TECHNIQUES FOR ARTIFICIAL MANIPULATION OF OVULATION IN DEER

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Introduction

In New Zealand there are two major reasons for artificially controlling ovulation in deer. These are to advance the onset of mating and hence bring forward the date of calving or to control ovulation in relation to an artificial insemination programme. Most effort has been directed towards the former objective which will form the major topic of this paper.

Generally the approach has been to utilise techniques based on those already employed successfully for other species of livestock such as sheep and cattle. This approach could be justified on the grounds that a variety of manipulative techniques work with similar degrees of success in quite unrelated species. It is probably too early yet to state whether this approach has been appropriate for deer, although this paper will endeavour to describe what has been achieved so far.

Use of progestagens

In seasonally breeding animals corpora lutea (or a single corpus luteum) are present during the breeding season, or just prior to its commencement since many such animals initially ovulate without an overt oestrus (i.e. a 'silent' ovulation) at this time. Subsequent ovulations, usually associated with an overt oestrus, occur following the demise of corpora lutea and it seems that the key event here is the removal of the source of progesterone. It is only in this circumstance that some ovarian follicles (called 'pre-ovulatory') can undergo rapid growth, thus escaping atresia, and secrete large amounts of oestrogens. High blood levels of these hormones produce oestrous behaviour and stimulate the release of a surge of pituitary luteinizing hormone (LH) which causes the follicles to ovulate. All of these events are suppressed under the inhibitory influence of progesterone but can proceed spontaneously when secretion of this hormone ceases.

To mimic the natural process the artificial technique consists of administering a progestagen (e.g. progesterone or a similar compound) for a few days, then rapidly withdrawing the source of hormone. In practice this is achieved by using polyurethane sponges or silastic rubber devices which have been impregnated with a progestagen and can be inserted intravaginally or subcutaneously. Withdrawal of hormone is achieved simply by removing the sponge or device. Experience with sheep in particular has shown that administration of an oestrogen, or LH, or follicle stimulating hormone (FSH), or gonadotrophin releasing hormone (GnRH), or of any compounds which behave like these hormones (e.g. pregnant mare serum gonadotrophin - PMSG, which has FSH-like properties), at about the time of progestagen withdrawal, will enhance the response ł

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TABLE I - Summary of trials utilising intravaginal progestagen treatment in red deer hinds.

Device and duration (days) of treatment	(days)	Addıtıonal treatment	No. of hınds	Result	Reference
		DURING BREEDING SEASON	IG SEASON		
MPA sponge ^a	14 đ	none	4	3 mated on same day, 4 calved	
MPA sponge	14 d	PMSG 1000 I.U. at 14 d after 1st induced oestrus	10	9 mated over 3 d period, 3 calved	Kelly et al., 1982
		FRIOR TO BRELDING SEASON	NG SEASON		
device not stated	15 đ	PMSG 500 1.U.	5	6 calved early	Moore & Kelly, 1982/83
device not stated	15 đ	PMSG 500 I.U.	ω	<pre>2 calved early but stag suspect</pre>	Moore, 1983/84
Cronogest sponge ^b	12 đ	PMSG 500 I.U.	٢	all ovulated, 5 mated successfully	
Cronogest sponge	12-14 d	PMSG 400 or 500 I.U.	24	all ovulated, 8 mated successfully	R.R.I., 1983
Cronogest sponge	12 đ	PMSG 500 I.U.	6 yearlıng	all ovulated, all reverted to anoestrus	
с.т.р.к. ^с с.т.р.к. с.т.р.к.	15 d 15 d 15 d	PMSG 125-500 I.U. GnRH ^d 15-60µq none	12 12 10	most (27) pregnant but very few calved early, best result - 3 out of 4 given 500 I.U. PMSG calved early	M.W. Fisher, P.F. Fennessy, and J.M. Suttie, pers. comm.
a sponge containing 100 mg medroxyprogesteron b sponge containing 30 mg flugestone acetate ^c C.I.D.R. (controlled internal drug release) d	100 mg m 30 mg f cd inter	sponge containing 100 mg medroxyprogesterone acetate sponge containing 30 mg flugestone acetate (Intervet) C.I.D.R. (controlled internal drug release) device (AHI Plastic Moulding Co.) containing 0.5 g progesterone) HI Plastic A	doulding Co.) containing 0.	5 g proqesterone

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to treatment.

Results for red deer where this technique has been used are varied but encouraging (Table I). These studies have been carried out at The Rowett Research Institute, Aberdeen by Dr Clare Adam and at Invermay Agricultural Research Centre in N.Z. by M.A.F. scientists. Generally 12 to 15 days of treatment with a progestagen administered intravaginally followed (at withdrawl) by 500 I.U. PMSG has given results which combine an advancement of calving (about 4 weeks) with an acceptable calving percentage. Although this type of procedure tends to induce oestrus and/or ovulations during the anoestrous period fertility can be low and treated hinds often revert to anoestrus following the induced cycle (C.L. Adam, High doses of PMSG (e.g. 1000 I.U.) given in pers. comm.). conjunction with progestagen treatment cause super-ovulations (up to 12 corpora lutea) but do not result in many successful calvings (Kelly et al., 1982). It is obvious from the preliminary nature of much of these data that it is too early yet to pass judgement on this avenue for advancing the date of calving in red deer. In addition the use of some of the other agents (mentioned above) which have proved useful in sheep and cattle deserve attention as adjuncts to progestagen treatment in deer.

Use of melatonin

In temperate latitudes seasonal changes in reproductive activity of mammals are regulated primarily by the annual changes in length of daylight - or daily photoperiod. These changes in photoperiod influence the pattern of secretion of melatonin from the pineal gland and this in turn modifies the activity of the reproductive system. The mechanism of this latter process is unclear but it is presumably that melatonin alters the release of hypothalamic and pituitary hormones which are involved in the regulation of reproduction (e.g. GnRH, LH, FSH).

Animals which become sexually active during autumnal months (i.e. when daily photoperiods are decreasing) can be stimulated to breed prior to their normal mating season by reducing their daily exposure to light or, alternatively, by altering the pattern of melatonin secretion. This latter can be achieved artificially by administering melatonin late in each afternoon during the summer months - a procedure which mimics the nightly onset of melatonin secretion during autumn. Results obtained with red deer from the use of this procedure are summarised in Table II. Once again the technique shows promise as a basis for the advancement of calving in deer but the impracticality of daily administration of a drug counts against it.

An alternative procedure is to use constant-release implants containing melatonin since these advance the onset of reproductive and associated events in rams (Lincoln & Ebling, 1985) and stags (Lincoln, Fraser & Fletcher, 1984). A trial has been conducted at Lincoln College in which red deer hinds (both dry and lactating) were implanted subcutaneously on 27 December 1983 with single tablets containing 80 mg of compressed crystalline melatonin (G.K. Barrell & P.D. Muir,

scasonal re	productive ac	scussonal reproductive activity in red deer hinds.	•sputi		
Melatonin treatment and duration	duration	No. of hinds	Result	Reference	
5 mg otally each p.m.	127 đ	4 dry	oestrus 2-8 weeks earlier than in control hinds	Adam & Atkınson, 1985	
3 mg orally cach p.m.		yearlings (no. not given)	puberty advanced by about 5 weeks <u>me</u> .	C.L. Adam, pers. comm. mean ± S.E.M.	÷
3.75 mg 1.m. each p.m.	83 đ	4 yearlıng	all calved early 11	11 Nov ± 3 d	
[8 hours light daily]	83 đ	3 yearling	all calved early 12	12 Nov ± 2 d Webster & Barrell, 1985	385
IMUC		5 אורנאייא 3	all כהועים הר הסבשם נו 13 the	1 13 Dec ± 8 d	
10 mg orally each p.m.	57 đ	8 dry			
10 mg orally each p.m.	57 d	6 lactating	0 secretion by 65th day	day } Rodway, 1985	
none		6 dry	2		

- Summary of trials utilising melatonin or shortened photoperiod treatment to advance the onset of TABLE II

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TABLE III - Effect of melatonin s.c. implant (implanted 27 December, 1983) on calving date in adult red deer hinds at Lincoln College.

Group	No.	No. calving/No. in group	tn group	Mean date of	ca]	Mean date of calving ± S.E.M.	
				(1984)			
control		5/5		9 December	+1	± 3 days	
lactating		4/4		3 December	+ı	± 3 days	
dry		5/5		30 November ± 5 days	Ħ	5 days	

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unpublished data). Results of this trial (Table III) indicated that any effects from this treatment were negligible. However Dr Clare Adam at Aberdeen (pers. comm.) has reported that subcutaneous implants of melatonin incorporated within silastic rubber envelopes will advance the onset of oestrus in red deer by about three weeks.

This year another trial has been carried out at Lincoln College utilising 24 lactating mixed age red deer hinds, 12 of which were each implanted subcutaneously with a silastic rubber block (prepared by courtesy of Dr R.A.S. Welch, Ruakura A.R.C.) containing 300 mg of crystalline melatonin (Sigma, U.S.A.) on 24 January. On 22 February 12 animals (6 melatonin implanted, 6 control) were implanted subcutaneously with progesterone containing devices (C.I.D.R., AHI Plastic Moulding Co) which were removed on 7 March (i.e. in for 13 Six days after C.I.D.R. removal (13 March) all hinds days). were examined by laparoscopy but no corpora lutea were seen. Other results (Table IV) show some evidence for effects due to treatments but it has to be accepted that none of these procedures induced an ovulation within six days of progesterone withdrawal.

Obviously more trials need to be conducted with constant-release administration of melatonin to hinds before any sound conclusions can be made about its use.

	MELAT		NO IMPLA	-
	C.I.D.R. ^a	No C.I.D.R.	C.I.D.R.	No C.I.D.R
No. of hinds with follicles on 13 March			-	
2 follicles	1	0	2.	0
1 follicle	5	5	4	4
0 follicles	0	1	0	2
No. of hinds marked by stag prior to 28 March	3	4	2	0
State of pelage at 28 March				
no. of hinds in summer pelage	0	2	4	5
no. of ninds part_ally moulted	2	2	2	1
no. of hinds fully moulted	4	2	0	0

TABLE IV - Effects of melatonin s.c. implants (implanted 24 Januar;, 1985) with and without progesterone treatment on reproduction and pelage changes in adult red deer hinds (n=6) at Lincoln College.

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, , , , Alternative approaches for the advancement of breeding in deer hinds.

It is not known whether there is a stag equivalent of the 'ram effect' where introduction of a ram can bring forward the onset of ovulation in ewes (see review by Knight, 1983), but this possibility warrants investigation in deer.

An alternative strategy is to hybridise red deer with other species which have earlier or less pronounced seasonal This possibility is being investigated at breeding patterns. both Ruakura and Invermay agricultural research centres by utilising Pere David's deer which begin their annual breeding season earlier than red deer. On the other hand their gestation length (about 280 days; Chaplin, 1977) is longer than that of red deer so it will be interesting to see if hybrid offspring occur which combine the required attributes of both species. Other species worthy of consideration for hybridisation with red deer include sambar and rusa (Short, These originated as tropical species and in N.Z. they 1985). appear to have different breeding patterns to those of red deer (G.W. Asher, pers. comm.). However even if the inter-species breeding approach does prove to be a viable proposition it must be seen as a very long term option for advancing calving in N.Z. deer herds.

Factors affecting the advancement of breeding in hinds.

Lactation stands uppermost as a factor which may influence attempts to advance seasonal breeding in adult deer hinds. In the trial reported by Nowak, Elmhirst and Rodway (1985) melatonin treatment was ineffective in lactating hinds. Lactation is confounded with nutrition and Loudon and co-workers (Loudon, McNeilly & Milne, 1983; Loudon & Kay, 1984) have put forward the view that when red deer calves are forced to suckle frequently because of nutritional constraints on the hinds, then the inhibitory influence of lactation on reproductive activity is exacerbated. In other words it is suckling frequency which is most important in maintaining lactational anoestrous. The message from this is clear. Early (pre rut) weaning is likely to be an important contributor to any procedure for advancement of the onset of breeding.

In the case of pubertal (yearling) hinds weight may be a crucial factor for determining the date of first mating. Kelly and Moore (1977) indicate a live weight of 65-70 kg as necessary for adequate fertility in pubertal hinds, so if growth during a hind's first year is limited at all, there is little chance of an early first conception. Presumably, if hind calves are born early they will have a greater opportunity to achieve adequate live weights before their first breeding season.

A final factor for consideration is the stag. Since calving dates up to eight weeks in advance of the usual time have been reported, it can be assumed that stags are reproductively competent earlier in the breeding season than hinds. Nevertheless stags are themselves seasonal animals and their fertility outside the breeding season must be suspect. For instance Haigh et al. (1984) found that in wapiti stags spermatozoa were abnormal until shortly before the breeding season when there was an abrupt improvement in spermatozoal morphology. In programmes where it is hoped to advance the breeding season by more than six weeks, consideration should be given to treatment of stags for this purpose (e.g. by using melatonin - Lincoln, Fraser & Fletcher, 1984).

Synchronisation of ovulation.

Detection of oestrus in red deer and wapiti has proved to be difficult and so far no thoroughly reliable techniques have This severely limits the application of artificial emerged. insemination procedures in deer and has raised interest in the possibility of oestrous synchronisation in these species. Ιt is worth noting that the progesterone/PMSG treatments described above have led to synchrony of mating (e.g. see Kelly et al., 1982) and can be considered as offering potential for this purpose. Another avenue involves the use of two prostaglandin injections 12 or 13 days apart. This has been reported anecdotally as being successful in red deer and is described in the literature as suitable for wapiti (Haigh, The latter paper reported that a dose of 500 μg 1984). cloprostenol was luteolytic after day 8 of the oestrous cycle in wapiti. In the case of red deer, 3 hinds injected with two lots of a prostaglandin (0.5 to 1.0 mg fenprostalene, 'Synchrocept', Syntex Laboratories N.Z.) 12 days apart in late March/early April conceived naturally to the ovulation induced by this procedure (N.S. Beatson, pers. comm.).

For artificial breeding purposes more studies need to be carried out to determine the time of occurrence of ovulation in relation to the synchronisation procedure, and on the optimal timing for insemination.

Induction of twinning.

Red deer and wapiti usually have a single calf and incidences of twinning in these species are low (generally much less than 1% of all calvings). Some deer farmers are confident that they have identified hinds which have borne twins. It is possible that by selecting for these animals and their progeny the natural incidence of multiple births may be increased within such herds. However insufficient data are available to answer adequately questions about the competence of all hinds to bear and rear twins, the possibility of free-martinism occurring, and the growth and subsequent productivity of calves raised as twins.

Although progesterone/PMSG treatment can induce multiple ovulations it does not necessarily result in multiple births. No twins resulted from the trial carried out at Invermay A.R.C. (Kelly <u>et al.</u>, 1982) but Dr Adam at The Rowett Research Institute has reported (pers. comm.) twins born to a PMSG-treated hind. Artificial induction of twinning in red deer and wapiti thus could become a feasible proposition.

Conclusion.

It is clear that considerable scope exists for the artificial manipulation of ovulation in deer. However, because most research activity in this field is very recent

and published information arising from these studies is still limited in volume, it is not possible to be categorical about any of the procedures described in this paper. In addition the high prices currently being paid for female deer will tend to limit the amount of reproductive research that can be carried out, especially if some of the treatments could in any way jeopardise fertility of hinds.

Advances in technology for the reproductive manipulation of deer will depend entirely on the acquisition of a large body of reliable data, so much more research has to be carried out before practitioners can expect to have a scientific basis for any procedure they may wish to use for these purposes.

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