

## ADVANCING THE CALVING SEASON IN RED DEER

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Advancing the calving season in red deer has been discussed at the last three seminars (Barrell 1985; Fisher & Fennessy 1985; Fennessy et al, 1986; Fisher & Fennessy 1987) yet there have been significant new developments particularly involving the use of melatonin. Melatonin is the pineal hormone whose production is increased during hours of darkness, providing the seasonal cue to changes in reproductive status in deer as in many other species.

### MELATONIN

#### Effects on the breeding season

As part of our ongoing research programme with melatonin, three experiments were conducted at Invermay in 1987, Experiment 1 with yearling hinds and Experiments 2 and 3 with hinds lactating during treatment and mating.

The melatonin-treated stags in the 3 experiments each received 2 implants of Regulin (Regulin Ltd, Melbourne) on each of 3 occasions at about 30 day intervals starting in mid-December. Except for two of the treatments in Experiment 1, the hinds also received 2 implants on each occasion they were treated. Apart from Experiment 2, all hinds were run with melatonin-treated stags.

Experiment 1 involved 6 groups each of 9 yearling hinds namely a control, December/January/February treatment (as for the stags), January/February plus 3 treatments of either 1, 2 or 3 implants in January alone. Three year old lactating hinds were used in Experiment 2, with 18 being controls and 18 being implanted in January/February. The 2 groups of hinds were then split and run with either control or melatonin-treated stags in a 2 X 2 experiment. In Experiment 3, 26 two year old lactating hinds were used with 8 controls and 3 groups each of 6 hinds, being treated in either January/February, January alone or February alone.

In the first experiment (Table 1) yearling red hinds received various melatonin treatments and were run with melatonin-treated stags. The standard December/January/February treatment advanced calving by about 7 days compared with the controls. This difference was not significant, with the higher standard deviation in this group indicating considerable variation in the calving pattern compared with the controls. This is possibly a real effect due to starting the melatonin treatment some 4 months prior to the normal breeding season. This variable response has been shown in a study with sheep treated early, where some ewes ovulated early, some at the normal time and some late (English et al, 1986).

The January/February and the January\*2 and January\*3 implant groups all advanced calving date significantly by about 12 days compared with the controls. However the effect of the single implant in January was less than that of the other treatments. The results of this experiment indicate clearly that much more work is required in order to define the effects of melatonin, and to make recommendations about the timing of first treatment, the dose and the duration of treatment.

TABLE 1. 1987, Experiment 1. Effect of various melatonin treatments of yearling red hinds (treatment started Dec. 15 or Jan. 14) on calving dates as 2 year olds; hinds were run with treated stags (Dec, Jan, Feb).

Group	Melatonin treatment <sup>1</sup>	(n) <sup>2</sup>	Calving dates		
			First calf	Mean $\pm$ SD	Median
1	Untreated	(9/9)	19 Nov	24 Nov $\pm$ 3.6	25 Nov
2	Dec, Jan & Feb	(8/9)	3 Nov	17 Nov $\pm$ 9.3	17 Nov
3	Jan & Feb	(8/9)	6 Nov	13 Nov $\pm$ 5.0	13 Nov
4	Jan*1	(8/9)	9 Nov	18 Nov $\pm$ 7.7	20 Nov
5	Jan*2	(8/9)	31 Oct	12 Nov $\pm$ 6.4	14 Nov
6	Jan*3	(7/9)	3 Nov	12 Nov $\pm$ 6.6	13 Nov

<sup>1</sup> Treatments: Group 2, two implants in Dec, Jan & Feb; Group 3, two implants in Jan & Feb; Group 4, single implant Jan; Group 5, two implants in Jan; Group 6, 3 implants in Jan.

<sup>2</sup> Number calving/number treated.

Overall the treated hinds in Experiment 1 calved on average 10 days earlier than the controls. However the control hinds in this experiment calved about 2 weeks earlier than yearling hinds at Invermay in previous years (Fisher *et al*, 1986; see Table 2).

TABLE 2. Calving dates of untreated yearling red hinds (calving as 2 year olds) in 1984, 1985 and 1987 at Invermay

Year	(n)	Calving dates		
		First calf	Mean $\pm$ SD	Median
1984	(7)	7 Dec	12 Dec $\pm$ 5.2	11 Dec
1985	(8)	27 Nov	7 Dec $\pm$ 8.4	8 Dec
1987	(9)	19 Nov	24 Nov $\pm$ 3.6	25 Nov

In 1987 the control untreated hinds were exposed to seasonally advanced (melatonin-treated) stags; this is in contrast to 1984 and 1985 where the hinds were run with untreated stags. It appears that the presence of the melatonin-treated stag and/or melatonin-treated hinds may have affected the onset of the breeding season and thus calving date in these untreated yearlings.

Further support for the interaction of hind and stag treatment on calving date was evident in Experiment 2, where 18 three year old lactating hinds were treated with melatonin and a further 18 hinds from the same group acted as controls. The hinds were split into groups and run with either treated or untreated stags (Table 3).

Melatonin treatment of either hinds or stags resulted in significant advancements of calving date by 17 and 20 days compared with the controls. Treating both hinds and stags resulted in a further significant shift of

7-10 days. That is, the combined effect of treating both hinds and stags was a shift in the mean and median calving dates of 27 and 32 days respectively.

TABLE 3. 1987, Experiment 2. Effect of melatonin treatment of stags (December, January, February treatment) and lactating hinds on calving date (hind treatment started 14 Jan with 2 implants in Jan and 2 in Feb).

	Calving dates		
	First calf	Mean $\pm$ SD	Median
<u>Untreated stags with (n)<sup>1</sup></u>			
Untreated hinds (9/9)	22 Nov	11 Dec $\pm$ 14.2	16 Dec}
Melatonin hinds (9/9)	15 Nov	24 Nov $\pm$ 6.7	22 Nov}
			} 4 Dec
<u>Melatonin stags with (n)</u>			
Untreated hinds (9/9)	13 Nov	21 Nov $\pm$ 6.9	19 Nov}
Melatonin hinds (9/9)	8 Nov	14 Nov $\pm$ 4.5	14 Nov}
			} 17 Nov

<sup>1</sup> Number calving/number treated.

It appears that the seasonal reproductive status of the stag may be affecting the onset of the breeding season in hinds. This is possibly different from the reported "stag effect" (Moore & Cowie 1986) where the introduction of a normal untreated stag prior to the normal breeding season results in a synchronised oestrus c. 18-25 days later. In fact, Experiment 2 provides very clear evidence that the presence of a seasonally-advanced stag can affect the onset of the breeding season. McComb (1987) has also shown that the presence of a vasectomised stag prior to normal mating advanced the calving pattern.

In Experiment 3, any effects of treatment were very small although again the calving date of the control hinds was earlier than expected (Table 4). For example, the controls calved on average 21 days earlier than the untreated hinds run with untreated stags in Experiment 2 in the same season, although the mean calving dates for the untreated hinds run with treated stags was virtually identical in the two experiments (21 Nov in Experiment 2; 20 Nov in Experiment 3). This again provides evidence that treating the stag alone may prove a very effective means of advancing calving, at least compared with the hind treatments imposed in this particular experiment.

TABLE 4. Experiment 3. Mean and median calving dates for 3 year old hinds treated during lactation as 2 year olds (2 implants per occasion), and run with melatonin-treated stags (Dec/Jan/Feb).

<u>Melatonin treatment (n)<sup>1</sup></u>	<u>Calving dates</u>		
	<u>First calf</u>	<u>Mean ± SD</u>	<u>Median</u>
Untreated hinds (7/8)	15 Nov	20 Nov ± 5.1	18 Nov
Jan, Feb (6/6)	14 Nov	20 Nov ± 5.8	19 Nov
Jan (6/6)	8 Nov	16 Nov ± 5.8	17 Nov
Feb (6/6)	15 Nov	23 Nov ± 4.9	24 Nov

<sup>1</sup> Number calving/number treated.

The variety of different treatment regimes and different animals used in the Invermay experiments and those in the literature indicates that there is a considerable amount of work required to examine timing, duration and dose of melatonin in both hinds and stags. However in order to help clarify the possible influence of the time of starting melatonin treatment on the advancement of the breeding season, the available data are presented in Fig. 1A. The graph indicates little obvious relationship between these two variables. However, as mentioned previously, control hinds run with treated hinds and/or treated stags may calve earlier than expected (eg, Experiment 2, Fisher & Fennessy this paper; Moore & Cowie 1986). Therefore in Figure 1B, the data for the control hinds in 3 of the 7 studies (4, 5 and 6) have been adjusted to the normal expected date of birth for hinds of the same physiological state (which were not run with treated stags) to account for this effect of melatonin treatment and/or mating activity on the onset of the breeding season in untreated hinds.

Using the adjusted data (Fig. 1B), there is an apparent relationship between the extent of the advancement in the breeding season and the date of the start of melatonin treatment (during the December–February period). The slope of the apparent relationship was such that for every 5 days earlier that melatonin treatment of hinds started, onset of the breeding season/calving apparently was advanced by about 1 day. While the relationship between the extent of the advancement and the date of starting melatonin treatment may be a real one, considerable caution is required in applying it to the practical situation. The data cover a wide range of melatonin treatments involving different treatment methods and duration of treatment. In addition the experiments involved hinds in different physiological states, namely non-lactating and lactating adult hinds and yearling hinds. There are certainly theoretical dangers in extrapolating the data for adult hinds to yearlings as with the latter there is the potential interaction of melatonin treatment with puberty. The only way to resolve these issues is to devise particular experiments which address the questions directly.



TABLE 5. Data used in Fig. 1. relating advancement of the breeding season to the time of starting melatonin or photoperiodic treatments.

Reference	N treated	Treatment			Advancement <sup>1</sup>	
		Start	Method <sup>3</sup>	Hinds <sup>4</sup>	Actual	Adjusted <sup>2</sup>
1 Adam & Atkinson (1984)	4	Dec 1 <sup>5</sup>	F	N	34	-
2 Adam <u>et al.</u> (1986)	12	Dec 18 <sup>5</sup>	F	L	32	-
	6	" "	F	N	35	-
3 Webster & Barrell (1985)	4	Jan 8	I <sub>-</sub> <sup>6</sup>	Y	31	-
	4	" "		Y	32	-
4 Barrell & Staples (1987)	8	Dec 20	R	L	16	22
	8	Jan 18	R	L	8	14
	12	Jan 24	S	L	6	14
5 Fisher & Fennessy (1987 Expt 1)	9	Dec 16	R	Y	7	23
	9	Jan 15	R	Y	11	27
	9	" "	R	Y	6	22
	9	" "	R	Y	12	28
	9	" "	R	Y	12	28
6 Fisher & Fennessy (1987 Expt 3)	6	Jan 14	R	L	0	21
	6	" "	R	L	4	25
	6	Feb 14	R	L	-3	18
7 Fisher & Fennessy (1987 Expt 2)	9	Jan 14	R	L	27 <sup>7</sup>	-
	9	" "	R	L	17 <sup>7</sup>	-

<sup>1</sup> Advancement is for mean date of calving except for 1 and 2 where it is mean date of first ovulation of the season.

<sup>2</sup> The control mean birth dates for data sets 4,5 and 6 have been adjusted to the expected normal birth dates for untreated hinds run with untreated stags; consequently the advancement due to melatonin has increased.

<sup>3</sup> Method of treatment with melatonin: F = melatonin given with feed; I = intramuscular injection; S = silastic implant; R = Regulin implants.

<sup>4</sup> N = non-lactating adults; L = lactating adults; Y = yearlings

<sup>5</sup> Dates altered by 6 months to Southern Hemisphere time.

<sup>6</sup> Short day treatment rather than melatonin.

<sup>7</sup> 27 days advancement with melatonin treated stags compared with 17 days advancement with untreated stags (Table 3); in all other studies treated stags were run with the treated hinds.

The practical aspects of melatonin treatment must also be considered. For example, to achieve a reasonable advancement in the onset of the breeding season in lactating hinds would require treatment of lactating hinds in December/early January. If the apparent relationship in Fig. 1 (and the results of Experiment 3) are supported in large scale trials, then the advantage of treating lactating hinds may be equivocal, particularly when the practical difficulties of yarding lactating hinds and their calves in December/January are considered. The best returns may well come from treating stags only; alternatively some hinds in the group could be given progesterone/PMSG treatment (Moore & Cowie 1986). With yearlings, which tend to calve later than adult hinds anyway, melatonin offers possibilities of significantly advancing calving. In this case, the most effective and practical treatment may well be to treat both stags and hinds. However, because of the possible variability in the response of yearlings treated early in the season this must be examined carefully in large scale trials in the near future.

Other effects on seasonal physiology

In addition to its effect on reproductive activity, melatonin may also modify the aspects of seasonal physiology such as patterns of liveweight gain and antler and coat cycles. In particular, the possible effects on growth of calves sucking treated hinds and on growth patterns of young hinds need to be considered.

Melatonin treatment of stags, designed to advance breeding, induced earlier casting of hard antlers in the following season, the effect being most pronounced in older stags (Table 6). However there was no effect on the interval between casting and velvet harvest nor on velvet antler weight. There are a number of management implications resulting from treating stags with melatonin to advance breeding. To minimise aggression between treated and untreated stags in February/March they should not be run together although problems may not necessarily occur if they are run together. The same applies later in the winter when treated stags are likely to cast their antlers earlier than normal stags resulting in their being harassed by the latter. Treated stags are rutting during summer and consequently it is essential that they have access to a good water supply and shelter from the heat.

TABLE 6: Date of antler casting in control and melatonin-treated (December, January, February) red deer stags (Fisher et al. 1988).

<u>Age of stag</u>	<u>Casting date</u>		
	<u>Control (n)</u>	<u>Melatonin-treated (n)</u>	<u>Difference, days</u>
≥ 5 years	23 Aug (4)	21 June (5)	63***
4 years	30 Aug (2)	28 July (3)	33 (NS)
3 years	2 Sept (2)	21 Aug (3)	12 (NS)

<sup>1</sup> NS - not significant; \*\*\* - P<.001

Although melatonin treatment appeared to have a very slight effect on the pattern of liveweight change in young, growing yearling hinds compared with controls, over the year there was no difference in overall liveweight gain between the two groups (Fisher et al. 1988). The effect of melatonin on

coat growth has been monitored closely since Lincoln & Ebling (1985) found that some melatonin-treated rams were in poor condition and more vulnerable to cold weather. In the studies with red hinds, melatonin treatment, commencing in mid-December, merely advanced the timing of the summer moult and winter coat regrowth by about a month (Table 7). Coat colour followed a similar trend changing from the reddish-brown of summer to the lighter greyish-brown of winter about a month earlier in the melatonin-treated hinds. Treated hinds tended to moult their winter coats earlier with 8/9 treated yearling hinds moulting in late September compared with only 2/9 controls. The seasonal coat changes involving moulting of the summer coat and growth of the winter coat in the control hinds are similar to those reported by Ryder (1977). However whether there are any potential adverse consequences of early moulting of the winter coat are as yet unclear although it would be advisable to provide good shelter for melatonin-treated hinds in late winter-early spring particularly in the event of adverse weather conditions.

TABLE 7: Mean coat length in control and melatonin-treated yearling hinds (Fisher et al. 1988).

<u>Season</u>	<u>Date</u>	<u>Mean coat length (cm)</u>		
		<u>Control</u>	<u>Melatonin</u>	<u>Difference</u>
Summer	15 Jan	4.4	4.6	0.1 <sup>NS</sup>
	12 Mar	4.1	2.8	1.3*
Autumn	9 Apr	2.2	4.7	2.5**
	4 Jun	3.8	4.0	0.2 <sup>NS</sup>
Winter	20 Aug	5.3	5.2	0.1 <sup>NS</sup>

<sup>NS</sup> - not significant; \* - P<.05; \*\* - P<.01

A further potential concern relating to the use of melatonin is the possible effect of treatment during lactation on the milk production of the hind and hence on calf growth rate. For this reason calf growth rate was recorded in Experiment 2, from the time of starting treatment in January until weaning. The growth rates of the calves sucking the melatonin-treated and control hinds were virtually identical (324 and 328 g/day respectively) with mean weaning weights of 55.0 and 55.7 kg on 23 April (Fisher et al. 1988). While lactation, measured indirectly as calf growth rate was unaffected by melatonin treatment started during lactation, the possible effects of melatonin administered during pregnancy or very early in lactation need to be examined. In this respect, melatonin treatment during pregnancy adversely affected the initiation of lactation in fallow does (G.W. Asher pers. comm).



GONADOTROPHIC STIMULATION

Gonadotrophic stimulation using PMSG or gonadotrophin-releasing hormone (GnRH) has been used to induce ovulation in hinds prior to the normal breeding season in several experiments over the last few years (Fennessy et al. 1986; Fisher et al. 1986; Moore & Cowie 1986). The results of some Invermay experiments involving comparisons of PMSG or GnRH (following pre-treatment with progesterone containing CIDRs) with CIDRs alone are shown in Table 8.

TABLE 8: Incidence of induced ovulation in yearling and and lactating hinds given various treatments

Treatment <sup>1</sup>	Hinds with induced ovulations <sup>2</sup>	
	Yearling hinds	Lactating hinds
Control	1/19	-
CIDR	5/18	0/13
CIDR/PMSG	13/16	11/13
CIDR/GnRH	12/26	8/13

<sup>1</sup> 12% (yearling hinds) or 9% (lactating hinds) progesterone CIDRs were inserted for 14 or 15 days with treatment at CIDR withdrawal (see Fisher et al. 1986; Fennessy et al. 1986).

<sup>2</sup> Number with induced ovulations (at laparoscopy)/number treated; only PMSG doses of  $\geq 250$  iu by intramuscular injection and GnRH doses of  $\geq 200$  ng/hour (for 7 days by osmotic minipump) are included.

In the absence of direct gonadotrophic stimulation, the proportion of hinds ovulating was very low. In fact, none of the lactating hinds ovulated following pretreatment with progesterone alone. The contrasting ovulatory response in the yearling and lactating hinds with the progesterone treatment alone (5/18 compared with 0/13 hinds ovulating) points to a difference in seasonal/pubertal anoestrus in the yearlings compared with seasonal/lactational anoestrus in the adult hinds. However, gonadotrophic stimulation, particularly using PMSG was highly successful in inducing ovulation. While PMSG induced ovulations in over 80% of hinds, GnRH was successful in only about 50%. However with the development of new GnRH preparations, further research is worthwhile. Despite the high incidence of ovulation prior to the normal breeding season, fertility at the associated oestrus has been poor (eg, 7 of 32 yearling hinds which had ovulated, calved to the induced ovulation). This low fertility is almost certainly due to the fact that stags are likely to be sub-fertile in February-early March prior to the onset of the normal breeding season in late March/April. Prior to the normal season, red deer stags appear to be able to copulate normally but they show much less interest in roaring or herding hinds compared with stags seasonally advanced by the use of melatonin (M. Quentin-Baxter and M.W. Fisher, unpublished data). When melatonin-treated stags have been used in experiments involving gonadotrophin-treated hinds, the fertility has been markedly improved

compared with using untreated stags. For example, in 1986, 33/56 (59%) of progesterone-PMSG treated hinds calved in response to treatment (Moore 1987) when run with melatonin-treated stags compared with only 6/48 (13%) in a similar experiment in 1985 when untreated stags were used (Moore and Cowie 1986).

In the 1986 experiment cited above (Moore 1987), 13 of the hinds had multiple births (12 twins, 1 triplet set). Although many of the multiple-born calves were successfully reared (21/27), the work is still considered experimental and cannot be recommended to farmers at this stage. Interestingly, no multiple births resulted in a group of 22 hinds which were treated with the same dose of PMSG 3 weeks later, still about 3 weeks before the normal season. This and other evidence (M.W. Fisher and P.F. Fennessy unpublished) has led us to suspect that the progesterone-PMSG treatment is only likely to result in a significant number of twins if hinds are treated about a month or more before the time of the normal breeding season. This is despite the fact that the higher PMSG treatments do induce multiple ovulations at all times.

#### CONCLUSIONS

Melatonin continues to show promise as a means of advancing the breeding season in red deer. However the complexity of the response means that well designed field trials are essential before recommendations for use by farmers can be made. The possibilities for the use of melatonin in stags alone rather than stags and hinds also need to be considered. Gonadotrophic induction of ovulation is also worth pursuing further, particularly now that the fertility at the induced ovulation can be greatly increased by the use of seasonally advanced stags.

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