Effects of melatonin implants on mating performance of Romney and Poll Dorset rams

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ABSTRACT

The effect of melatonin (Regulin®) implants on the early season performance of Romney and Poll Dorset rams was investigated in experiments carried out over three consecutive years. In each experiment, treated and untreated rams were introduced to groups of 100 or more ewes which had been synchronised with CIDR's. Ram to ewe ratios ranged from 3.5% to 5.5%. In Experiment 1, melatonin treatment of Poll Dorset and Romney rams prior to a January mating significantly improved ram performance measured as the proportion of ewes mated ($P < 0.0001$) but not the proportion of ewes conceiving. In Experiment 2, untreated Romney rams were already active at a January mating and melatonin treatment was found to have no effect. In Experiment 3, there was a significant interaction between ram breed and melatonin treatment in December but not in January or February. Moreover, at the December mating, the more active ram groups not only mated more ewes which were in oestrus, but also stimulated significantly more CIDR treated ewes to ovulate. Since more active ram groups stimulated more ewes to ovulate the results may explain the variation in results obtained when CIDR's are used at an early season mating.

Keywords: Rams, Poll Dorset, Romney, Regulin, melatonin.

INTRODUCTION

In New Zealand, as well as in many other countries, it is necessary to join ewes at times earlier than the natural peak of reproductive activity in autumn. This early joining extends the pattern of lamb supply and, in some locations, enables lambs to be slaughtered before the onset of summer drought conditions.

The onset of the annual breeding season in the ewe is entrained by exposure to long days (Woodfill et al. 1991) and hormonally mediated by melatonin which is released from the pineal gland during darkness (Karsch *et al.*, 1984). Melatonin implants can over-ride the natural photoperiod (Arendt *et al.*, 1983) and a single controlled release dose has been used to successfully advance the seasonal pattern of ovulation in ewes (Staples *et al.*, 1986; Staples *et al.*, 1992). Melatonin can also alter the hormonal and physiological characteristics of fertility in rams (Lincoln and Ebling, 1985). Following the development of a subcutaneous implant as a practical controlled delivery dose form of melatonin (Regulin®; Staples 1989; Staples *et al.*, 1991; Staples *et al.*, 1992) melatonin treatment has been proven to improve reproductive performance of ewes in extensive field studies in Australia (Williams *et al.*, 1992), New Zealand (Knight *et al.*, 1992), UK (Haresign *et al.*, 1990) and France (Chemineau *et al.*, 1991).

Most ram breeds also exhibit seasonal breeding patterns and early matings have therefore traditionally relied on the use of rams with an extended breeding season. No previous studies have examined whether a further advantage may be obtained from the concurrent treatment of rams with melatonin. These experiments aimed to compare the effects of "Regulin" on the libido and fertility of Romney and Poll Dorset rams. These breeds were chosen as they were considered to be representative, respectively, of the most and least seasonal examples of New Zealand sheep breeds.

MATERIALS AND METHODS

Three experiments were carried out in Hawkes Bay, New Zealand, over the summers of 1987-90. Experiments 1 and 2 were conducted with the same flock of ewes at Crownthorpe and Experiment 3 at the Poukawa Research Farm. The Romney and Poll Dorset rams used were all mature (aged 5 years or greater) and each breed originated from the same stud flock. Rams were allocated to treatment groups on the basis of liveweight and testis size such that each treatment group was balanced with rams of the same average liveweight and testis diameter. Implants, each containing 18mg melatonin (Regulin Ltd, Melbourne, Australia now marketed by HOECHST) with a delivery phase of approximately 10 weeks (Staples *et al.*, 1991) were subcutaneously implanted at the base of the ear. Rams were fitted with harnesses and crayons. In order to give the maximum opportunity to measure treatment effects, oestrus was synchronised in ewes using a 10 - 14 day CIDR® treatment (Type S, Alex Harvey Industries) and rams were introduced to ewe groups at ram to ewe ratio's of 3.5% to 5.5%, somewhat lower than the typical figure of 10% rams usually used for early season mating (Knight *et al.*, 1989). Results were analysed by fitting a linear model to the logits using SAS. Where a significant breed x treatment interaction existed, treatment effects within a breed have been compared using χ^2 analyses.

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Experiment 1 (1987-88)

This study assessed the effects of melatonin treatment on ram performance at a January mating. Twelve Poll Dorset and 12 Romney rams were each allocated to two treatment groups and six rams of each breed treated with a single melatonin (Regulin) implant on the 1 November 1987. Eight hundred and eighty mature parous Romney and Border Leicester x Romney ewes (440/breed) were allocated to four treatment groups on the 5 January and 100 ewes weighed to determine average liveweights. CIDR's were inserted into half of the ewes (110) in each group and removed on the 19 January. On the day of CIDR withdrawal (80 days after ram treatment) each group of 6 rams was joined with a group of 220 ewes. Raddle marks were recorded 7 days after CIDR removal (1st cycle). Ram crayons were changed and raddle marks again recorded 17 days later (2nd cycle) so that ram performance could be measured over the first two induced cycles. Pregnancy was diagnosed (April 22) by ultrasonography in all ewes which mated over the first two cycles.

Experiment 2 (1988-89)

This experiment was conducted to assess the effects of timing and duration of melatonin treatment on ram performance. Fifteen Romney rams were allocated to three treatment groups of five rams in late September 1988. Group 1 rams were implanted with a total dose of 36 mg melatonin given in two sequential doses of 1 implant each on the 5 October and the 16 November. Group 2 rams received a single implant (18 mg) on the 16 November and rams in group 3 were left as untreated controls. On the 10 January CIDR's were inserted into 360 mixed age Romney ewes (mean liveweight, 51 kg). CIDR's were removed on the 20 January and harnessed rams introduced to the respective groups, 106, 65 and 0 days after start of melatonin treatment. Raddle marks were recorded after 1 week and ram crayons changed. Raddle marks were recorded after a further 17 days.

Experiment 3 (1989-90)

To assess the effects of melatonin treatment on ram performance in December, January and February, 10 Poll Dorset rams and 10 Romney rams were allocated to groups of 5 treated and 5 control rams of each breed. All rams in the treated group received a total dose of 36 mg melatonin as two implants, given on the 1 November and 27 November. Total duration of treatment was therefore approximately 14 weeks. On the 11 December (40 days after start of melatonin treatment), each group of harnessed rams was joined for 7 days with 140 Romney ewes which had previously been synchronised and progesterone primed by means of 10 days of CIDR treatment. Individual rams in each group were allocated different coloured crayons to identify variation in libido between rams. Eight days after CIDR removal, tup marks were recorded. Two days later unmarked ewes were laparascoped (it was assumed that all marked ewes had ovulated) and ovulations recorded. This procedure was repeated on further groups of ewes on the 12 January and the 14 February, although ewes were not laparascoped after the February mating.

RESULTS

Experiment 1

Ewes averaged 55.6 kg at mating. Only CIDR treated ewes were marked during the 7 days following CIDR removal and melatonin treatment of rams of both breeds had a marked effect on mating performance at the first synchronised oestrus following CIDR removal (Table 1). There was a significant effect of melatonin on number of ewes mated ($P < 0.001$) but not on conception rate (Table 1). There was a significant breed effect in terms of both ewes mated ($P < 0.02$) and ewes conceiving ($P < 0.001$).

TABLE 1: Effect of melatonin treatment on the early season mating performance (January) of Poll Dorset and Romney rams (Expt 1)

a) First Cycle (matings recorded in CIDR sychronised ewes within 7 days of CIDRwithdrawal)

Breed	Ram Treatment	Ewes mated Ewes treated (+ CIDR)		Ewes conceiving Ewes mated (+ CIDR)	
Poll Dorset	Melatonin	77/105	(73%)	58/77	(75%)
	Control	51/104	(49%)	39/51	(76%)
Romney	Melatonin	68/104	(65%)	39/59	(66%)
	Control	36/105	(34%)	17/36	(47%)
Significance					
Poll Dorset v Romney		0.02		<0.001	
Melatonin v Control		<0.001		0.24	
Interaction		0.57		0.16	

b) Second Cycle (matings recorded in untreated ewes within 7-24 days of CIDR withdrawal)

Breed	Ram Treatment	Ewes mated Ewes treated (- CIDR)		Ewes conceiving Ewes mated (- CIDR)	
Poll Dorset	Melatonin	95/108	(88%)	86/95	(91%)
	Control	93/108	(85%)	82/93	(88%)
Romney	Melatonin	93/108	(86%)	82/93	(88%)
	Control	85/108	(79%)	66/85	(78%)
Significance					
Poll Dorset v Romney		0.21		0.11	
Melatonin v Control		0.21		0.11	
Interaction		0.52		0.41	

Ram performance generally improved in all groups during the second cycle although there was no effect of ram breed or melatonin treatment on mating performance or conception rate.

Experiment 2

Ewes had a mean liveweight of 51.0 kg at mating. There were no significant differences between groups of rams in terms of ewes mated (range 72% - 75%) or ewes conceiving (range 60% - 72%; Table 2).

TABLE 2: Effect of 0, 65 and 102 days of melatonin treatment on performance of Romney rams at a January mating (Expt 2)

Treatment	Ewes mated Ewes treated	Ewes conceiving Ewes mated
0 Days Melatonin	89/118 (75%)	60/89 (67%)
65 Days Melatonin	86/118 (73%)	62/86 (72%)
102 Days Melatonin	86/119 (72%)	52/86 (60%)

Experiment 3

Romney and Poll Dorset rams had mean weights of 86.3 and 81.0 kg, respectively, at the December mating. Ewes weighed 55.0 kg on the 1 December and maintained this weight throughout the experiment. At the December mating there was a significant interaction ($P < 0.001$) between ram breed and melatonin treatment (Table 3). Treatment of Romney rams with melatonin gave a marked positive response, with untreated rams marking an average of only 3.6 ewes and treated rams marking an average of 37.6 ewes each at the December mating. In contrast, within the Poll Dorset rams treated with melatonin, two of the five rams performed poorly, the net result being that treated rams marked an average of 17.8 ewes and untreated rams marked an average of 43.2 ewes (Table 4). As a result melatonin treated Romney rams mated 53% of ewes compared with 11% by untreated Romney rams ($\chi^2 = 55.90$, $P < 0.001$). Conception rates also differed significantly between treated and untreated Romney rams, being 60% and 33%, respectively ($\chi^2 = 22.3$, $P < 0.001$). On the other hand, treated Poll Dorset rams mated only 37% of ewes compared with 65% by the untreated rams ($\chi^2 = 22.53$, $P < 0.001$) but there was no difference in conception rate.

TABLE 4: Effect of melatonin treatment on December, January and February mating performance of individual Poll Dorset and Romney rams (Expt. 3)

Group	Ram ID	Numbers of ewes mated Dec	Jan	Feb
Romney (control)	377	2	25	29
	378	4	52	3
	379	5	59	33
	376	3	46	40
	382	4	46	52
		3.6 ± 1.1	45.6 ± 12.1	31.4 ± 18.1
Romney (melatonin treated)	353	30	56	26
	389	45	54	33
	363	24	50	34
	356	53	23	2
	381	36	39	29
		37.6 ± 11.6	44.4 ± 13.6	24.8 ± 13.1
Poll Dorset (control)	259	59	46	30
	257	20	12	42
	385	38	41	24
	391	47	35	19
	393	52	32	40
		43.2 ± 15.1	33.2 ± 13.0	31.0 ± 9.9
Poll Dorset (melatonin treated)	392	0	7	2
	395	18	48	35
	368	31	59	45
	384	7	16	22
	387	33	57	19
		17.8 ± 14.5	37.4 ± 24.2	24.6 ± 16.4

A relationship was observed between ram activity and number of ewes ovulating at the December mating (Table 3) with the more active ram groups stimulating more ewes to ovulate. There was no significant effect of melatonin treat-

TABLE 3: Effect of melatonin treatment on mating performance of Poll Dorset and Romney rams joined to CIDR synchronised Romney ewes in December, January and February (Expt. 3)

Date of joining and days after start of melatonin treatment of rams	Breed	Treatment group	Ewes mated/ Ewes treated	%	Ewes ovulating/ Ewes treated	%	Ewes mated/ Ewes ovulating	%	Ewes conceiving/ Ewes mated	%
11 December (40 days)	Poll Dorset	Melatonin	51/513	(37)	81/138	(59)	51/81	(63)	30/51	(59)
		Control	91/139	(65)**	126/139	(91)**	91/126	(72)^{NS}	58/91	(64)^{NS}
	Romney	Melatonin	73/139	(53)	97/139	(70)	73/97	(75)	44/73	(60)
		Control	15/139	(11)**	58/138	(42)**	15/58	(26)**	5/15	(33)**
Significance	Poll Dorset v Romney		<0.001		<0.001		0.003		<0.001	
	Melatonin v Control		0.011		0.07		<0.001		0.04	
	Interaction		<0.001		<0.001		<0.001		0.75	
12 January (71 days)	Poll Dorset	Melatonin	90/142	(63)	132.142	(93)	90/132	(68)	61/90	(68)
		Control	77/139	(55)	122/139	(88)	77/122	(63)	42/77	(55)
	Romney	Melatonin	114/140	(81)	136/140	(97)	114/136	(84)	62/114	(54)
		Control	103/140	(74)	132/140	(94)	103/132	(78)	67/103	(65)
Significance	Poll Dorset v Romney		<0.001		0.02		<0.001		0.76	
	Melatonin v Control		0.04		0.08		0.14		0.79	
	Interaction		0.75		0.88		0.71		0.02	
14 February (96 days)	Poll Dorset	Melatonin	71/141	(50)	-	-	-	-	43/71	(61)
		Control	90/142	(63)	-	-	-	-	58/90	(64)
	Romney	Melatonin	87/142	(61)	-	-	-	-	58/87	(67)
		Control	98/141	(70)	-	-	-	-	53/98	(54)
Significance	Poll Dorset v Romney		0.04						0.71	
	Melatonin v Control		0.01						0.42	
	Interaction		0.63						0.12	

TABLE 5: Effect of melatonin implants on reproductive performance of Romney rams joined in January to CIDR synchronised Romney ewes over 3 consecutive years

Date	Treatment group		Ewes mated / Ewes treated	%	Ewes conceiving / Ewes mated	%
19 Jan 1988	Romney	Melatonin	68/104	(65)	45/68	(66)
(Expt. 1)		Control	36/105	(34)	17/36	(47)
20 Jan 1989	Romney	Melatonin	86/118	(75)	62/86	(53)
(Expt. 2)		Control	89/118	(75)	60/89	(44)
12 Jan 1990	Romney	Melatonin	114/140	(81)	62/114	(54)
(Expt. 3)		Control	103/140	(74)	67/103	(65)

ment at the January mating (Table 3) although there was a significant breed effect with Poll Dorset rams mating significantly more ewes than Romney rams ($P < 0.001$). Melatonin treated rams mated significantly fewer ewes in February ($P < .01$) but there was no effect on conception rate.

DISCUSSION

The seasonal breeding pattern of Romney rams is well illustrated in Experiment 3 where untreated Romney rams mated only 11% of progesterone primed ewes in December, compared with 74% in January and 70% in February. Onset of the breeding season also varied between years with the results of three consecutive January matings with Romney rams summarised in Table 5. In Experiment 1, melatonin treatment significantly improved ram libido (% of ewes mated) and fertility (% of ewes conceiving) but in Experiments 2 and 3, untreated rams were already active in January. However the difference between trials may also have resulted from differences in the time between start of treatment and exposure to ewes and also between trials in the absolute date of treatment. Recent data have shown that it is important to correctly time the flock mating to the peak response to melatonin and to avoid both the delay phase to response and the refractory phase after response (Staples *et al.*, 1992; and Williams *et al.*, 1992). Since no measurements of testis size were conducted in the present experiments it is not possible to judge whether the time between start of treatment and exposure to oestrus ewes was optimum. This factor may require further study in view of the transitory nature of the ram response to continuous melatonin (Lincoln and Ebling, 1985) and also since the premature development of refractoriness follows melatonin treatment in ewes (Staples *et al.*, 1992). In Experiment 3, refractoriness may have commenced by the time of the February mating as melatonin treated rams had a significantly reduced mating performance.

At those early season matings (Experiment 1 - January; Experiment 3 - December) when overall ram performance was poor (55% and 42% of ewes mated, respectively) melatonin treatment of rams improved performance in three out of four groups. The group in which melatonin treatment appeared to have a negative effect had significant between ram variability and it is possible that between ram variability may, in some cases, be greater than any treatment effect. The observation that more active ram groups stimulated more ewes to ovulate following progesterone priming (December, Experiment 3) is particularly significant as the effect was irrespective of melatonin treatment. This effect did not occur at the January mating and, presumably, was only observed at the very beginning of the breeding season or while ewes are still in anoestrous. This may explain why Knight and Gibb (1990) failed to detect differences in stimulatory activity between melatonin treated and untreated Romney rams at a February mating even though libido differences existed. If the active rams stimulate early season ovulation, this may help explain some of the large variation in response which has been observed from use of CIDR's at an early season mating.

In the present experiments the proportion of ewes mated was generally low. Even when 93% of ewes were ovulating (January mating, Experiment 3) the proportion of ewes mated ranged only between 55% and 81%. This was possibly a result of the low ram:ewe ratios (3.5% - 5.5%) used in these studies to test the effects of melatonin. More usually a 10% ram:ewe ratio is used (Knight *et al*; 1989) and recommended for a synchronised mating and may still be a practical precaution even after melatonin treatment.

If more active rams are able to stimulate ewes to ovulate (present study) and early season treatment of ewes with melatonin can increase ewe fertility by 20 to 30% (Knight *et al*; 1992) it is possible that in some cases a combination of ram and ewe treatment with melatonin could be justified as a means of maximising the benefits of melatonin treatment at an early season mating of British breed sheep particularly when ram % is low as in the present designs.

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REFERENCES

Arendt, J.; Symons, A.M.; Laud, C.A. and Pryde, S.J.; 1983. Melatonin can induce early onset of the breeding season in ewes. *Journal of Endocrinology* **97:** 395-400.

Chemineau, P.; Vandaele, E.; Brice, G. and Jardon, C.; 1991. The use of melatonin in improving reproduction performance in sheep. *Recueil de Medicine Veterinaire Special Reproduction des Ruminants* Mars-Avril p 227.

Haresign, W.; Peters, A.R. and Staples, L.D. 1990. The effect of melatonin implants on breeding activity and litter size in commercial sheep flocks in the UK. *Animal Production* **50:** 111-121.

Karsch, F.J.; Bittman, E.L.; Foster, D.L.; Goodman, R.L.; Legan, S.J. and Robinson J.E.; 1984. Neuroendocrine basis of seasonal reproduction. *Recent Progress in Hormone Research* **40:** 185-232.

Knight T.W. and Gibb M. 1990. Effect of social facilitation and Regulin implants on the ram's ability to stimulate ewes. *Australian Society for Reproductive Biology. Proceedings of 22nd Annual Meeting,* University of Western Australia p13.

Knight T.W.; McMillan W.H.; Kannegieter S.G.; Sorenson E.S.; Ridland C.J. and Gibb M.; 1989. Mating Romney ewes in November-December using CIDR's and pregnant mares serum gonadotrophin. *Proceedings of the New Zealand Society of Animal Production.* **49:** 255-260.

Knight, T.W.; Muir, P.D.; Smith, J.F.; Scales, G.H.; Reid, T.C.; McPhee, S.R. and Staples, L.D.; 1992. Effects of long acting melatonin implants on the reproductive performance of Corriedale, Romney, Coopworth and Perendale ewes in New Zealand. *New Zealand Journal of Agricultural Research* **35:** 185-193.

Lincoln, G.A. and Ebling,F.J.P.; 1985. Effect of constant-release implants of melatonin on seasonal cycles in reproduction, prolactin secretion and moulting in rams. *Journal of Reproduction and Fertility* **73:** 241-253.

Staples, L.D.; McPhee, S.; Ayton, B.; Reeve, J. and Williams, A.; 1986a. Optimum melatonin treatment to improve reproductive performance of Merino, Border Leicester x Merino and Romney ewes joined in spring and early summer. *Proceedings of the Australian Society of Reproductive Biology* **18:** 26 (Abstr).

Staples, L.D.; 1989. The development of "Regulin" from concept to international market place. *Australian Journal of Biotechnology* **3:** No 3 164-175.

Staples, L.D.; McPhee, S.R.; Reeve, J. and Williams A.H.; 1991. Practical applications for controlled release melatonin implants in sheep. *Advances in Pineal Research* **6:** 199-208.

Staples, L.D., McPhee, S.R., Kennaway, D.J. and Williams, A.H. 1992. The influence of exogenous melatonin on the seasonal patterns of ovulation and oestrus in sheep. *Animal Reproduction Science* **30:** 185-223

Woodfill, C.J., Robinson, J.E., Malpaux, B. and Karsch, F.J. 1991. Synchronisation of the circannual reproductive rhythm of the ewe by discrete photoperiodic signals. *Biology of Reproduction* **45:** 110-121.

Williams, A.H., McPhee, S.R., Reeve, J.L. and Staples, L.D. 1992. Optimum use of subcutaneous melatonin implants to enhance the reproductive performance of seasonal and non-seasonal sheep joined in spring and early summer. *Animal Reproduction Science* **30:** 225-258.

Yeates, N.T.M.; 1949. The breeding season of the sheep with particular reference to its modification by artificial means using light. *Journal of Agricultural Science* **39:** 1-43.