MANIPULATION OF REPRODUCTION OF RED DEER

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ABSTRACT

Red deer (Cervus elaphus), being a species of temperate origin, have a highly seasonal reproductive cycle with calving concentrated in the early summer period.

In temperate pasture situations, there would be an advantage in calving earlier to take advantage of high spring pasture growth and to minimise disruption due to low summer rainfall during some years. Several methods to advance the breeding season are currently being investigated both in New Zealand and the United Kingdom. Most involve hormonal manipulation using melatonin or progesterone plus gonadotrophins. In some studies both hinds and stags have been treated while in others only the hinds have been treated. We review the prospects for advancing the breeding season with particular emphasis on procedures likely to be practicable on farms.

There is considerable interest in New Zealand in both embryo transfer and artificial insemination to increase the use of superior animals, in overcoming problems with importing live animals and with hybridisation across species of deer. The techniques used are based on those used with domestic species. The current status of some of the work in these areas will be described and the prospects reviewed.

INTRODUCTION

Red deer (Cervus elaphus) and their close relation the wapiti (C. elaphus) and sika deer (Cervus nippon) are species of temperate origin. They have a highly seasonal reproductive cycle with an intense rut in the autumn, followed by a 230-250 day gestation period resulting in calving in the early summer. In temperate pasture situations, where high quality pasture is grown in spring, there are advantages in calving

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earlier to take advantage of this quality feed. In this paper we describe some of our studies and review some other relevant work, while also briefly mentioning ongoing work with embryo transfer and artificial insemination.

THE HIND

CONTROL OF OVULATION

In order to identify the possibilities for manipulation, an understanding of the hypothalamic-pituitary-ovarian regulation of ovulation is useful. Such a simplified scheme is shown in Figure 1. Where the objective is to advance the breeding season, promote superovulation or manipulate the oestrous cycle, the possibilities include:

- reducing daylength
- treatment with melatonin
- treatment with gonadotrophin releasing hormone (GnRH)
- treatment with luteinizing hormone (LH) or follicle stimulating hormone (FSH).

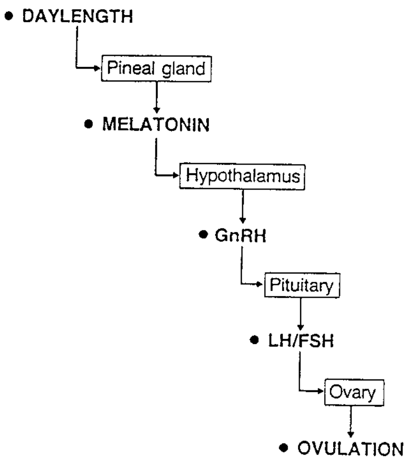

Figure 1: Simplified diagram of the regulation of ovulation in female red deer

With any of these procedures, treatment with progesterone may also be necessary. A further possibility involves the utilisation of pheromonal influences by altering the timing of stag introduction to a mob of females or perhaps introducing cycling hinds to a group of anoestrous hinds. The presumed site of action of such a pheromonal influence is via the phyothalamus.

PUBERTY

Generally puberty (defined as the onset of oestrus and ovulation) in the red deer female occurs at about 16 months of age and is related to the nutritional status of the animal as reflected in body condition and weight (Table 1).

TABLE 1 - Relationship between mating weight as yearlings and subsequent calving or pregnancy rates as 2 year olds

Liveweight (kg)	Calving Invermay (1975-80; n=130)[1]	Pregnancy rate[2] Invermay (1984,85; n=92)	
62-65	51%	0/2	0%
66-69	81%	0/2	0%
70-73	78%	10/11	91%
74-77	91%	15/17	88%
78-81	97%	14/16	88%
82-85	73%	18/20	90%
86-69	85%	15/16	94%
90		6/8	75%

[1] Kelly et al., 1982b

[2] Data from ultrasound diagnosis of pregnancy at day 70-110 post mating

From the table it is evident that for red deer at Invermay the threshold weight for attainment of puberty, ascertained from pregnancy or calving data is about 65-70 kg. In fact, it would appear that puberty actually occurs at a threshold weight of about 70% of the mature body weight.

TABLE 2 - Influence of treatment with melatonin or manipulated photoperiod on seasonal reproductive parameters in red deer

Treatment	Days	Period	N	Age/status	Result
Adam and Atkinson (1984, UK)					
Control			4	2-3 yr Dry	
Oral melatonin 5 mg daily pm	127	1/6-5/10	4	2-3 yr Dry	Advanced first oestrus by 2-8 weeks
Webster and Barrell (1985, NZ)					
Control			3	Yearlings	Mating: 24 Apr ± 13.7 (sd)
3.75 mg melatonin i.m. daily pm	83	8/1-31/3	4	Yearlings	29 Mar ± 6.8
8 h light/16 h dark	83	8/1-31/3	4	Yearlings	23 Mar ± 4.6
Nowak et al. (1985, UK)					
Control			6	Dry	Ovarian activity[1] - 2/6
Oral melatonin 10 mg daily pm	57	27/7-21/9	6	Lactating	" " - 0/6
" "	57	27/7-21/9	8	Dry	" " - 7/8

[1] Ovarian activity up to day 73 (6 Oct) deduced from plasma progesterone concentrations

SEASONALITY AND PHOTOPERIOD

Seasonality has been likened to an annual puberty and photoperiodic changes have been implicated in the control of both events. Hinds subjected to earlier seasonal photoperiods by shortening daylengths or treatment with melatonin (see Figure 1) have been induced to ovulate and subsequently calve earlier in the season (Table 2) although lactation may create some difficulties inhibiting induction of ovulation in some hinds (Nowak et al., 1985).

PROGESTERONE AND GONADOTROPHINS

The essential requirement for the development of a practical technique for advancing the breeding season is simplicity. Therefore in the Invermay studies we have concentrated on simple techniques. In our work, Controlled Internal Drug Releasing devices (CIDR's; AHI, Hamilton) containing progesterone are inserted into the vagina of the hind for 12-15 days (a period of progesterone priming is required to ensure that oestrogen produced by the developing follicle induces oestrus). At the time of CIDR withdrawal, the hind is treated with GnRH or pregnant mare's serum gonadotrophin (PMS) which has both LH- and FSH-like activity. Although the use of progesterone alone will cause ovulation and oestrus in a small proportion of hinds, gonadotrophic stimulation is necessary to induce ovulation in a significant proportion prior to the start of the normal breeding season. Some of the Invermay work with yearling hinds is summarised in Table 3.

Similar results to those with the yearlings have been achieved in non-lactating adults where the hinds have been treated in February with calving in October (Moore, et al., unpublished data).

TABLE 3 - Induction of ovulation prior to the breeding season in yearling red hinds[1]

Treatment			N	Hinds Ovulating
Controls		1984	10	0
		1985	9	1
CIDR[2]		1984	9	1
		1985	9	2
CIDR + PMS[3] (IU)	125	1984	4	1
	250		4	2
	500		4	4
CIDR + PMS4 (IU)	250	1985	8	7
CIDR + GnRH[5] (ng/h)	63	1984	4	1
	125		4	0
	250		4	2
	200	1985	7	4
	400		8	3
	800		7	3

1 Of 13 hinds induced to ovulate in 1984, 7 calved prior to the beginning of the normal calving season. In 1985, ovulations were induced in 19 hinds.

2 14 day treatment with CIDR containing 12% progesterone (Alex Harvey Industries, Hamilton) from February 27.

3 Pregnecol (Commonwealth Serum Laboratories, Australia) given at CIDR withdrawal.

4 Folligon (Intervet, Australia) given at CIDR withdrawal.

5 Sigma Chemicals (USA) delivered in a 7-day osmotic minipump (Alza, USA) implanted subcutaneously at CIDR withdrawal.

However as indicated previously in the work with melatonin, lactation may affect the induction of oestrus and ovulation. This is akin to those natural situations where lactating hinds under conditions of poor nutrition either do not get pregnant or alternatively get pregnant late in the season as on Rhum in Scotland (Clutton-Brock et al., 1982). Although it appears that there may be problems with melatonin induction of ovulation in lactating hinds, such does not appear to be the case where gonadotrophic stimulation is used; Table 4 presents data from a recent small Invermay experiment.

TABLE 4 - Induction of ovulation prior to the breeding season in lactating 3 year old red hinds

Treatment	N	Number of hinds ovulating
CIDR[1]	6	0
CIDR + 300 IU PMS[2]	7	6
CIDR + 500 ng/h GnRH	6	3

1 14 days treatment with CIDR containing 9% progesterone starting 25 February.

2 Folligon (Intervet, Australia) given at CIDR withdrawal.

3 Sigma Chemicals (USA) delivered in a 7 day osmotic mimipump implanted subcutaneously at CIDR withdrawal.

SYNCHRONY AND SYNCHRONISATION OF OESTRUS

In any situation where it is necessary to precisely time the onset of oestrus and ovulation (eg. artificial insemination, embryo transfer) the factors influencing synchrony must be understood at least in a general sense.

Several studies have implied that mating/conception within a population of red deer hinds is highly synchronous as calving is often concentrated within a relatively short period (Lincoln and Guiness 1973; Kelly and Whateley 1975; Clutton-Brock et al., 1982), despite both the stag and the hind being capable of breeding over an extended period. More recently, studies at Invermay have shown that such synchrony can be induced. Over a period of 3 years various groups of adult non-lactating hinds have been treated with progesterone and PMS to advance the breeding season. Such treatment had the effect of synchronising oestrus in untreated control hinds run with the progesterone-PMS treated hinds with the result that the hinds calved approximately one cycle later than the treated hinds but still prior to normal calving season (Moore et al., unpublished data). The exact mechanism is unknown but may involve either a 'hind effect' (ie. an oestrous hind may stimulate other hinds to ovulate) or a 'stag effect', probably mediated by pheromonal influences.

The possibility of such a stag effect has prompted further work (Moore and Cowie, 1985). A total of 79 non-lactating hinds, run together in a group at least 250 m away from stags prior to allocation to their groups on March 7, were used. One group of 40 hinds (teased) was run with 2 vasectomised stags for 15 day from March 7-22. The second group (non-teased) were run at least 250 m away from stags for the same period. On March 22, the vasectomised stag were removed and all hinds joined with experienced stags for single sire mating. A March 22 mating would give an expected calving on November 11. Should a 'stag effect' similar to a 'ram effect' been operating, then a higher proportion of the teased hinds would have been expected to calve over the period of November 11-20, having been synchronised by the introduction of the vasectomised stags. The results summarised in Table 5, clearly indicate that this was the case (difference $P < .01$).

TABLE 5 - Calving data for groups on teased and non-teased red deer hinds

Mating group	No. of hinds	Calving				
		Median date	Mean date ± SD	Hinds calving 11-20 Nov	Spread	Total hinds calving
Teased						
1	20	20 Nov	22 Nov±6.5	11	13 Nov-5 Dec	20
2	19	21	20 Nov±6.0	9	11 Nov-4 Dec	17
Non-teased						
1	21	26	28 Nov±6.6	1	21 Nov-12 Dec	19
2	19	26	25 Nov±5.8	4	15 Nov-3 Dec	17

Therefore it would appear that manipulation of the oestrous cycle by the subtle use of cycling hinds or the timing of stag introduction is a possibility worthy of much further investigation.

As indicated previously in the discussion on the advancement of the breeding season, progesterone-containing CIDR's have been used to artificially synchronise the oestrous cycle. With CIDR's, oestrus usually occurred 48-96 hours following CIDR withdrawal (Table 6). Prostaglandin treatment has also been used successfully to synchronise oestrus in wapiti females (Glover, 1985).

TABLE 6 - Occurrence of oestrus[1] following CIDR withdrawal in mixed age red hinds

		Hours after progesterone withdrawal 0-24	24-48	48-72	72-96	96-120	Not Detected
Untreated							
N	63	0	15	20	10	4	14
%	100	0	24	32	16	6	22
Treated[2]							
N	54	11	15	9	3	1	15
%	100	20	28	17	6	2	27
Total							
N	117	11	30	29	13	5	29
%	100	9	26	25	11	4	25

1 Oestrus was detected by using a greased stag and checking daily for mating marks.

2 Includes hinds treated with PMS, FSH and GnRH as part of superovulation and early induction of oestrus experiments.

THE STAG

To date in our work at Invermay, we have concentrated on manipulating the hind and have carried out no work with stags. However it has become clear that in order to make any technique of advancing the breeding season practical on a large scale it will be necessary to advance the breeding season in stags to ensure that they are ready and willing at the appropriate time.

PHOTOPERIOD AND SEASONALITY

As with the female red deer, the male is highly seasonal with the antler and sexual cycles being closely linked. A simplified description of the endocrine regulation in male deer relevant to advancing the breeding season is shown in Figure 2. In essence the principles of regulation are similar to those operating in the female.

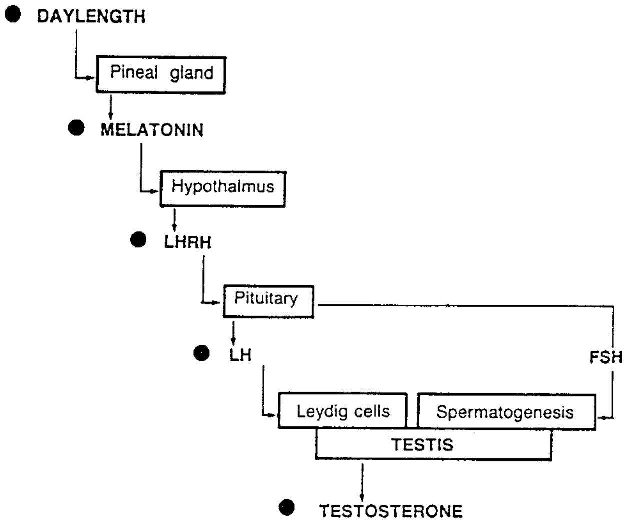

Figure 2 - Simplified description of the endocrine regulation of the reproductive cycle in male red deer

Several studies have shown that it is the change in daylength, specifically a decline in daylength, which operates as the cue to stimulate testicular development. For example, stags under artificial photoperiods with alternate 2 monthly periods of hours light:8 hours dark (16L/8D) and 8L/16D exhibited cyclical changes in testis diameter, antler status, and feed intake with a lag of about 8 weeks after the daylength change (Suttie et al., 1984). Similarly, a melatonin implant has proved effective in inducing premature testicular development about 6 weeks earlier than controls, when the stags were treated about one month prior to the summer solstice (Lincoln et al., 1984). This effect of melatonin is dependent on LH releasing (LHRH) secretion since any melatonin effect was blocked by active immunisation against LHRH. Therefore the simplest methods for advancing spermatogenesis and the onset of the rut in stags are altering daylength or treatment with melatonin.

ADVANCING THE BREEDING SEASON

PRACTICAL APPROACHES

From the foregoing discussions some tenative recommendations as to the most practical methods of advancing the breeding season by about 4-6 weeks in red deer can be put forward.

Treatment of the hinds would involve intravaginal progesterone for up to 15 days in early March followed by an intramuscular injection of about 250 IU PMS at progesterone withdrawal. A stag:hind ratio of about 1:15 in a single sire situation would be appropriate. It is also important to ensure that the stag is functional. Therefore stags should also be treated. One simple method is to run the stag into darkened pens in late afternoon from about the beginning of December. An alternative is melatonin treatment starting at about the same time.

PROBLEMS

The major problem so far has been the extended spread of calving resulting from the treatment. This is due to the fact that some of those hinds not getting in calf at the early induced oestrus do not cycle again 18 days later but return to anoestrus while others do continue to cycle and take the stag at varying intervals. Practically, the simplest solution is to run the stag with the hinds to cover the induced cycle and then to withdraw the stag until the time of the normal breeding season.

TOWARDS EMBRYO TRANSFER

There is considerable interest in embryo transfer in New Zealand as it potentially offers a considerably cheaper and safer method of importing new strains of red deer and wapiti. However before such procedures can be practical on any sort of scale there is a need for much more research into superovulation and recovery and transfer of fertilised embryos. Currently, there is a very long way to go before embryo transfer in deer can be considered as a practical technique.

SUPEROVULATION AND EMBRYO RECOVERY

Both PMS and FSH have been used to induce superovulation in progesterone synchronised hinds. The results of Invermay studies are summarised in Table 7. Although some very high ovulation rates have been recorded, fertilisation rates and embryo recovery at surgery have been variable. With FSH, part of the problem is the suppression of oestrus which in the natural situation results in a failure to mate with the stag. Timed artificial insemination should help overcome this problem.

In 1984, 12/12 expected embryos were recovered from 6 hinds and of these 11 were fertilised. However, in 1985, 26/61 were recovered from 5 hinds, of which only 9 were assessed as viable on microscopic examination.

TABLE 7 - The ovulatory responses of adult red deer to PMS and FSH. Treatments were administered at or around CIDR withdrawal or several days prior to the day of expected oestrus.

Treatment		Year	N	Number ovulated	Range of ovulations	Number with >1 ovulation
Controls		1984	4	4	1	0
		1985	10	10	1	0
PMS[1] Pregnecol	500	1984	4	2	0-1	0
	1000		10	9	0-5	7
	1500		3	3	1-2	1
Folligan	400	1984	5	4	0-3	2
	800		8	8	1-4	3
	1600		5	5	1-3	2
FSH Single injection (mg)		1984				
	15		4	4	1	0
	30		4	2	0-1	0
	60		4	3	0-2	1
FSH[2] x daily over 4 days		1985				
	4		4	4	1	0
	8		3	3	1	0
	15		8	8	1-28	6
	30		5	4	0-10	3
	60		5	5	1-5	2

1 In 1984, PMS treatment resulted in the birth of one set of viable twins.

2 FSH: BURNS - Biotech, Nebraska, USA

PROBLEMS

The major problems with embryo transfer in deer relate to the unpredictable response in ovulation rate and to the effects of surgery, with any resultant reproductive tract adhesions being the major difficulty. In the smaller species, such as the NZ red deer, surgical recovery of embryos is the only practical method. However with the larger species such as the wapiti, the less traumatic non-surgical recovery is preferred. Any substantial future research effort will depend on the use of the larger animals.

ARTIFICIAL INSEMINATION

As with embryo transfer, artificial insemination (AI) offers an alternative method to natural mating for increasing the rate of genetic progress by the use of superior sires, either imported or identified local animals. The interest in artificial insemination in New Zealand has resulted in some small research programmes being set up (see Haigh, 1985). Good quality semen has been collected using electroejaculation of stags restrained in a chute/crush system or under xylazine/fentanyl/azaperone anaethesia. Semen has been used successfully either fresh or following freezing. In Poland, Krzywinski and Jaczweski (1978) have collected semen in an artificial vagina using very tame female deer or dummies (see also Jaczewski et al., 1984). Although AI has been used successfully in New Zealand, further research is necessary to make it a viable alternative to importing or purchasing superior stags.

CONCLUSIONS

It is expected that useful techniques for advancement of the breeding season in red deer will be developed within the next 1-2 seasons. Successful artificial insemination techniques will also likely be developed within the next 2 years. However, embryo transfer has a long way to go before it can be regarded as a viable technique for increasing the rate of multiplication of high quality deer.

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