibly because of a failure of such breeds to be able to fully interpret the pharmacological short-day signal before they have experienced an adequate period of long-day priming. For all breeds,

Correspondence to: A.H. Williams, Victoria Institute of Animal Science, Werribee, Vic. 3030, Australia.
treatments given late in summer at a time of decreasing natural daylight were also ineffective since, by this time, ewes were already experiencing a naturally occurring inductive photoperiodic signal.

Overall, the studies reviewed in this paper showed that melatonin pretreatment of spring and early summer joined ewe flocks resulted in both a modest decrease in the number of barren ewes and an increase in the number of multiple births. The effects were seen in flocks of high or low basal performance and in both maiden and mature ewes, but in no case were the responses associated with the induction of supraphysiological litter size. Mean increases in litter size, for different breeds of ewe, ranged from 14.3 to 28.6 additional lambs born per 100 ewes treated, with variation between trials largely accounted for by expected sampling errors associated with the precise measurement of the discontinuous variable of litter size in relatively small sample sizes of 50–200 animals per group. Treatment of mothers with melatonin prior to joining had no effect on the sex ratio, birth weight or appearance of offspring.

Melatonin pretreatment of breeding ewes was also associated with an earlier mean conception (lambing) date and a compaction of the conception (lambing) patterns but the magnitude of these measured effects was dependent upon the extent to which untreated control flocks were responsive to the pheromonal influence of rams.

Overall the data extends, to a practical field level, the proper use of melatonin to at least partially overcome the seasonal constraint to breeding in sheep, and provides a sound basis for future research and clinical experimentation in this area.

INTRODUCTION

Obligate herbivores have evolved with an annual reproductive rhythm which is entrained to the annual pattern of day length. This has proven to be a successful strategy, firstly because the annual day-length pattern is a very stable terrestrial event, and secondly because day length is closely linked to the long-term seasonal pattern of feed supply. However, the precise timing of reproductive processes in relation to day length and the extent to which other climatic, physiological or social factors influence breeding patterns varies greatly between species and breeds. Species factors which determine optimum breeding times include the life expectancy of the animal, the length of gestation, and the rate of growth and onset of puberty in the offspring. Optimum breeding strategies are also linked to environmental events which might enhance or prejudice the feed supply or survival of the parents and of the offspring (Bronson, 1988).

In temperate climates the preferred birth period for herbivores like sheep, goats and deer, is during spring. This minimises the risk of exposure of newborn animals to the cold of mid-winter. Also, in spring, rising temperatures, adequate rainfall and increasing day length combine to permit the growth of the high-quality pasture which best meets the nutritional support for late pregnancy, for lactation and for weaning. Since the gestation period is about 5 months for sheep, the natural period for mating is in autumn.

With increasing distance from the equator and higher altitude, the spring pasture growth season is more pronounced but shorter, and winters are more severe. It is therefore generally true that the time of commencement of the
autumn breeding period is later and the duration of the breeding period is shorter, for those breeds which have evolved at polar latitudes and at high altitude, than for those which have evolved in the lowlands or nearer to the equator (Hafez, 1952; Williams, 1970; Lincoln, 1985). To achieve this timing, different species and breeds have evolved mechanisms to delay the time at which the neuroendocrine control centres become receptive and responsive to an inductive short-day signal (Reiter, 1991). Thus, although all breeds of sheep show a natural autumn peak of reproductive activity which is characterised by a peak in both the occurrence of oestrous cycles and in ovulation rate (Radford, 1959; King, 1976), there are major differences in the width of these peaks between breeds.

It is the change in day length from long to short days, and not the absolute day length, which triggers the transition from reproductive quiescence to seasonal activity (Robinson and Karsch, 1987). The endocrine transducer for the conversion of day-length signals into chemical information is the pineal gland. The pineal regulates the daily production of melatonin in proportion to the period of darkness (Klein et al., 1981; Bittman et al., 1983). The daily melatonin profile modulates the seasonal pattern of activity of the hypothalamic-pituitary-gonadal axis (Karsch et al., 1984) although the precise mechanism by which this modulation is achieved is still under study (Karsch et al., 1988; Reiter, 1991).

Since the melatonin profile determines the time of the transition to reproductive activity in sheep (Bittman et al., 1983), a number of research groups have recognised the potential for exogenous melatonin to be used to advance the onset of the normal seasonal breeding period (Nett and Niswender, 1981, 1982; Kennaway et al., 1982, 1987; Arendt et al., 1983; Williams, 1984; English et al., 1986; Poulton et al., 1986).

As might be expected, the extent to which the onset of breeding periods can be influenced by melatonin-based therapy varies greatly between sheep breeds (Staples et al., 1992). In addition a number of other factors such as lactation, nutrition, and social interactions such as the pheromonal influence of rams (Pearce and Oldham, 1984) may influence the response to melatonin under field conditions.

USE OF MELATONIN IMPLANTS UNDER FIELD CONDITIONS

The recent development of the sustained-release melatonin implant, Regulin®,a (Staples, 1989), has made possible the large-scale evaluation of the effects of melatonin under field conditions. In a companion review (Staples et al., 1992) we have shown that the continuous delivery of melatonin has the

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aRegulin is a Registered trademark of Hoechst Veterinaer GmbH, Munich, Germany. Regulin is also known as Melovine in some countries.
same effect as short days in stimulating the premature transition from reproductive quiescence to activity in sheep. It was also shown that, while the period of the melatonin-induced ovarian activity is shorter than the normal breeding season, it is nevertheless of a similar amplitude. Thus peak seasonal ovulation rates can be induced, by melatonin treatment, to occur several weeks or months earlier than the time of the normal peak in autumn.

In order to take the maximum advantage of the melatonin-induced advancement of the seasonal pattern of ovulation, it was necessary to optimise the timing of commencement of treatment, the dose and duration of treatment, and the time of introduction of the rams, so that conception occurred at the time of peak ovarian response to melatonin.

This paper reviews an extensive programme of field testing to measure the responses to continuous melatonin treatment applied in spring or summer and defines the time for effective treatment for various breeds of sheep. The data provides the basis for the recommendations for the safe and effective use of melatonin treatments under field conditions. The experimental work collated in this review commenced in 1984 at the Victorian Institute of Agricultural Science at Werribee. Subsequently certain preliminary reports have been released (Reeve et al., 1986, 1987; Staples et al., 1986a,b, 1990; Croker et al., 1987; McPhee et al., 1987). This paper provides a more detailed account of the developmental and clinical testing of the effects of the Regulin implant.

MELATONIN TREATMENTS

In the experiments described below, melatonin was administered by means of a membrane-coated subcutaneous implant (Regulin) which contained 18 mg melatonin. This implant was placed at a subcutaneous site near the base of the ear. The implant continuously released melatonin at a rate sufficient to maintain contralateral jugular venous daytime plasma melatonin levels in 40-60 kg sheep in the range of 300–900 pmol l\(^{-1}\) for a period of approximately 10 weeks following treatment (Staples et al., 1992).

The extent to which it is necessary to exactly mimic the night-time jugular venous levels of melatonin to achieve a 'short-day' effect has not been defined. However, it may be calculated that the gradual and continuous release of 18 mg of melatonin from the subcutaneous implant over approximately 10 weeks is equivalent to a daily treatment of approximately 250 \(\mu g\) day\(^{-1}\). Although the melatonin implant is administered at a peripheral site, and is thought to exert its effect within the brain via the blood circulation, we consider that the implant treatment is physiologically equivalent to the effects of an abrupt extension of the daily period of exposure to endogenous melatonin which follows a reduction in day length. This is supported by studies which have shown that the testicular response of Suffolk rams to an abrupt reduction in day length from 18L/6D to 9L/15D is both temporally and quantita-
tively identical to that observed following the administration of a Regulin melatonin implant to animals maintained on long days (Hanif and Williams, 1991).

OPTIMUM TIMING AND DURATION OF MELATONIN TREATMENTS

Four large field trials were conducted in spring–summer of 1985–1987, firstly to determine the optimum timing of the commencement of treatment in relation to the introduction of fertile rams, and secondly, to test whether it was necessary to continue melatonin treatment for extended periods. Three separate trials were conducted in pure-bred Merino, Border Leicester × Merino and in pure-bred Romney ewes to determine whether the optimum time of treatment varied between breeds of low, moderate and high seasonality, respectively. Trials were conducted on three farms near Melbourne Australia (latitude 38°S).

In the first trial, maiden (1.5-year-old) Merino ewes (N=819) were allocated at random to seven groups of 115–120 ewes. Ewes in Groups 1, 3 and 5 received a single implant so that a continuous treatment of approximately 10 weeks duration commenced at 63, 42 or 21 days (respectively) prior to the introduction of rams (joining). In this study ewes were joined to untreated fertile Merino rams (average three rams per group) on 8 November 1985. Two further treatment groups (Groups 2 and 4) also commenced treatment with a single implant at 63 days or 42 days, respectively, prior to ram introduction but received a second implant 4 weeks after the first, so that the total period of continuous exposure to exogenous melatonin in Groups 2 and 4 was approximately 14 weeks.

Since it was not possible to isolate all five treatment groups from each other, two untreated control groups were used to assess any impact of social facilitation within the trial. The first control group (Group 6) was grazed with Treatment Groups 1–5. The second control group (Group 7) was grazed at the same stocking rate on similar pasture but maintained in isolation from the main groups. The isolated control group (Group 7) was also joined to 3% fertile Merino rams on the same day as the main flock containing Groups 1–6. A summary of the treatment strategies tested in Trials 1, 2 and 3 is shown in Fig. 1.

Rams were fitted with harnesses and crayons which were changed to new colours fortnightly. Rams were left with the ewes for 8 weeks, during which time all ewes were observed at 2- to 3-day intervals for evidence of mating marks. Ewes which returned to service were also recorded. Liveweights were measured at 9 weeks before joining, at joining and at the time of removal of rams. Pregnancy status and fetal age were determined by mid-pregnancy ultrasonography with an accuracy validated in separate individually lambed flocks of 99.1% for non-pregnant, 94.4% for single lambs, 93.4% for twins and
Fig. 1. Schematic representation of the trial design to evaluate the effects of continuous melatonin treatments commencing at 9, 6 or 3 weeks prior to joining on the reproductive performance of Merino (Trial 1), Border Leicester × Merino (Trial 2) and Romney ewes (Trial 3) joined in spring or summer. The design allows for comparison of performance in treatment groups which received one implant (Groups 1, 3 and 5) or a sequence of two implants (Groups 2 and 4) with the performance of control (untreated) ewes run either with the treated ewes (control, Group 6) or in isolation from the treated ewes (separate control, Group 7). Arrows indicate the time of administration of implants in Groups 1–5 and the length of the treatment bar represents the approximate duration of treatment.

75.8% for triplets (S.R. McPhee, unpublished data, 1988). Statistical comparisons of differences in litter size distributions between treated and control groups was by $\chi^2$ analysis of quantitative contingency tables (Bhupkar, 1968).

The second trial was conducted with 944 parous (3- to 4-year-old) Border Leicester × Merino ewes. This breed shows intermediate seasonality between the Merino and British breeds. The experimental design for treatments and observations was the same as that described above for Merinos in Experiment 1, except that group sizes were 123–143 and the ewes were joined to untreated fertile Dorset (terminal sire) rams on 18 November 1985.

The third trial was conducted with 694 mature pure-bred Romney Marsh ewes in seven groups of 93–104 ewes. These ewes were joined to untreated Romney Marsh rams (3%) on 28 January 1986. Experimental design and observations for Trial 3 were similar to those in Trials 1 and 2.

**Trial 1 — Merino ewes joined 8 November 1985**

Analysis of mating and conception patterns showed that the majority of Merino ewes (Fig. 2a) were already experiencing spontaneous oestrous cycles at the time of ram introduction in early November. Thus, conceptions in all treatment and control groups were largely confined to a 20 day period following ram introduction. Although the mean conception dates did not differ
Fig. 2. Effect of melatonin treatments given at different times relative to joining in (a) Merino ewes joined on 8 November, (b) Border Leicester × Merino ewes joined on 18 November and (c) Romney Marsh ewes joined on 28 January in Trials 1, 2 and 3, respectively. Top panel shows day length (hours from sunrise to sunset) at 38°S. Points show the cumulative percentage of ewes which conceived by the end of each week after ram introduction. Symbols are as follows for all breeds: O, denotes 10 week treatment commencing 9 weeks before joining (Group 1); ●, denotes 14 week treatment given at 9 weeks before joining (Group 2); ▼, denotes 10 week treatment given 6 weeks before joining (Group 3); ▼, denotes 14 week treatment given at 6 weeks before joining (Group 4); △, denotes 10 week treatment commencing at 3 weeks before joining (Group 5); □, denotes within-flock control (Group 6); ■, denotes separate control (Group 7). Time axis shows days from previous winter solstice, calendar month and season.
**TABLE 1**

Effect of melatonin treatments commencing at different times prior to joining on the reproductive performance of Merino (Trial 1), Border Leicester × Merino (Trial 2) and Romney Marsh ewes (Trial 3), joined in spring or early summer. Refer to text for description of Treatments 1–7.

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<th>Breed Group</th>
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<th>Pregnancy diagnosis (%)</th>
<th>Foetuses per 100 ewes in group</th>
<th>Conception Mean date</th>
<th>Conception Mean days after joining</th>
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<td>7.8 86.1&lt;sup&gt;a,b&lt;/sup&gt; 6.1&lt;sup&gt;b&lt;/sup&gt; 0.0 98.3&lt;sup&gt;c&lt;/sup&gt; 1.066&lt;sup&gt;b&lt;/sup&gt;</td>
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<td><em>Border</em></td>
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<td>6/3 36.8&lt;sup&gt;a&lt;/sup&gt;</td>
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Note: a ≠ b ≠ c, etc. within columns and within trials, P<0.05.

greatly between groups (range 13–18 days post ram introduction; Table 1), significantly (P<0.05) more ewes conceived during the first 14 days of joining in Treatment Groups 2–5 than in either control group. This latter finding suggests that treatment with melatonin prior to joining has a slight effect in advancing oestrus in some ewes, even when the majority of the flock is cycling spontaneously at the time of ram introduction.

When compared with the isolated control group, litter size was increased (P<0.05) when treatments commenced at 42 or 21 days prior to joining, but not when treatment commenced at 63 days prior to joining. Optimum results in Merinos occurred in Treatments 4 and 5 in which 19.1 and 19.6 additional lambs, respectively, were observed per 100 ewes treated (Table 1). The increased lambing performance of Merino ewes resulted from a reduction of
the occurrence of dry (non-pregnant) ewes and by an increase in the occurrence of twin pregnancies, but was not associated with a concurrent induction of triplets (Table 1).

**Trial 2 — Border Leicester × Merino ewes joined 18 November 1985**

The moderately seasonal cross-bred ewes were not spontaneously cycling in mid-November so very few untreated ewes mated or conceived in the first 2 weeks after ram introduction (Fig. 2b). The majority of ewes in all treated groups responded to the ram effect so that the first overt oestrus (second ovulation) for most ewes occurred during the period from 20 to 28 days after ram introduction. More ewes \((P<0.05)\) mated in the first 14 days after ram introduction when the two-dose sequential treatment commenced 63 days prior to joining (Group 2) but otherwise the mating patterns were similar in all treated and control groups (Groups 1–6) which were grazed together as one mob. All groups in the main flock showed a more compact conception pattern than the isolated control group (Group 7) which responded poorly to the ram effect. The marked difference in mating pattern between the two control groups (Fig. 2b) (contrast Groups 6 and 7) is thought to illustrate the effect of social facilitation of oestrus between the treated and control ewes in the main mob. However, the data does not permit a distinction between possible ewe–ewe, ram–ewe or ewe–ram–ewe interactions. Mating patterns in the cross-bred ewes (Fig. 2b) also suggest that the early melatonin treatment in Group 2 induced some ewes to cycle prior to the introduction of rams as shown by the occurrence of early matings and a broader mating pattern, in Group 2 than in the other treated groups. The significance of these early matings for possible social facilitation between the experimental groups cannot be defined in these trials, but such effects may be important for the compaction of mating patterns in some treatment groups.

As for the previous trial in Merino ewes, melatonin treatment of cross-bred ewes which commenced at 21 or 42 days prior to joining resulted in 28.4\% (Group 4) and 25.7\% (Group 5) increases \((P<0.05)\) in number of lambs present per 100 ewes treated when compared with the untreated isolated control group (Table 1). The increase in expected lambing percentages in Groups 4 and 5 resulted from a reduction of dry ewes to less than half of control and from a trebelling of the occurrence of twin-bearing ewes, but did not involve the induction of triplets. Despite the good response to ram effect in the within-flock control (Group 6) there was no increase in lambing percentage without melatonin treatment. Also, those groups in which treatment commenced 63 days prior to ram introduction (Groups 1 and 2) showed significantly \((P<0.05)\) lower lambing response than the later treatment groups (Table 1). This could be explained either by a failure of ewes in this trial to fully respond to a short-day signal commencing 63 days before 18 November, or by a tran-
sient ovarian response followed by the commencement of a refractory state prior to rams having an opportunity to exert a stimulatory effect and to mate. Both possibilities are consistent with the known ovarian responses to melatonin (Staples et al., 1992).

**Trial 3 — Romney ewes joined 28 January 1986**

The mating and conception patterns for Romney ewes joined on 28 January (see Fig. 2c) were similar to the patterns seen for cross-bred ewes joined more than 2 months earlier (compare Figs. 2b and 2c). Treatments which commenced 63 days prior to joining induced some ewes to conceive within the first 2 weeks after ram introduction and rendered the remainder of these early-treated groups responsive to the ram effect. The treatments at 42 or 21 days prior to joining did not enable ewes to conceive within the first 2 weeks of joining but did result in a mating pattern characteristic of the ram effect with a peak of conception at 24–28 days. The mating patterns of treated ewes were more compact than those of the isolated control group (Fig. 2c).

The conception pattern for the within-flock control (Group 6) again showed the effects of social facilitation since this group was more ram responsive than the isolated control (Group 7). In fact the isolated controls showed a mating and conception pattern which was both late and gradual, with conceptions spread over 2 months; a characteristic which results in the typical industry reports of spread lambings in British breed ewes when joining is attempted in summer.

Both the proportion pregnant and litter size of Romney ewes were increased by treatment, particularly in Group 4 where treatment commencing at 42 days prior to joining resulted in an increase of 24.2 fetuses per 100 ewes treated ($P<0.05$) due both to a reduction in dry ewes and increased twinning (Table 1). Despite the effects of social facilitation on the conception pattern of the within-flock control (Group 6), the lambing percentage of this group was identical to that observed in the isolated control (Group 7). A single-implant treatment commencing 63 days prior to joining (Group 1) resulted in a significantly lower ($P<0.05$) lambing percentage than groups treated later (Table 1).

Overall these three trials showed that treatments which commenced between 21 and 42 days before joining (Groups 3, 4 and 5) were effective in increasing the litter size by 20–28 fetuses per 100 ewes joined and resulted in a compaction of the conception pattern compared with that observed in an isolated control group joined at the same time.

Treatments commencing at 63 days prior to ram introduction were too early to achieve an improvement in litter size, and may even be detrimental. Single-dose treatments commencing 42 days before joining (Group 3) were slightly less effective than a sequence of two doses commencing at this time.
MELATONIN ADVANCES SEASONAL BREEDING PATTERNS IN SHEEP

(Group 4) for all three breeds. However, this effect was only significant on one occasion (Romneys) and, overall, the data suggested that a single-implant treatment was associated with a good response provided that treatment commenced at approximately 3–6 weeks prior to introduction of rams.

Overall, the compaction of joining patterns achieved with the single-implant treatment commencing 21 days before joining (Group 5) is graphically illustrated for all three breeds in Fig. 3. The minimum number of days over which 65%, 80% or 95% of the flock conceived and the mean conception date (arrowed) for treated group were less and earlier than in the isolated control group.

Fig. 3. Compaction of joining patterns following ram introduction to (a) Merino, (b) Border Leicester × Merino and (c) Romney Marsh ewes which were treated with a single melatonin implant at 21 days prior to joining (top three bars), compared with joining patterns in isolated untreated control groups joined at the same time (bottom three bars). Data is taken from Groups 5 and 7 of Trials 1, 2 and 3. The horizontal bars show the minimum period over which 65, 80 or 95% of ewes conceived, and the location of the bar relates this minimum interval within the joining period of 8 weeks. The length of each period in days is indicated at the right hand end of each bar and the mean conception date for each group is shown by the arrow.
Comparison of the effects of subcutaneous implant treatments commencing at 3, 4, or 6 weeks prior to joining on the reproductive performance of Border Leicester × Merino ewes joined on 17 December 1987 (Trial 4)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total dose of melatonin (mg)</th>
<th>n</th>
<th>% of ewes bearing</th>
<th>Foetuses per 100 ewes</th>
<th>Lambs marked per 100 ewes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1: Control</td>
<td>0</td>
<td>87</td>
<td>9a</td>
<td>66a</td>
<td>25a</td>
</tr>
<tr>
<td>2: 3 weeks before joining</td>
<td>18</td>
<td>87</td>
<td>7a</td>
<td>62a</td>
<td>31a,b</td>
</tr>
<tr>
<td>3: 4 weeks before joining</td>
<td>18</td>
<td>87</td>
<td>3a</td>
<td>60a</td>
<td>38a,b</td>
</tr>
<tr>
<td>4: 6 and 2 weeks before joining</td>
<td>36</td>
<td>88</td>
<td>9a</td>
<td>50a</td>
<td>41b</td>
</tr>
</tbody>
</table>

a ≠ b within columns are significantly different by Chi-square on quantitative contingency tables (P<0.05).

(Group 7). Thus for all three breeds the conception pattern was both advanced and compacted by this treatment.

**Trial 4 — Merino×Border Leicester ewes joined 17 December 1987**

The efficacy of a single implant given at 4 weeks prior to joining was confirmed in a fourth experiment, conducted in Border Leicester × Merino ewes joined to 2% Dorset rams on 17 December 1987. In Trial 4, single-dose treatments which commenced at 3 (Group 2, n=87) or 4 weeks (Group 3, n=88) prior to joining were compared with a two-dose sequential treatment given at 6 and 2 weeks prior to joining (Group 4, n=88) and with untreated controls (Group 1, n=87). The double-dose sequential treatment did not result in significantly different fertility or fecundity than either of the single-dose treatments (Table 2).

**EFFECT OF MELATONIN TREATMENTS AT DIFFERENT TIMES OF THE YEAR**

While the above studies described an effective treatment for a given breed and at a particular time, it was not valid to assume that such treatments could be applied with equal efficacy at different times of the year. A further study (Trial 5) was therefore conducted in Border Leicester × Merino ewes at Rutherglen in Victoria (latitude 36°S) to assess the response to melatonin in mating flocks joined at different times throughout spring, summer and autumn. Trial 5 utilised a flock of 1016 mature parous ewes which was maintained in isolation from rams. At intervals of 6 weeks, commencing 12 weeks
before, and continuing until 12 weeks after the summer solstice, groups of 200–205 ewes were transferred from the main flock to a separate paddock where they were joined with 3% Dorset rams. Half of the ewes in each joining group were treated with a sequence of two melatonin implants given at 42 and 14 days prior to joining (see Fig. 4). Ewes were observed daily for raddle marks. At the end of a 6 week period with the rams, the mating flocks were progressively transferred to a third paddock where pregnancy was diagnosed by ultrasound at 10 weeks post joining. Just prior to expected parturition, treatment and control groups were separated again to allow accurate records of birth weights, sex ratios and incidence of abnormalities in lambs, lamb growth and weaning.

For flocks joined on 30 September, 11 November or 23 December, conception patterns in both treated and control groups were characterised by a ram-induced onset of oestrus with most matings observed at 14–28 days after ram introduction (Figs. 5a, 5b, 5c). Slightly more ewes mated during the first 2
Fig. 5. Effect of melatonin treatments in Border Leicester × Merino flocks joined at various times before, at, and after, the summer solstice. The top panels show prevailing hours of day length during the joining period for each group. The middle panels show cumulative percentage of ewes which had conceived at the end of each week after ram introduction. The bottom panel shows the cumulative number of foetuses present per 100 ewes joined related to the week of conception. In all panels, the treated group is shown as filled circles (●) while the control untreated group is shown as open circles (○). Figures 5a-5e show results for flocks joined on 30 September, 11 November, 23 December, 3 February and 10 March, respectively, in Trial 5.

weeks of joining in the treated groups, which indicated that treatment induced early ovulation in some ewes. Since the untreated ewes were in close contact with the treated ewes in each mating flock, it was possible that the successful induction of mating patterns by rams which was observed in the control ewes was partially due to effects of social facilitation. At the later joinings, on 3 February and 17 March, an increasing proportion of both treated and control groups were already cycling at the time of ram introduction, as indicated by the proportion of ewes which mated and conceived within the first 2 weeks of exposure to rams (Figs. 5d, 5e).

Treatments which commenced prior to the September, November or December joinings resulted in significant increases in fecundity with 19.6 ($P<0.001$), 26.1 ($P<0.001$) and 26.2 ($P<0.005$) additional foetuses pres-
TABLE 3

Effect of melatonin implants given at 6 and 2 weeks prior to joining on reproductive performance of Border Leicester × Merino ewes joined at various times of spring, summer and early autumn (Trial 5).

<table>
<thead>
<tr>
<th>Treatment date</th>
<th>Joining date</th>
<th>Group size</th>
<th>Allocation</th>
<th>No. of ewes bearing foetuses</th>
<th>Foetuses per ewe pregnant</th>
<th>Foetuses per 100 ewes</th>
<th>Change in number of foetuses per 100 ewes</th>
</tr>
</thead>
<tbody>
<tr>
<td>19/8/1985</td>
<td>30/9/1985</td>
<td>102</td>
<td>Control</td>
<td>18</td>
<td>70</td>
<td>14</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treated</td>
<td>17</td>
<td>52</td>
<td>33</td>
<td>1.39</td>
</tr>
<tr>
<td>30/9/1985</td>
<td>11/11/1985</td>
<td>102</td>
<td>Control</td>
<td>6</td>
<td>84</td>
<td>12</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treated</td>
<td>5</td>
<td>59</td>
<td>39</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treated</td>
<td>12</td>
<td>53</td>
<td>36</td>
<td>1.44</td>
</tr>
<tr>
<td>23/12/1985</td>
<td>3/2/1986</td>
<td>100</td>
<td>Control</td>
<td>18</td>
<td>46</td>
<td>36</td>
<td>1.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treated</td>
<td>13</td>
<td>39</td>
<td>48</td>
<td>1.58</td>
</tr>
<tr>
<td>3/2/1986</td>
<td>10/3/1986</td>
<td>102</td>
<td>Control</td>
<td>8</td>
<td>42</td>
<td>52</td>
<td>1.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treated</td>
<td>12</td>
<td>45</td>
<td>46</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n.s., not significant.
* Significant at $P<0.05$.
** Significant at $P<0.01$.
*** Significant at $P<0.005$.
**** Significant at $P<0.001$.

ent per 100 ewes joined respectively (Table 3). There was a progressive increase in the number of foetuses per 100 ewes joined in the untreated control flocks, from 96.1 for ewes joined in September to 143 for ewes joined in March. This was consistent with the expected seasonal increase in ovulation rate in autumn. Treatments given to the ewes joined in February resulted in a non-significant ($P>0.05$) increase of 16.7 foetuses while treatment of ewes to be joined in March resulted in a non-significant reduction of eight foetuses per 100 ewes joined (Table 3).

Thus, this trial showed that melatonin treatment of Border Leicester × Merino ewes at 36°S was effective over a wide period from early spring (September) to early summer (late December). The treatment was only slightly less effective in the flock joined in February since at this time the performance of the untreated ewes approached the natural seasonal peak. However, for ewes joined in mid-autumn at a time of natural decline in day length, melatonin treatment in February was ineffective.
Effect of maternal melatonin treatment on offspring

In vitro studies of the effects of melatonin, or the major metabolite, 6-hydroxy melatonin, on growth and replication of *Salmonella typhimurium* have shown that neither substance has mutagenic properties in this test system (Neville et al., 1989). Nevertheless, since some exogenous melatonin is expected to be present at around the time of ovulation, conception, and early organogenesis, it was of interest to determine whether the offspring of treated mothers were normal at birth.

Trial 5 was therefore extended to monitor the sex ratio, birth weights and incidence of abnormalities in the lambs born from cross-bred ewes treated at different times of the year. Pooled lamb data was obtained for 466 lambs born to 480 untreated mothers and from 491 lambs born to 476 of the ewes which had been treated with a total of 36 mg of melatonin prior to joining.

The ratio of male/female lambs born to untreated mothers was 1.026 and this did not differ significantly from the sex ratio of 1.063 in offspring from treated mothers (Table 4).

Birth weights of lambs were heavier for single lambs than twin lambs and for male lambs than female lambs in both groups, but there was no significant effect of maternal treatment on any birth-weight category (Table 4). Similarly, there was no effect of maternal treatment on the incidence of abnormalities in the lamb offspring since only two abnormal lambs were born from

<table>
<thead>
<tr>
<th>Maternal treatment</th>
<th>Number of ewes for which lambing data was obtained</th>
<th>Lambs born</th>
<th>M/F ratio</th>
<th>Mean birth weights (kg)</th>
<th>Incidence and type of abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>480</td>
<td>Male</td>
<td>236 (50.6)</td>
<td>5.00 4.28 (187) (49)</td>
<td>One hermaphrodite</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>230 (49.4)</td>
<td>4.88 3.87 (166) (64)</td>
<td>One with malformed front leg</td>
</tr>
<tr>
<td>Melatonin treatment given at 6 and at 2 weeks prior to joining (36 mg total dose)</td>
<td>476</td>
<td>Male</td>
<td>253 (51.5)</td>
<td>5.26 4.15 (157) (96)</td>
<td>One hermaphrodite</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>238 (48.5)</td>
<td>4.87 3.90 (150) (88)</td>
<td>One with malformed lower jaw</td>
</tr>
</tbody>
</table>

Sex distribution, birth weights and incidence of abnormalities in lamb offspring of Border Leicester × Merino ewes treated with two melatonin implants prior to joining. Data were pooled for ewes treated in August, September, November, December or February (Trial 5)
the untreated mothers (0.42%) and two from the treated mothers (0.41%) (Table 4). This incidence of abnormalities is normal. Both the male and female offspring of treated mothers grew normally, reached puberty and were fertile at the expected times (A.H. Williams, unpublished data, 1990).

This data is now supported by extensive clinical observations in several breeds of sheep, goats and deer where no effect of maternal treatment on the birth, growth or development of offspring has been reported.

RESULTS OF CLINICAL TRIALS IN DIFFERENT BREEDS AND AT DIFFERENT TIMES OF THE YEAR

The studies in the five experiments above, together with the related series of studies on the effects of melatonin on seasonal patterns of ovulation rate, have enabled the recommendation of a treatment strategy based on the administration of a single implant at 4–6 weeks prior to joining (Staples et al., 1992).

In order to confirm this proposed usage strategy under field conditions a clinical trial programme was established in flocks of sheep on farms throughout Australia, New Zealand and the UK. The data shown below includes data collected from field trials conducted from 1985 to 1988. The general format of each clinical trial in Australia and New Zealand was to treat a group of 100–200 ewes and compare lambing in these ewes with that observed in 100–200 untreated controls. Trials were conducted in post-pubertal maiden (1.5-year-old) and in mature animals. Ewes were allocated randomly to treatment and control groups.

Treated ewes in all Australian and New Zealand trials received a single melatonin implant, at a subcutaneous site near the base of one ear. The implant was given at 28–35 days prior to the introduction of 2–3% fertile rams. In some UK trials, comparison was made between a single dose and a double dose (36 mg of melatonin) in order to assess the dose dependence of effects in high fecundity breeds of high mature liveweight. Group size for UK trials usually ranged from 25 to 50 ewes, owing to the smaller flock size in the UK sheep industry. Treatments always commenced at 1 month prior to joining.

In all trials care was taken to keep ewes isolated from rams for at least 6 weeks prior to joining but it was not always possible to join treated and control groups in mutual isolation. It is possible that social facilitation, between treated and some control groups of the type observed in Trials 1–3 occurred in the clinical trials. Accordingly, results in control groups may have been somewhat better than might have been achieved in isolated joining groups. The results observed in many trials may therefore represent a conservative estimate of the true effects of treatment.

All ewes were joined for a 6 or 8 week joining period. Ewes underwent mid-pregnancy ultrasonography to determine pregnancy, fetal number and fetal
age (with a validated accuracy of \( \pm 5 \) days). In UK flocks, accurate records of lamb birth date and lamb survival were also obtained but this was not possible under extensive group lambing conditions in Australia and New Zealand.

Effect of treatment on foetal number or on lambs born

The results of each Australian and New Zealand trial were summarised by plotting the number of additional foetuses present per 100 ewes treated as a single histogram bar located on a date axis (within \( \pm 3 \) days for clarity) of the joining date for the trial (see Fig. 6, adapted from Staples et al., 1990). Similar data in respect of additional lambs born per 100 ewes joined for Suffolk (Fig. 7a) and for Mule-based breeds (Fig. 7b) is presented for the UK trials. Some of the UK trials have already been described in more detail by Dr. Will Haresign of Nottingham University (Haresign et al., 1990) but the present presentation includes additional data from trials supervised by Dr. Huw Williams of the Royal Veterinary College.

Single-implant treatments were effective in Merino and cross-bred ewes joined at any time between early October and mid-January in Australia and New Zealand (Figs. 6a, 6b, 6c). Within the joining times tested, there was no evidence of a consistent change in responsiveness with time of year in Merino or cross-bred flocks. For mature ewes in Australia and New Zealand the mean (\( \pm \)SEM) increases in foetal numbers per 100 animals treated were 17.8 \( \pm \) 2.6 for Merinos (25 trials) and 17.6 \( \pm \) 1.3 additional foetuses per 100 cross-bred ewes treated (20 trials) (Table 5). Overall, results were similar in maiden and mature animals (Table 5). The median (and range) of observed responses were an extra 14.7 (−2 to +62) for mature Merino ewes and an extra 17.2 (8–27) for mature cross-bred ewes.

On the basis of Trial 5 described previously, and the known ovarian responses to melatonin in Merino and cross-breeds (Staples et al., 1992), it is predicted that responses would be lower in these breeds if treatment was attempted in flocks joined earlier than October (Southern Hemisphere), when animals may not be receptive to a 'short-day' signal. Similarly response may be poor if Merino and cross-bred flocks are joined later than February, when these breeds may already be under the influence of the natural decline in day length during autumn.

The marked contrast to the broad period of effective treatment in Merino-based breeds, the British breed trials in Australia and New Zealand showed a restricted ‘window’ of effectiveness with positive responses obtained only in the Southern Hemisphere trial flocks treated in December and joined during January (Fig. 6d). Attempts to apply treatments to flocks joined earlier than December were either unsuccessful or resulted in significant depressions in reproductive performance. Also treatment of British breed ewes after mid-January for a joining in mid-February gave no response. These field trials
Fig. 6. Distribution of results for clinical trials conducted on farms in Australia and New Zealand during 1986–1989. Figure 6a shows data from maiden and mature Merino ewes (35 trials, 10,000 ewes); Figs. 6b and 6c show Merino cross-bred ewes in Australia and New Zealand (27 trials, 8169 ewes); Fig. 6d shows data for British breed ewes (25 trials, 5433 ewes). Each histogram presents the data from one trial involving 100–200 ewes per treatment group and is plotted on the calendar axis according to joining date (± 3 days for clarity). Histogram height indicates the difference in number of foetuses per 100 ewes in the treated groups in comparison with the control group for that trial.
highlighted the importance of using melatonin treatment at a time commensurate with the underlying photoperiodic control mechanisms which have evolved in that breed.

Data from British breed trials conducted in the Northern Hemisphere (UK) were broadly consistent with the above results. Responses were usually positive in Suffolk flocks joined in July (Fig. 7a) and for Mule flocks joined in July and August (Fig. 7b). Good responses were obtained despite the much higher basal fecundity of these UK breeds. It is notable, however that the
TABLE 5

Summary of the effect of melatonin implant treatment commencing 1 month prior to joining on the reproductive performance of various breeds of sheep. Data show the mean (±SEM), median and range of responses observed in all trials expressed as the difference between lambing percentages in the treated versus control flocks in each trial.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Location</th>
<th>Age</th>
<th>Dose¹</th>
<th>No. of trials</th>
<th>Mean increase ±SE²</th>
<th>Median increase</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merino</td>
<td>Australia</td>
<td>Maiden</td>
<td>1</td>
<td>10</td>
<td>17.5± 5.4</td>
<td>17.3</td>
<td>-4--59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mature</td>
<td>1</td>
<td>25</td>
<td>17.8± 2.6</td>
<td>14.7</td>
<td>-2--62</td>
</tr>
<tr>
<td>Cross-bred</td>
<td>Australia</td>
<td>Mature</td>
<td>1</td>
<td>20</td>
<td>17.6± 1.3</td>
<td>17.0</td>
<td>12--64</td>
</tr>
<tr>
<td></td>
<td>N.Z.</td>
<td></td>
<td>1</td>
<td>7</td>
<td>28.6± 6.7</td>
<td>17.2</td>
<td>8--27</td>
</tr>
<tr>
<td>British</td>
<td>Australia³</td>
<td>Mature</td>
<td>1</td>
<td>5</td>
<td>14.3± 4.8</td>
<td>20.7</td>
<td>3--24</td>
</tr>
<tr>
<td>breeds</td>
<td>N.Z.</td>
<td></td>
<td>1</td>
<td>8</td>
<td>21.0± 6.1</td>
<td>17.1</td>
<td>3--64</td>
</tr>
<tr>
<td>Suffolk</td>
<td>UK</td>
<td>Mature</td>
<td>1</td>
<td>8</td>
<td>15.8±10.4</td>
<td>0.8</td>
<td>-15--81</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>5</td>
<td>15.0±7.3</td>
<td>15.5</td>
<td>-13--34</td>
</tr>
<tr>
<td>Mule</td>
<td>UK</td>
<td>Mature</td>
<td>1</td>
<td>14</td>
<td>21.5±5.4</td>
<td>16.2</td>
<td>-11--65</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>6</td>
<td>24.7±7.8</td>
<td>25.4</td>
<td>-11--48</td>
</tr>
</tbody>
</table>

¹Dose consisted of a single 18 mg implant given 1 month before joining, or in some UK trials, a dose of two implants (36 mg) given together at 1 month before joining.
²Responses are shown as the increase in number of additional foetuses present (Australian and New Zealand trials) or lambs born (UK trials) per 100 treated ewes after subtraction of the equivalent figure for untreated ewes in the same flock. Data are pooled with trials as replicates and without weighting for differences in group size between trials.
³Only includes trials in mating period 10 January to mid-February, since treatment was shown to be ineffective for flocks joined earlier than this date (see text). Breeds include Romney, Border Leicester and Borderdale.

Magnitude of responses in the Mule and Suffolk flocks was generally more variable due to small group sizes than those obtained in the Southern Hemisphere trials (see Table 5) and that data was not available for the effect of treatments on ewes to be joined in May or June.

Effect of treatment on litter size distribution

In all clinical trials, the increases in lambing performance resulted from the combined effects of a reduction in the proportion of non-pregnant animals and an increase in the proportion of multiple births, regardless of breed or location of trials (Fig. 8). The relative contributions of each factor depended on the importance of each factor to the basal performance of the untreated flock. Thus, for example, in the Australian cross-bred flocks, the proportion of non-pregnant ewes averaged 10.7±1.3% (20 trials, range 0.5--25.3, median 9.7%). The proportion non-pregnant after treatment was reduced to a mean of 7.2±0.9% (range 2.1--16.7, median 6.3%). However this contribu-
Fig. 8. Effect of administration of a single melatonin implant at 1 month prior to joining (■) on the distribution of litter size compared with control untreated flocks (■■) under field conditions. Data show the mean (±SEM with trials as replicates) proportion of ewes non-pregnant (0), or bearing single (1), twin (2) or triplet (3) litters assessed by foetal ultrasonography (Australian and New Zealand trials) or by lambing observations (UK trials). Data is collated from only those British breed trials in Australia and New Zealand in which treatment was commenced at a time when ewes were responsive to a short-day signal (n = number of trials).

In contrast to flock performance was quantitatively outweighed by an increase in the occurrence of twins from a mean of 24.0 ± 2.5% in controls (range 8.6–50.6, median 24.6%) to 38.0 ± 2.3% in the treated flocks (range 24.5–66.7, median 37.2%). Thus, although the treatment resulted in a 32.7% reduction in the proportion non-pregnant and a 58.3% increase in the occurrence of twinning, these effects added 3.5 and 14.1 foetuses per 100 ewes treated, respectively, assuming that one extra lamb was obtained from each type of transition. Thus for the cross-bred ewes the major end benefit was derived from the increased fecundity.

In contrast, for the maiden Merino ewes, which under Australian conditions are known to have a high and variable occurrence of non-pregnant ewes, the effects of treatment on the reduction of non-pregnant ewes was more important. In ten trials conducted in maiden Merino ewes, melatonin treatment reduced the percentage non-pregnant from 17.1 ± 5.9% in control (range 3.3–71.9, median 12.1%) to 10.2 ± 2.4% after treatment (range 3.4–30.0, median 8.0%). Twinning was increased from 6.5 ± 1.5% (range 2.1–16.7, median 6.1%) to 17.2 ± 3.4% (range 2.0–35, median 17.0%). The 59.6% relative re-
duction in proportion non-pregnant contributed 6.9 extra foetuses per 100 ewes whereas the 264.6% relative increase in twinning contributed just 10.7 extra foetuses per 100 ewes treated.

Melatonin treatment had almost no effect on the occurrence of triplets in the low fecundity Merino or cross-bred ewes. This is a desirable outcome under Australian and New Zealand conditions where mortality of triplets under extensive lambing systems is very high.

The converse was true for Mule and Suffolk flocks in the UK (data pooled from 22 trials). In these trials, a single implant treatment reduced the proportion of ewes which did not rear a lamb from 15.8 ± 3.8% to 9.9 ± 1.8%, had little effect on the number bearing single or twin lambs, but doubled the occurrence of triplet-bearing ewes from 7.92 ± 1.75% to 15.93 ± 2.16%. Under UK conditions, where intensive lambing lowland flocks is common practice, the birth of triplets is not usually accompanied by large increases in lamb mortality, and so this effect on the occurrence of triplets is acceptable.

The data illustrate the importance of distinguishing between relative and absolute changes in relation to evaluation of benefits, and also highlight the nature of the changes brought about by the treatment. In general, it can be said that the treatment is of most value in decreasing the proportion of the flock which is non-pregnant where these are a problem, whereas treatment is most useful in modestly increasing litter size where multiples are tolerated. This is an ideal outcome from a practical viewpoint.

**Responses obtained in flocks of different basal performance**

In Australian and New Zealand Merino and cross-bred flocks joined in spring and early summer, the typical lambing percentage is 80–130 lambs born per 100 ewes joined. Acceptable responses, ranging from approximately 5 to 30 additional foetuses per 100 ewes treated were achieved across this range of normal performance (Fig. 9a). Higher responses of up to 60 extra lambs per 100 ewes treated were seen in poor performing flocks, even though the final performance in these treated groups was not extraordinary. In these latter flocks, the response to treatment was largely characterised by a reduction in the proportion of dry ewes following a 6–8 week joining period. This reduction may have resulted from an improvement in the proportion of ewes responding to ram effect and thus conceiving. The results suggest that melatonin treatment may be beneficial in limiting the risk of catastrophic failure of conception in flocks where few ewes respond to the rams and mate during a restricted joining period. Similar effects were observed in the British breed trials in Australia and New Zealand (Fig. 9b).

In contrast to the Australian and New Zealand cross-bred flocks, the basal performance in most of the UK Suffolk and Mule flocks was between 140 and 200 lambs born per 100 ewes joined (Fig. 9c). Responses to melatonin treat-
Fig. 9. Distribution of responses observed to single-implant melatonin treatments in relation to the basal performance of the flock. The number of additional foetuses per 100 ewes treated in Australian and New Zealand trials (a and b), or additional lambs born per 100 ewes treated for UK trials (c) is plotted against the basal performance (lambing percentage) of the untreated flock. Data is pooled from Australian and New Zealand Merino and cross-bred ewe flocks joined at any time from October to February, from Australian and New Zealand British breed flocks joined in mid-January to mid-February only and from UK Mule and Suffolk ewe flocks joined in July and August.

Response as additional lambs per 100 ewes treated

Response as additional foetuses per 100 ewes treated

Lambing Percent of Control Flock

Fig. 9. Distribution of responses observed to single-implant melatonin treatments in relation to the basal performance of the flock. The number of additional foetuses per 100 ewes treated in Australian and New Zealand trials (a and b), or additional lambs born per 100 ewes treated for UK trials (c) is plotted against the basal performance (lambing percentage) of the untreated flock. Data is pooled from Australian and New Zealand Merino and cross-bred ewe flocks joined at any time from October to February, from Australian and New Zealand British breed flocks joined in mid-January to mid-February only and from UK Mule and Suffolk ewe flocks joined in July and August.

ment, although variable due to small sample size, ranged from approximately 15 less to 30 more lambs born per 100 ewes treated, for flocks with basal performance in the normal range (Fig. 9c). Responses were generally higher, (ranging from 20 to 80 extra lambs) for those UK flocks in which control performance was below 120 lambs per 100 ewes joined (Fig. 9c) and this was particularly evident if the joining period was restricted (Haresign et al., 1990).

Taken together, these field results show that melatonin treatment offered adequate benefits in flocks of average performance and gave substantial benefits in poor performing flocks which were encountered during the trial programme. However, it must be stressed that this does not mean that melatonin treatment can be used effectively in all poor performing flocks, where infertility or poor fecundity may arise from causes other than seasonal factors.
An assessment of the range of response is of interest in an evaluation of clinical efficacy, as this defines both the typical and extreme responses which may be expected in the field. Since the research literature seldom provides for comparisons of effects in large numbers of related trials, it is frequently difficult for research groups to compare responses obtained in individual studies with the general expectations, and previous experimental experience for a particular treatment strategy. The present series of related trials therefore provides a rare opportunity to evaluate the likely consequences of melatonin implant treatments in the field.

The frequency distribution of observed response for Merino and cross-bred trials conducted in Australia and New Zealand (Fig. 10a) showed that responses were mostly grouped around an overall mean of 18.9 additional lambs born per 100 ewes treated (overall 62 trials in both maiden and mature ewes, mean response with trials as replicates unweighted for animal numbers). Higher responses were associated with significant reductions in dry ewes in those flocks which had low pregnancy rates as a result of a poor response to ram effect (as discussed above). The distribution of results for British Breed trials in Australia and New Zealand was similar to that observed for the Merino-based breeds provided that data was only included for those trials in which treatment was given in December for a January joining (Fig. 10b). Results from UK trials in Mule and Suffolk ewes were distributed around a mean of approximately 18 extra lambs born per 100 ewes treated (Fig. 10c).

For comparative purposes Fig. 10d shows the theoretical distribution of expected trial outcomes for 200 mock trials when the 'true' effect of 'treatment' was 20 additional lambs per 100 ewes treated. These theoretical distributions were generated by random selection of 'treated' and 'control' animals from two data bases, each containing 100,000 records. The change from a mock performance level of 100 lambs born per 100 ewes joined in control to 120 lambs per 100 ewes in the mock population of treated ewes was achieved by reducing the proportion of non-pregnant from 10 to 5% and increasing twinning from 10 to 25% after treatment. Mock 'trials' were then conducted by comparing lambing percentages in random samples of 50, 100 or 200 animals selected from the 'treated' and 'control' data bases.

Reducing group size from 200 to 50 had a dramatic effect on the observed frequency distribution of responses for the 200 mock trials (Fig. 10d). The spread of mock trial outcomes was similar to the frequency distributions achieved in the real trial programmes, and demonstrated the need for caution in the interpretation of data from individual trials, especially where group size is small and where sampling error can contribute to the measured trial outcome (Brown, 1988).
Fig. 10. Frequency distribution of responses observed in (a) Merino and cross-bred ewe trials in Australia and New Zealand, (b) British breed trials in Australia and New Zealand and (c) Mule and Suffolk trials conducted in the UK. The responses were pooled into five-lamb intervals (0–4, 5–9, 10–14, etc.). For comparative purposes Fig. 10d shows the frequency distributions for the results of 200 computer generated ‘mock’ trials for samples of 50, 100 or 200 treated and control ewes taken at random from 100 000 record data bases with ‘control’ performance of 100 (10% dry, 80% single and 10% twin) and ‘treated’ performance of 120 lambs per ewe joined (5% dry, 70% single and 25% twins). The figure demonstrates that the distribution of observed outcomes is at least partially attributable to the difficulty of accurately determining changes in a discontinuous variable such as litter size in individual trials when experimental samples are small.

Response in relation to dose and liveweight

Most studies reported herein, used a treatment dose of one Regulin implant. However, in Trials 1–5 a sequential dose of two implants given 4 weeks apart, was also consistently shown to be effective. Most of the sheep trials in Australia and New Zealand involved animals whose liveweight was in the range of 40–60 kg. However, in the UK trials Suffolk and Mule ewes fre-
quently weighed more than 70 kg. In order to evaluate whether dose rates needed to be increased for the heavier UK breeds an assessment was made of the effects of a double-dose (two-implant) treatment in those UK trials where flock size was sufficient to permit a dose–response comparison. The results showed no consistent advantage of the double-dose treatment when results of all UK trials were compared, or when paired comparisons were made between dose levels for just those trials where both dose levels were tested (Table 5) (see also Haresign et al., 1990). However, the median response was higher for the double-dose groups (+15.5 for Suffolk and +25.4 for Mule) than in the single-dose groups (+0.8 for Suffolk, +16.2 for Mule). This may suggest greater reliability of response for the higher dose groups. Data from Australian trials has also been analysed after classification of treated and control groups into 5-kg liveweight categories. The results indicated a consistent response to treatment across all liveweights in the 40–60 kg range despite the general trend for higher lambing percentages as liveweight increased (data not shown).

The observations are consistent with the ovulation rate studies (Staples et al., 1992) in which the inductive phase ‘short-day’ response in Romney ewes was largely independent of dose when dose rates of one or four implants were compared. The data suggest that provided a critical threshold level of melatonin is reached, the continuous melatonin treatment acts as a ‘switch’ on the other neuroendocrine mechanisms to drive the transition to autumn reproductive activity. Provided the ‘switch’ is held on for a period exceeding 40 days, and possibly for 70 days (Staples et al., 1992), an inductive short-day response will be induced. For ewes which are responsive, there appears to be no advantage in ‘pushing harder on the switch’ by increasing the dose rate of melatonin above the response threshold. However, while it appears that a single implant releases sufficient melatonin to exceed the threshold level for response in most animals the use of higher doses may increase the population of animals which respond.

It should be stressed that much of the available data is limited to animals of less than 60 kg liveweight and that further work is needed to clarify whether there may be a relationship between threshold dose and liveweight in larger breeds, such as some British and Middle Eastern animals and in large rams.

Effect of treatment on mating patterns

The use of melatonin pretreatment in conjunction with ram effect in Merino and Merino cross-bred flocks resulted in some advancement and tightening of the mating pattern (e.g. see Figs. 2 and 3). In most cases, both the treated and control groups responded typically to ‘ram effect’ with a peak of conceptions occurring at about 3 weeks after ram introduction. Under these
circumstances the effect of treatment in advancing or compacting the lambing pattern is small, since the lambing pattern is primarily a consequence of the oestrous pattern which results from the pheromonal influence of the rams. Nevertheless, it was a frequent observation of flock masters and owners that the lamb drop from treated flocks 'was compacted' compared with that seen in untreated ewes.

In British breed flocks the effect of treatment on mating patterns was more variable. Some flocks showed a response to ram effect in both treated and control animals and therefore only a small advancement in mean conception date and compaction of the lambing pattern could be achieved with treatment. However, in those flocks in which control groups responded poorly to ram effect, the treatment resulted in a significant advancement of mean mating date. This was particularly evident in UK flocks in which rams were removed after a joining period of just 6 weeks. In this circumstance, those ewes which did not commence cycling early in control flocks were deprived of an opportunity to mate, and the melatonin pretreatment increased the proportion mated from 56–68% to 90–93% following treatment (Haresign et al., 1990).

DISCUSSION AND CONCLUSIONS

Following the demonstration that continuous release of melatonin from subcutaneous silastic sachets mimicked the effects of short days in depressing prolactin levels (Kennaway et al., 1982), a number of laboratory studies confirmed that subcutaneous or intravaginal continuous melatonin treatment, could advance the occurrence of spontaneous oestrus in ewes (Arendt et al., 1983; Hanrahan et al., 1983; Nowak and Rodway, 1985) and of testicular activity in rams (Lincoln and Ebling, 1985). Preliminary trials using subcutaneous silastic sachets, in which treatments commenced at different times, showed that Mule and Suffolk cross ewes were not uniformly responsive to melatonin at all times of the year. A period of long-day exposure was necessary to 'prime' the circannual rhythm of the neuroendocrine axis to a state of long-day refractoriness and/or short-day receptivity (Arendt et al., 1983; Nowak and Rodway, 1985). However, in order to avoid the possible role of pheromones in the commencement of breeding patterns, rams were excluded from the above experiments and oestrous activity was determined by analysis of serial blood samples for progesterone. As a result, previous studies were not able to empirically test various strategies in the practical situation, when rams must be introduced to the flock for natural mating.

Since the introduction of rams is frequently associated with a pheromonal effect on the production of gonadotrophin releasing hormone and thus on ovulation, it was important to take into account the effects of both pheromones and melatonin on the dynamic pattern of ovarian function in order to
optimise practical treatment strategies for melatonin in sheep. In a companion review (Staples et al., 1992) we have defined the ovarian response of different breeds to continuous melatonin given prior to the start of the normal breeding season. From this data it was predicted that the optimum treatment strategy required administration of a melatonin implant at 30-40 days prior to joining (Staples et al., 1992).

The present studies were designed to validate this treatment strategy under field conditions. The results of trials reported in this review demonstrate that melatonin may be used in conjunction with ram effect in sheep, to obtain the dual benefits of a tighter and slightly earlier mating pattern in response to rams and also an improvement in fecundity.

The trials have further shown that the correct timing of the commencement of melatonin treatment, both in terms of the stage of season for a particular breed and in terms of the timing of introduction of rams, is critical for successful use of melatonin in early joining flocks.

The duration of treatment achieved with a single Regulin implant is about 70 days (Staples et al., 1992) and this has been shown to be effective in all breeds tested so far, provided that treatment commences at a time of receptivity to short days. This is consistent with the work of Rodway et al. (1985) who showed that British breed ewes treated on 4 July (Northern Hemisphere) failed to respond to subcutaneous silastic sachets if they are removed after 16 or 36 days but that spontaneous oestrus was advanced if treatment continued for 93 days. In separate studies in Merino ewes we have also found that treatments which commence at a time of short-day receptivity in October (Southern Hemisphere) do not elicit an ovarian response if discontinued after 20-40 days (Staples et al., 1992). We conclude that a continuous treatment exceeding 40 days duration is necessary for a full response although there is obviously some latitude between trials since the present data (e.g. Group 5 in Trial 1 above) show that fecundity responses can be observed in Merinos when the mean conception date was just 36 days from the start of treatment.

If the melatonin treatment is given prior to the time of receptivity to short days then either no signal will be interpreted or only the later part of the treatment will be recognised as the animals reach a state of short-day receptivity. If only the later part of the melatonin treatment is interpreted and the treatment is of a defined duration, it is possible that the perceived length of exposure is inadequate to obtain a full inductive response.

This could result in either no response or in suppression of reproductive activity and such mechanisms would explain the poor or negative responses observed in the Southern Hemisphere British breed trials where British breed ewes were treated in November for an attempted joining in December (Fig. 6d). Clearly these studies demonstrate that the available 'window' for effective treatment is much longer for the less-seasonal Merino or Merino-based cross-breed than for the more seasonal British breeds.
With respect to the timing of melatonin treatment in relation to the time of introduction of rams, the results of Trials 1, 2, 3 and 4 confirmed that it is necessary to commence treatment at 30–40 days prior to ram introduction. Earlier treatments are either ineffective because they start prior to the time at which the ewe becomes receptive to treatment (as discussed above) or because the inductive response is short lived and followed by an early and transient return to reproductive quiescence (or 'melatonin refractoriness', see Staples et al., 1992). In the latter case the majority of conceptions may occur after the peak ovarian response to the inductive melatonin signal. Thus the rams are introduced too late to capitalise on the early peak in ovulation rate.

Observations of mating patterns in flocks which are maintained in the continuous presence of rams show that melatonin treatment alone will advance the onset of first oestrus but does not synchronise first oestrus (Staples et al., 1992). The present data, obtained in trials in which rams are introduced after a period of absence, suggest that the melatonin pretreatment also brings forward the time of the year at which the ewes become responsive to ram effect. However, the present studies showed that, while melatonin treatment advanced and tightened the conception pattern in all breeds, the effects were only slight when control flocks were already responsive to the ram effect. Thus the tighter lambing pattern is primarily a consequence of the slightly improved response to the rams and not a direct consequence of melatonin treatment per se. This is perhaps surprising since the administration of implants would be expected to yield an abrupt short-day cue on the same day to all animals. The fact that not all animals show first oestrus on the same day following such an abrupt photoperiodic cue may arise if not all animals are receptive to the cue at the same time, or if the lag phase between photoperiodic cue and ovarian response differs between animals. Either mechanism would ensure that precise synchrony of breeding is avoided in the wild, and this may have evolutionary significance.

Since the rams were not treated with melatonin in the present trial programme, the question arises as to whether mating patterns in Merino, cross-bred and British breeds could be further improved by concurrent treatment of males. Such a treatment of rams for early season joining could be advantageous since preliminary data from New Zealand (P. Muir, personal communication, 1988) has shown that Romney and Poll Dorset rams treated with melatonin implants on 1 November showed substantially improved mating performance when joined to British breed ewes in January. This aspect of melatonin usage requires more study.

In addition to the data reviewed in this paper, a number of clinical and research trials which used the Regulin melatonin implant as the melatonin delivery vehicle, have now confirmed the efficacy of melatonin treatments to enhance the reproductive performance of sheep in a variety of situations. Melatonin implants can be used in both high and low fecundity breeds (Moore
and Miller, 1988) and melatonin implants have been shown to act synergistically with immunisation against androstenedione to boost prolificacy to supraphysiological levels (Dunstan et al., 1988). The same type of implants have also been shown to advance the seasonal pattern of testes growth, androgen production, pelage changes and rutting behaviour in stags and conception in hinds in both fallow (Asher et al., 1988) and red deer (Wilson et al., 1988) which are joined early in the season. Related studies in mink (Hulsebos and L.D. Staples, unpublished data, 1989) and fitch (Pearson et al., 1989) have also shown that continuous melatonin treatments achieved by means of a subcutaneous biocompatible implant can advance the time of occurrence of winter pelt in these fur-producing animals. However, a different delivery profile is needed in mink to avoid the risk of induction of premature shedding of winter pelts.

Thus it appears that continuous melatonin therapy has the potential to be used to overcome seasonal constraints in the production of both offspring and animal products in a number of species. Since melatonin treatment is the first pharmaceutical strategy to be effective in manipulating seasonal processes in both males and females, it has the potential to make a substantial contribution to enhanced agricultural production. However, before the technology is applied to new usage situations, new breeds, and new species, we stress the need to thoroughly define the optimum parameters for dose rate, duration of dose and timing of dose so that effects on the desired production trait are optimised. Since the melatonin treatment will potentially affect all seasonal events we also stress the need to fully evaluate potential side-effects on appetite, milk production and pelage to ensure that the positive effects of treatment on one trait are not counterbalanced by a negative effect on another.

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