MELATONIN IN MALE DEER - EFFECTS ON SEASONAL CYCLES

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INTRODUCTION

Advancing the breeding season in farmed red and fallow deer offers many potential advantages for the deer manager. This has especially been the case in New Zealand where dry summers can result in limited supplies of pasture with dramatic effects on the growth of sucking calves. Treatment with melatonin offers one possible approach to advancing the breeding season and this paper is concerned principally with the effects of melatonin on the seasonal cycles in male red deer but the available information suggests that the general conclusions probably also apply to fallow deer.

SEASONAL CYCLES

Seasonal patterns

Red and fallow deer are temperate species, exhibiting a very marked seasonal pattern of reproduction with an intense autumn breeding season (rut). The sexual and antler cycles are synchronised by the annual cycles of luteinising hormone (LH) and testosterone (Suttie et al, 1984; Asher et al, 1989). The hard antler is cast and the new velvet antler grown during the period of low circulating testosterone during the spring; with the increasing levels of testosterone in the late summer the antlers calcify and are cleaned of velvet. Consequently the antlers are hard during the high testosterone period of the rut. Testosterone levels subsequently decline and the antlers are cast in the early spring (Fennessy et al, 1988). Several sources indicate that it is the changes in the circulating levels of testosterone (and/or its metabolites) which are responsible for the annual cycle of antler growth (see Fennessy and Suttie, 1985). Similarly several studies have shown that it is seasonal changes in daylength which are responsible for entraining this putative circannual rhythm to a period of one year (see Goss, 1983). The role of the pineal gland (the source of melatonin) in this synchronisation is evident from studies where it has been surgically removed (pinealectomy - Brown et al, 1978; Snyder et al, 1983) or denervated (removal of the superior cervical ganglion or SCGX - Lincoln, 1985). In addition, replacement studies with melatonin treatment of pinealectomised animals have confirmed that it is melatonin from the pineal gland which is the active compound involved in synchronising the cycle, and that it is the duration of the nocturnal secretion of melatonin which codes for day length (Wayne et al, 1988). Consequently over the last 15 years there has been considerable interest in the role and possible use of melatonin in manipulating the breeding season in seasonally breeding species. This interest led to the development of the melatonin implant, Regulin (Regulin Ltd; Melbourne; Staples et al, 1986) which has been licensed for use in deer in New Zealand.

Melatonin rhythms and seasonality

Plasma melatonin concentrations reveal a marked circadian pattern of secretion with very high levels during darkness and low or undetectable levels during daylight in many species including both fallow (Asher et al, 1988) and red deer (Webster et al, in press). The precise nature of the mechanism whereby a circadian rhythm of melatonin secretion can influence a circannual pattern of pituitary LH secretion (and hence testosterone secretion) is not known, although it is clear that the melatonin effects are mediated via effects on the release of LHRH (the LH releasing hormone from the hypothalamus; Lincoln et al, 1984). However it is believed that, either the length of time that melatonin is secreted daily, or whether or not melatonin is secreted during a "photo-sensitive phase" of the day, provides information to the animal about changes in daylength.

MELATONIN TREATMENT

Several experiments involving melatonin treatment of male deer of temperate species have been carried out. Administration has been via various routes, including intramuscular injection, oral (in the feed) or by various forms of implants (eg, silastic envelopes, capsules or Regulin implants).

Early work concentrated on advancing the normal darkness levels of melatonin (simulated short day) by either injection (Webster and Barrell, 1985) or feeding (Bubenik, 1983; Adam and Atkinson, 1984; Bubenik and Smith, 1985; Asher et al, 1987) with treatment starting about 3 to 7 weeks before the summer solstice. In all cases the breeding season as evidenced by rutting behaviour or testis size was advanced by about 1 to 2 months.

Studies in sheep have shown that although it is the circadian nature of melatonin secretion which controls the timing of reproductive rhythm (Bittman et al, 1983), it is not necessary to mimic the circadian melatonin rhythm to advance the reproductive rhythms, and in fact melatonin implants which resulted in high levels throughout the day and night were effective (Lincoln and Ebling, 1985). Interestingly, Lincoln et al, (1984) implanted melatonin in both intact and SCGX stags There was no difference in the response of the two groups indicating that the difference in melatonin between daylight and darkness (which based on other studies would still be apparent in melatonin-treated intact stags) was not important in advancing the seasonal rhythm This study also indicates that exogenous melatonin operates independently of the pineal to stimulate breeding activity, and that it was the loss of the melatonin influence which led to the delay in breeding activity in the SCGX stags (Lincoln, 1985).

The development of the small Regulin implant containing 18 mg melatonin has provided a practical method for treatment of animals, and as a result, several studies have been carried out in red stags and fallow bucks in New Zealand. Table 1 presents details of 6 experiments, selected aspects of which will be discussed in detail, particularly in relation to the effects on live weight patterns, antler cycles and other aspects of seasonal physiology.

Seasonal cycles

Regulin treatment has very marked effects on the sexual cycle of male red and fallow deer. For example, in the study of Webster et al (Table 1, Expt 1), melatonin treatment advanced the sexual cycle by 1 to 2 months as evidenced by changes in both scrotal circumference and live weight (Fig. 1) As expected, there were also marked effects on the antler cycle, with antlers of treated stags hardening and being cleaned of their velvet earlier than controls. The results of Expt 2 are particularly interesting. Compared with the controls, testicular regression and antler casting were delayed in the melatonin-treated stags where treatment started in June and August. In contrast, the groups where treatment started in September or October had cast their antlers by the time of treatment. However, in these two melatonin-treated groups, subsequent reproductive development was advanced by about a month whereas in the three groups where treatment started in June and August, reproductive development was delayed by about a month compared with the controls The results of these two experiments on red deer stags demonstrated that treatment with melatonin implants from mid-September to early December advanced reproductive development. However, when red deer stags were treated with melatonin implants from June to August, reproductive development was delayed indicating a change in response to melatonin treatment during the year. This change in response to melatonin treatment between late winter and early spring was interpreted as a resetting of an endogenous circannual rhythm caused by a photoperiodic cue responsible for initiating the final stages of reproductive regression. In red deer stags it would appear that the increasing photoperiod in late winter and early spring is particularly important in the entrainment process. Similar results to those of Expt 1 with red deer were reported by Asher and Peterson (Table 1, Expt 6) in studies of adult fallow bucks, although in this work the bucks were polled so that antler data were not available. Fisher et al (1988) and Fisher and Fennessy (1990) (Table 1, Expt 4) have conducted studies principally concerned with reproduction with melatonin treatment starting in December; observations indicated that melatonin-treated stags tended to roar and herd their hinds much more actively than untreated stags in the first few weeks following joining in late February. In a further Invermay study, Suttie (Table 1, Expt 3) treated 2 year old red stags with various Regulin treatments; all groups which received melatonin implants showed evidence of a melatonin response (Fig 2) which was particularly apparent in the timing of testicular regression post-rut.

Antler growth

The melatonin treatments in Experiment 1 had a dramatic effect on the timing of the antler cycle (Table 2). The extra antler cycle is apparent with the treated stags first casting their hard antlers in May instead of in the spring. These stags then went through a complete cycle with antler cleaning in June-July-August followed by a second casting in August-September-October. By the following late summer-autumn, it appears that the treated stags were again synchronous with the controls as evidenced by the data on scrotal circumference. Although the velvet antler yield following the first casting in May was significantly lower in the treated stags than in the controls following normal casting (2.24 and 2.72 kg ± 0.22, SED), overall the annual yield was markedly higher from the melatonin-treated stags with their double antler cycles (3.95 and 2.72 ± 0.36, SED).

Table 1. General details of New Zealand studies involving Regulin treatment of male red and fallow deer

			Regulin treatment regime			
Expt[1]	Species	Age	Number of treatments	Implants per treatment	Start date	Number treated
1	Red	Adult	0	0	-	12
			4	3	Nov 8 (early)	6
			3	3	Dec 9 (late)	6
2	Red	Adult	0	0	-	5
			6	3	Jun 22	5
			6	3	Aug 4	5
			6	3	Sep 16	5
			6	3	Oct 23	5
			12	3	Jun 22	5
3	Red	2y	0	0	-	5
			3	2	Dec 17	5
			2	2	Dec 17	5
			1	2	Dec 17	5
			1	2	Jan 17	5
4	Red	3,4y	0	0	-	8
			3	3	Dec 16	11
5	Red	3,4y	0	0	-	14
			2	2	Dec 20	12
6	Fallow	6y	0	0	-	4
			5	1	Nov 4	4

[1] Experiments
1. Webster et al, (in press)
2. Webster et al, (in press)
3. Suttie (unpublished data)
4. Fisher et al, (1988)
5. Jopson and Fennessy (unpubl. data)
6. Asher and Peterson (in press)

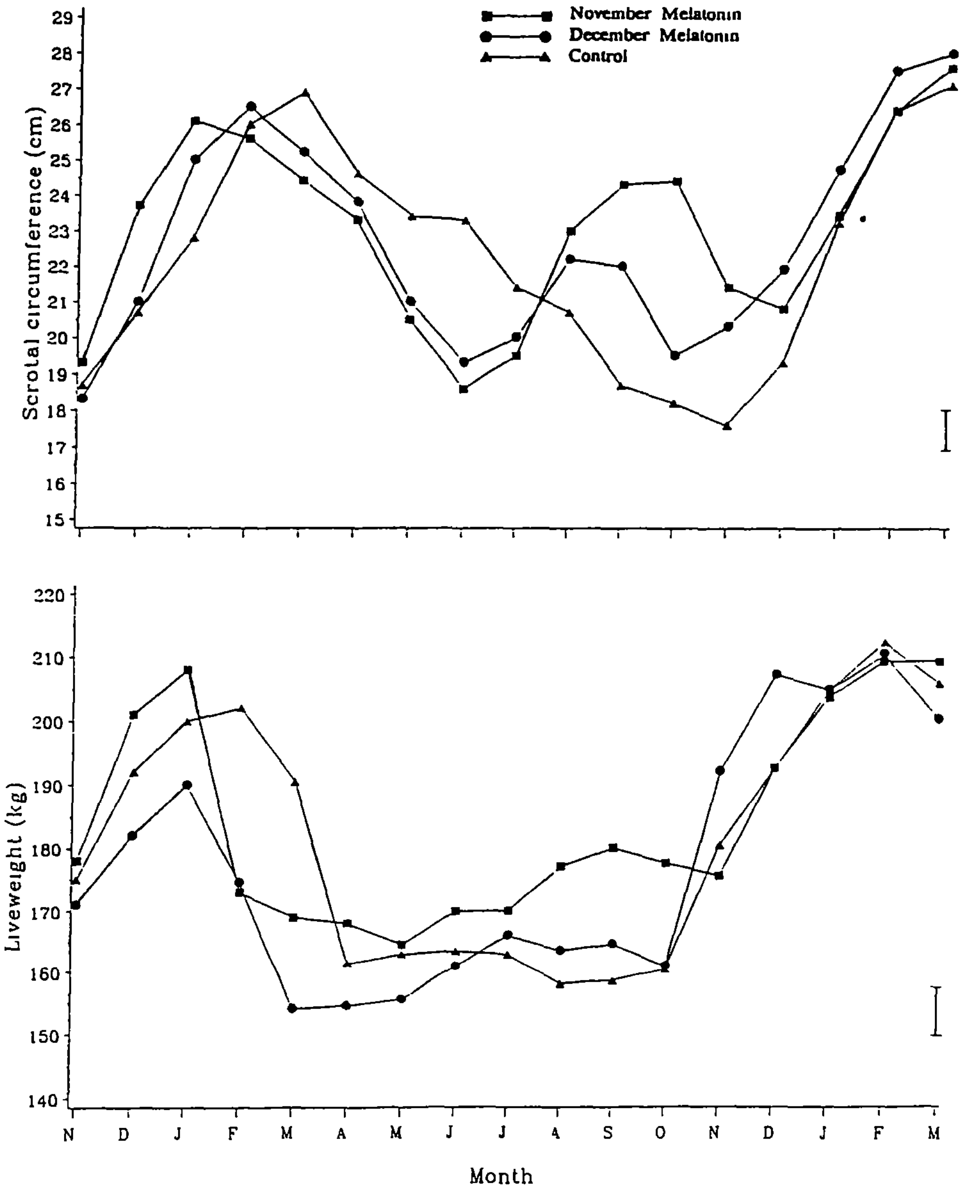

Fig 1. **(Expt 1) Mean scrotal circumference and live weight patterns for the groups of red stags; melatonin treatment started in November (■) or December (●); controls (▲). The bar indicates the approximate SEM.**

Table 2. **(Expt. 1). Mean values for casting dates of hard antler for control and melatonin-treated stags both pre-treatment (1985) and post-treatment (1986). Treatment started in November 1985 or December 1985.**

	Casting date			Difference in casting date from controls (days)	
Melatonin treatment	1985 pre-treatment	1986 autumn	1986 spring	1985 pre-treatment	1986 autumn/spring
Control	30 Aug	-	23 Aug	0	0
November start	27 Aug	7 May	10 Oct	-3	-108/+48
December start	26 Aug	8 May	11 Sept	-4	-107/+19

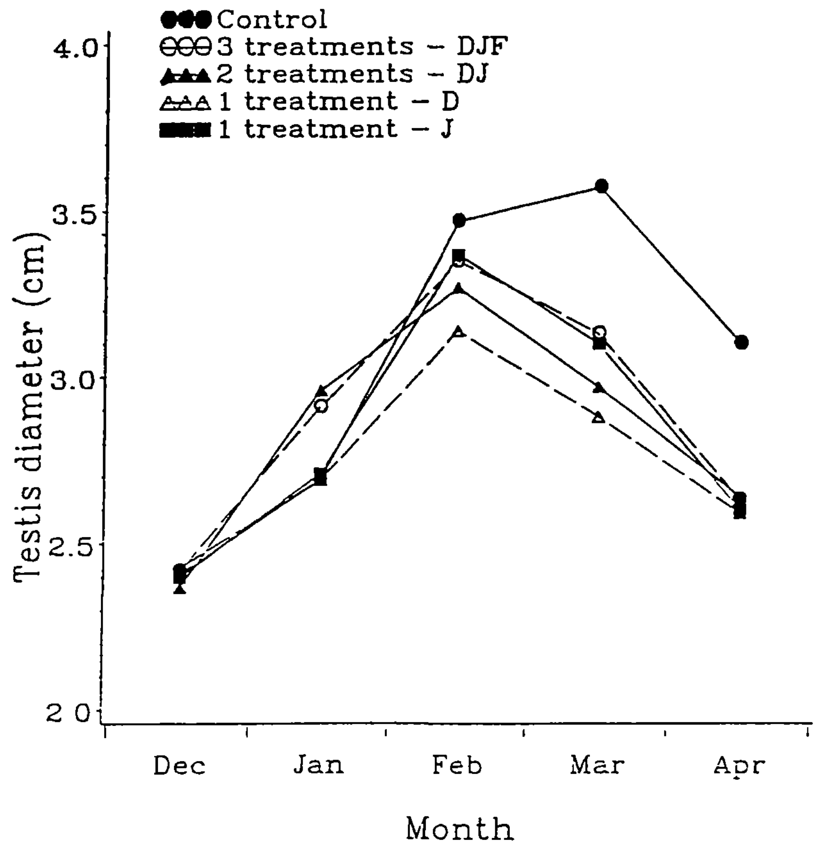

Fig. 2. (Expt. 3). Mean testis diameter for the groups of 5 two-year old stags given the various melatonin treatments (DJF - December, January, February treatment).

Table 3. (Expt. 4 and 5). Mean date of hard antler casting of control and melatonin-treated red deer stags by age at time of first melatonin treatment.

Expt	Age (yrs)	Mean casting date ± SD (n) Control	Melatonin-treated	Difference between groups (days)	Range in casting date of melatonin treated stags
4	5+	23 Aug ± 4.4 (4)	21 Jun ± 17 9 (5)	63	8 Jun-13 Jul
	4	30 Aug ± 3 5 (2)	28 Jul ± 21.1 (3)	33	6 Jul-17 Aug
	3	2 Sep ± 7 8 (2)	21 Aug ± 2.9 (3)	12	19 Aug-24 Aug
5	4	6 Sep ± 9 5 (5)	19 Jul ± 22.4 (4)	49	23 Jun-16 Aug
	3	13 Sep ± 5.4 (9)	16 Aug ± 39.3 (8)	28	2 Jul-22 Sep

Table 4. (Expt. 5). Mean values for velvet antler growing period (days ± SD, casting to harvest) and first-cut yield (± SD) for control and melatonin-treated stags.

Age (yrs)	(n)	Control Growing period (d)	Control Yield (kg)	(n)	Melatonin-treated Growing period (d)	Melatonin-treated Yield (kg)
4	(5)	66.0 ± 4.6	2.15 ± 0.39	(4)	67.5 ± 8.3	2.41 ± 0.72
3	(9)	63.3 ± 2 2	2 09 ± 0.40	(8)	63.9 ± 6 1	1.86 ± 0.14

Experiments 4 and 5 also provided detailed information on the effects of Regulin treatment on velvet antler growth and yield. The data are summarised in Tables 3 and 4. There was a very marked effect of Regulin treatment on the date of hard antler casting with the effect being much greater in older stags. For example in Expt 5, casting was apparently advanced by 49 days in the stags which were 4 years old at the time of first treatment and 28 days in the stags which were a year younger. There was no effect of treatment on days of growth or velvet antler yield at the first cut in either experiment. However in Expt 5, 6 of the 12 melatonin-treated stags produced marketable regrowth velvet antler (an average of 1.13 kg over 6 stags) while none of the 14 controls produced any.

PRACTICAL CONSIDERATIONS

Regulin treatment offers a useful method for advancing the breeding season in red deer stags and fallow bucks. At Invermay, treatment of red stags in November-early December advances the reproductive cycle by 1-2 months as evidenced by the effects on the antler cycle (i e cleaning), testis size, the patterns of live weight change and behaviour.

While the effects of Regulin in advancing the breeding season in stags and bucks are particularly marked, the other effects of the treatment, both positive and negative, must also be considered. The increase in velvet antler production which may occur though a higher yield of marketable regrowth is unlikely, in itself, to constitute a reason for Regulin treatment

There are also some other aspects of treatment with Regulin, which if not taken account of, can result in difficulties for the deer farmer. The susceptibility of rutting stags to heat stress is important; this is particularly so with seasonally advanced stags which are rutting in the February heat. Consequently, the availability of shade and a good supply of water are critical The advanced breeding season also means that Regulin-treated and untreated stags must be separated from about January onwards, due to the aggressive behaviour of the seasonally-advanced stags.

The post-rut variability between stags on the same or apparently similar Regulin treatments is considerable. Most treated stags undergo premature testicular regression resulting in premature hard antler casting. Some stags will cast their hard antlers as early as May, following their first Regulin treatment in November or December. Consequently these stags will likely have impaired fertility at the time of the normal rut in April-May, although there is no evidence of any problems with their fertility at their advanced breeding season in February-March. Therefore we recommend that Regulin-treated stags be used only for breeding at their advanced rut and that they not be relied on during the normal rut.

There have also been reports of stags not being resynchronised in time for the rut in the following breeding season (i.e. 16 months after the start of a 3 month Regulin treatment). While these cases are apparently unusual, this is a particularly serious problem, when a valuable stag is involved.

The premature testicular regression means that stags will be growing velvet antler during winter. Their docile nature at this time means that Regulin-treated and untreated stags must be separated. However the variability among treated stags also means that there is usually a considerable range in the stage of the antler cycle within a group of stags at any particular time, although this can be reduced by treatment of stags of only one age group.

The effect of the timing of the treatment (i e. the start) and the treatment regime (3 implants x 3 occasions compared with 2 implants x 2 occasions) has not been examined in any of our experiments. However, it seems likely that these treatment variables may have an impact on the carryover effects of Regulin treatment. In the practical situation we now use 2 implants on 2 occasions in early December and early January with both red deer stags and Canadian wapiti x red F1 hybrid stags.

In the final analysis the decision as to whether to use Regulin in a particular situation must depend on the farmer's evaluation of its potential overall usefulness. This involves consideration of the expected advance in the breeding season and its expected economic value, plus the risk factor in terms of upsetting the seasonal cycle of the stag beyond the next breeding season, and management of seasonally advanced stags both during their early rut and during their velvet growing period during winter.

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