MANIPULATION OF REPRODUCTION IN RED DEER

P.F. Fennessy, M.W. Fisher*, J.R. Webster, C.G. Mackintosh

J.M. Suttie, A.J. Pearse and I.D. Corson

Invermay Agricultural Centre, Agricultural Research Division,

Ministry of Agriculture & Fisheries, Private Bag, MOSGIEL

*Alpine Helicopters Fellow

SUMMARY

Recent studies concerned with advancing the breeding season and artificial insemination (AI) and pregnancy diagnosis in red deer are described. Treatment with melatonin offers a real possibility for advancing the breeding season in yearling hinds. There is also potential for using pheromonal/behavioural factors such as the "stag effect" to advance the breeding season. In addition to treating hinds, there is evidence that stags must be treated to ensure satisfactory fertility when mating is intended for early March.

Research into AI is underway; there are considerable problems including the necessity to develop satisfactory procedures for collection of semen, synchronisation of oestrus, detection of oestrus and timing of insemination to ensure good fertility.

INTRODUCTION

An understanding of the control of the reproductive cycle is essential background in any attempt to manipulate reproduction in deer. Although there have been considerable advances in knowledge, translating these into practical on-farm approaches to manipulating reproduction will take longer.

A considerable amount of work was reviewed in the papers presented to the 1985 seminar (Fisher and Fennessy 1985; Fennessy *et al.*, 1985a; Barrell 1985). This paper, which updates information where appropriate, discusses four important areas:

* puberty in hinds
* advancing the breeding season
* artificial insemination
* pregnancy diagnosis

PUBERTY IN HINDS

Generally, puberty in red deer hinds (defined as the onset of oestrus and ovulation), occurs at about 16 months of age and is related to the nutritional status of the hind as reflected in body condition and weight. In the absence of any reproductive manipulation, yearling hinds at Invermay have never calved prior to 1 December, indicating an absence of fertile oestrus prior to about 12 April (233 day gestation).

TABLE 1: Relationship between mating weight as yearlings and subsequent calving or pregnancy rates in Invermay red deer as 2 year olds.

Liveweight (kg)	Calving rate % (1975-80, n=130)[1]	Pregnancy rate % (N) (1984, 1985, n=92)[2]
<62	0	-
62-65	51	0 (0/2)
66-69	81	0 (0/2)
70-73	78	91 (10/11)
74-77	91	88 (15/17)
78-81	97	88 (14/16)
82-85	73	90 (18/20)
86-89	85	94 (15/16)
>89	-	75 (6/8)

[1] *Kelly et al (1982)*

[2] *Data from ultrasound diagnosis of pregnancy at day 70-110 post mating; 2 of the hinds diagnosed pregnant failed to produce a calf at term.*

For red deer at Invermay, the threshold weight for attainment of puberty, ascertained from calving or pregnancy data, is in the range of 65-75 kg (Table 1). Therefore it appears that the threshold weight is about 70% of the mature body weight.

ADVANCING THE BREEDING SEASON

Regulation of reproduction

Red deer are "short-day" breeders, requiring stimulation by decreasing day length to stimulate their reproduction. Simplified schemes for the regulation of the reproductive cycles are shown in Figs 1 and 2 for the female and male respectively.

An understanding of the principles of regulation can indicate the possible routes for manipulating reproduction in deer. Where the aim is to advance the breeding season, induce superovulation or synchronise oestrus in hinds, the possibilities may involve:

(i) manipulating daylength,
(ii) treatment with melatonin,
(iii) treatment with gonadotrophin releasing hormone (GnRH),
(iv) treatment with luteinising hormone (LH) or follicle stimulting hormone (FSH); pregnant mare's serum gonadotrophin (PMSG) has both LH-like and FSH-like activity, and/or
(v) treatment with progesterone

Similarly, where the objective is to ensure that stags are sexually active and fertile, the possibilties include

(i) manipulating daylength, or
(ii) treatment with melatonin.

From Fig. 2, it is apparent why testosterone treatment itself would not be a useful approach - testosterone is essentially a result of the changes and not the primary cause.

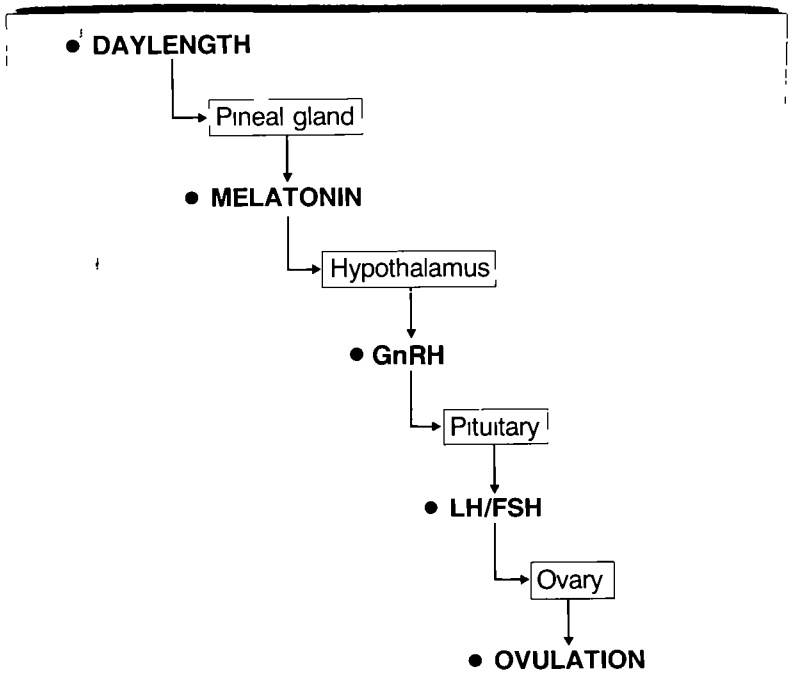

FIG 1: Simplified description of the endocrine regulation of the reproductive axis in female red deer.

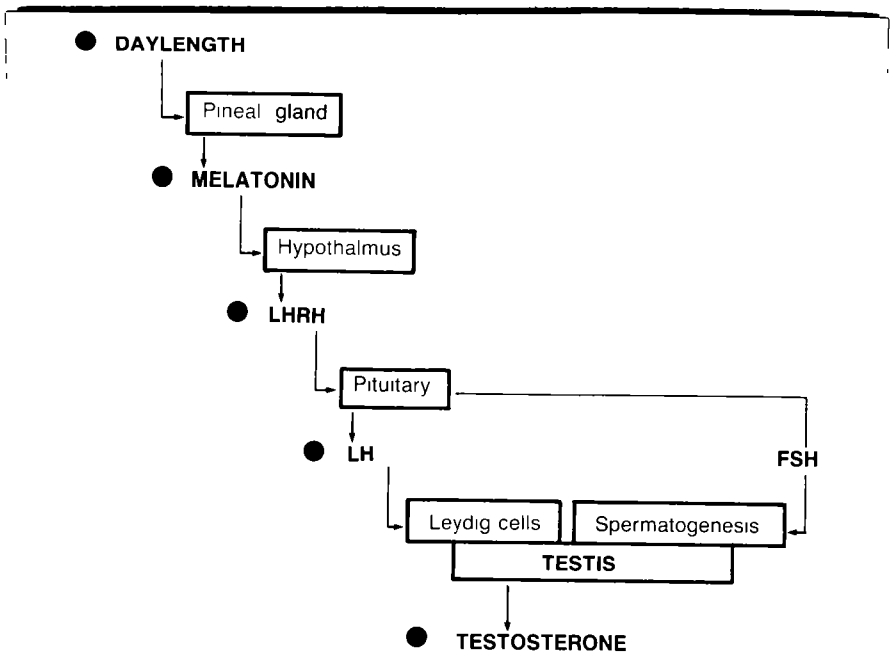

FIG. 2: Simplified description of the endocrine regulation of the reproductive axis in male red deer.

Hinds

The principles relating to treatment of hinds to advance the breeding season have been outlined. From a practical point of view, the technique must be simple and involve a minimum of extra handling of the animals, while still having a high rate of success.

Melatonin

Melatonin could fulfill the above criteria. Some of the relevant studies using melatonin were described by Barrell (1985) with the work of Webster and Barrell (1985) at Lincoln College being particularly pertinent. Yearling hinds were given daily injections of melatonin or subject to short days for a period of about 12 weeks from January through March. The results are summarised in Table 2 and indicate that treated hinds calved about 4 weeks earlier than controls.

TABLE 2: Influence of melatonin and manipulated photoperiod on mating and calving date in yearling red deer hinds (from Webster & Barrell 1985)

Treatment	N	Mating mean ± SD	Calving mean ± SD
Control	3	24 Apr. ± 13.7	13 Dec ± 7.9
Melatonin[1]	4	29 Mar. ± 6.8	11 Nov ± 3.2
Manipulated photoperiod	4	23 Mar. ± 4.6	12 Nov ± 1.7

[1] *Melatonin (3.75 mg/day) was given by intramuscular injection at 1500 h N.Z. standard time from 8 Jan to 31 March.*

[2] *Manipulated photoperiod: 8 hours dark/16 hours light daily from 8 Jan to 31 March.*

Two other methods of administering melatonin have been investigated this season, namely a new experimental long acting implant or feeding the melatonin directly to the hinds. In the experiment, 42 yearling red hinds were used: 6 were untreated controls (run some distance from the treated hinds), 20 were offered the melatonin mixed with a daily ration of pelleted feed and 16 received the melatonin implants ('Regulin', Gene Link, Australia). The treatments were applied from December to March.

Of those offered the melatonin in the feed, only 11/20 regularly ate the supplement. For those hinds which ate the supplement and for those given the melatonin implants, the treatments were highly successful. When laparoscoped in early March, following an oestrous synchronisation procedure, 80% (21/27) of the melatonin-treated hinds had ovulated whereas none of the controls nor any of the 9 which did not eat the supplement had ovulated (Table 3).

TABLE 3: Induction of ovulation (recorded by laparoscopy on 12 March) in control and melatonin-treated yearlings synchronised using progesterone or prostaglandin treatment.

Treatment	N	Hinds ovulating[1]		
		Total	Synchronisation Treatment	
			Progesterone	Prostaglandin
Control	6	0	0/6	-
Fed melatonin[2]				
Did not eat supplement	9	0	0/7	0/2
Ate supplement	11	9	4/5	5/6
Melatonin implants[3]	16	12	7/8	5/8

1 *Hinds were treated with progesterone (controlled internal drug releasing devices, CIDRs, containing 9% progesterone, AHI, Hamilton) from 17 February-4 March or with 250 mg of a prostaglandin F2 analogue (Cloprostenol, Estrumate, ICI) on 21 February and 3 March.*

2 *The supplement was offered daily at 1500 hours NZ standard time from 10 December to 6 March; a solution of melatonin in ethanol - 0.1% w/v - was mixed with a portion of the ration of pelleted feed. The feed was spread on the ground for the hinds at a rate of 0.5 kg/head/day to provide 5 mg of melatonin/head/day. 9/20 hinds offered the supplement did not eat it regularly; therefore they can be regarded as contiguous controls.*

3. *Hinds received experimental subcutaneous implants of melatonin at 28 day intervals in December, January and February.*

Although melatonin looks very promising, there are some concerns, particularly over the influence of melatonin on the pattern of weight change. Basically, it appears that the melatonin treatment has convinced the hind that autumn and winter have arrived; this is reflected in the earlier development of winter pelage in treated hinds.

Gonadotrophins in yearling hinds

Gonadotrophic stimulation, following progesterone priming (progesterone priming is necessary to ensure that the oestrogen produced by the developing follicle actually results in overt oestrus) is an alternative means of advancing the breeding season. The full range of treatments used in yearling hinds at Invermay in 1984 and 1985 has been described (Fisher *et al* 1986) and the ovulation data are summarised in Table 4. Suffice to say that although a satisfactory procedure for inducing ovulation in yearling red hinds is available (progesterone + PMSG), fertility at the resulting oestrus is poor, with only 2/14 PMSG-treated hinds calving to the induced ovulation. It is likely that stag fertility and/or behaviour is a major contributor to the problem, especially considering that yearling hinds may have a relatively short period of oestrus (12 hours).

The GnRH treatment is more complicated to administer than the PMSG treatment, and to date the ovulation response is less reliable. However, despite the low fertility at the PMSG or GnRH induced oestrus, a number of hinds did calve earlier than would normally be expected. The overall calving data are shown in Table 5.

TABLE 4: Summarised data for rising 2 year old hinds calving to an induced ovulation prior to the start of the normal breeding season (hinds treated in March at 15 months of age[1]).

Treatment	N	Ovulated at laparoscopy	Calved to induced ovulation[2]	Calved prior to 26 November[3]
Control	19	1	0	0
CIDR	18	5	2	2
CIDR/PMSG	20	14	2	4
CIDR/GnRH	34	13	3	8

1 *1984 and 1985 experiments combined; see Fisher et al (1986) for further details.*

2. *Calved about 4 November, calculated gestation lengths of < 240 days.*

3. *13/14 of these hinds ovulated in response to treatment as detected at laparoscopy.*

TABLE 5: Calving results for untreated and treated yearling red deer hinds calving as 2 year olds.

Treatment	Number calving	Calving Date	
		Mean ± SD	Spread[1]
Control	17/19	10 Dec ± 8	27 Nov - 26 Dec
CIDR	14/18	9 Dec ± 15	7 Nov - 26 Dec
CIDR/PMSG	16/20	6 Dec ± 13	7 Nov - 21 Dec
CIDR/GnRH	28/34	2 Dec ± 15	30 Oct - 22 Dec

1 *Stags in on 14 March, withdrawn on 9 May.*

Lactating hinds

Generally, lactating hinds have been regarded as a special case, because any treatment has to overcome both the seasonal anoestrus and any lactational anoestrus. Experiments conducted in 1985 and 1986 have indicated that gonadotrophic stimulation is essential to induce ovulation, with no hinds ovulating in response to progesterone alone (Table 6). The calving data (Table 7) indicate that 3/9 hinds calved to the induced ovulation in 1985. There were probably stag problems in this experiment so the calving results were not encouraging.

TABLE 6: Induction of ovulation prior to the breeding season in rising 3 year old lactating red hinds (1985 and 1986)

Treatment	N	Ovulated at laparoscopy
CIDR[1]	13	0
CIDR/PMSG[2]	13	11
CIDR/GnRH[3]	13	8

[1] *14 days treatment with CIDR containing 9% progesterone from c.23 Feb.*

[2] *300 iu Folligon (Intervet, Australia) at CIDR withdrawal*

[3] *500 ng/hour GnRH (Sigma Chemicals, USA) delivered in a 7 day osmotic minipump (Alza, USA) implanted at CIDR withdrawal.*

TABLE 7: Calving results for lactating red hinds treated to advance calving[1] (1985 results only, see Table 6)

Treatment	N	Ovulated at Laparoscopy	Hinds Calving	Calved to induced ovulation	Calving Date Mean ± SD	Spread
CIDR	6	0	6	0	29 Nov ± 12	4 Nov-7 Dec
CIDR/PMSG	7	6	6	1	20 Nov ± 17	26 Oct-4 Dec
CIDR/GnRH	6	3	5	2	18 Nov ± 19	24 Oct-7 Dec

[1] *Calves were weaned from the hinds on 13 March at the time of laparoscopy.*

Pheromonal/behavioural factors

Natural synchrony of oestrus can be induced in hinds. Moore and Cowie (1986) treated a number of hinds from a group with progesterone + PMSG to advance the breeding season. This had the effect of synchronising oestrus in the untreated hinds with these hinds calving to matings approximately 18-25 days after the induced oestrus in the treated hinds. The mechanism is unknown but almost certainly involves pheromonal/behavioural factors - it may involve a "stag effect" similar to the "ram effect" where rams are apparently necessary for stimulation of anoestrous ewes by oestrous ewes (Knight 1985).

However, there are still a considerable number of unknowns with regard to the regulation of the onset of the breeding season, with some evidence that running stags and hinds together over a considerable period may induce earlier breeding. A recent observation from a Southland farm highlights this, where 7 of 27 rising 2 year old hinds calved over about a 10 day period in late October - early November. The hinds had been part of a mixed sex group since weaning the previous year. In mid-March, the yearling stags were separated from the hinds and it was observed that at least one stag was very interested in the hinds. The real questions,

though, relate to whether the stag was a 'genetic freak', being a sexually active yearling so early in the season, and what factors actually stimulated these yearling hinds to start ovulating and exhibit oestrus in early March.

Stags

Part of the problem with low fertility at the induced oestrus prior to the normal breeding season is due to the stag, there being considerable variability between stags in terms of rutting behaviour at this time. Therefore the use of melatonin to advance the breeding season in stags is being investigated. The experimental melatonin implants, as used in the hinds, were implanted in groups of stags treated from November (early melatonin) or December (late melatonin) until February. Two groups of controls have also been used, one run with the treated stags (contiguous controls) and the other run in an area well away from the treated animals (remote controls).

Several physical measures as well as hormonal and semen changes were recorded in the groups of stags. Scrotal circumference, an indication of testicular growth, revealed that the effect of the melatonin implants was apparent by 4 weeks after the start of treatment (Table 8). Maximum scrotal circumference was recorded for the early melatonin group in January, the late melatonin group in February and for the 2 control groups in March. The treated stags started to roar in January. Antler regrowth was completely inhibited in all early melatonin stags but in only 1/6 late melatonin stags. All of the melatonin treated stags cast their hard antlers in late April/early May and grew velvet.

TABLE 8: Scrotal circumference (cm) in control (mean of 2 groups) and melatonin implanted stags (maximum scrotal circumference*)

	Control	Late Melatonin	Early Melatonin
November	18.6	18.4	19.3
December	20.6	20.9	23.7
January	22.8	25.0	*26.2**
February	26.0	*26.5**	25.7
March	*26.9**	25.2	24.4
April	24.5	23.8	23.4
May	23.4	20.9	20.5
June	23.2	19.2	18.6

The melatonin-implanted stags showed normal sexual behaviour 2-3 months after the start of treatment. However, as with the hinds there are some concerns. From the management point of view it is advisable to keep treated and untreated stags separate to minimise fighting, while a good water supply is essential where stags are rutting during the heat of summer. In addition, there is the question of possible carryover effects into next season.

Breeding in imported animals

There has been considerable interest in ensuring that deer imported from the Northern Hemisphere start breeding in New Zealand as soon as possible. However, the time of the year the animals are imported (which determines the daylength change) and their body condition at the time must be considered. Successful breeding of these animals can be regarded as a special case of advancing the breeding season.

Hinds

Hinds may not present the problem that stags do, it being possible to induce a hind to ovulate after a relatively short period of short day stimulation (it is not clear how much is actually necessary). In fact, hinds imported in November could reasonably be expected to ovulate and exhibit oestrus at about the normal time in the autumn. Similarly, those imported in April could be expected to mate successfully 2-4 months later, still within the normal breeding season of the local deer. In this case, the resultant progeny would be born during the following autumn. However, imported hinds could be treated to ensure that they ovulate, the best treatment probably being progesterone/PMSG.

Stags

Stags present a somewhat more difficult case than hinds, since spermatogenesis is estimated to require a period of 60 days. We have recently outlined a method, involving running the stags into darkened sheds each day, to improve the chances that stags imported in November shipments will exhibit a reasonable level of fertility in the following autumn (Fennessy *et al* 1985).

Although some stags imported in November shipments do apparently exhibit a reasonable level of fertility, the critical factor is the variability between animals. Fertility is often very low and the stags become sexually active late in the season (May-June). The low fertility situation is akin to that of the local red stags in February. The basic problem is that stags seem to vary to their sensitivity to daylength changes.

The probable reason that some of the imported stags are fertile so soon after importation is itself very interesting, and is related to this sensitivity to daylength changes. While red deer are short day breeders (i.e. they require a change from long to short days to stimulate their reproductive systems) some stags also respond to the reverse situation (short to long days) similarly; e.g. the occasional stag has 2 antler cycles in a year. Usually the hormonal changes in response to a short-long daylength shift are less marked that the normal short day response and are incomplete (e.g. LH responds but not testosterone, Suttie *et al.*, 1986). It seems likely that those imported stags who are fertile during the local breeding season without manipulation, may well be responding to the short to long daylength change they were subject to on importation.

TOWARD ARTIFICIAL INSEMINATION

As with embryo transfer, artificial insemination offers an alternative method to natural mating for increasing the rate of genetic progress by the use of superior sires. In Poland, Krzywinski and Jaczewski (1978) have

collected semen in an artificial vagina using very quiet stags and tame or dummy hinds (see also Jaczewski *et al.*, 1984). To make AI a viable alternative to importation or purchase of superior stags, a considerable amount of further research is necessary. Most of the relevant work has been described by Haigh (1985) and only a few points from our own recent experiences are described.

Semen Collection

Although some workers prefer to collect semen from stags physically restrained in a crush, our preference is to collect from stags under anaesthetic (xylazine/fentanyl/azaperone reversed with yohimbine/ lethidrone).

As part of the studies on the use of melatonin in stags, semen has been collected from stags by electroejaculation at monthly intervals. From this, a number of points are evident. Although there is considerable variability between stags in their response to the procedure, there is an internal consistency within stags from month to month. For example, some stags will regularly urinate in response to stimulation, while others never do so. To minimise the problem of urine contamination, it is advisable to collect semen as soon as possible after the stag is recumbent; it appears this may reduce the risk of urination. The influence of the actual area stimulated is also being investigated.

Ejaculate quality, described in terms of gross motility, individual motility, % normal sperm and sperm concentration improved markedly through the December to March period. Some of the February ejaculates were of reasonable quality with some values for individual motility and % normal sperm being greater than 70%, although the concentration was usually low at about 0.5×10^9/ml. Later samples have been as high as 2.6×10^9/ml. However, there is considerable variability between stags, possibly a consequence of the electroejaculation procedure and the particular anatomical areas stimulated.

Storage of semen in egg yolk-citrate extender for up to 8 hours prior to freezing has proved to be satisfactory. In another case semen from 2 stags was extended and held for up to 30 hours at 4°C - as judged by gross motility at 30 hours, the semen from one stag was in excellent condition, while that of the second stag was dead.

The major problem is that of obtaining a consistently high quality sample at moderately frequent intervals. The frequent collection of semen probably precludes the use of those anaesthetics currently available. Without anaesthetics, the use of the electro-ejaculator is questionable on humane grounds. Therefore practical approaches to collection via an artificial vagina (AV) must be pursued. Although the AV approach has been used by Jaczewski and his colleagues, some of their techniques cannot be regarded as practical on even a moderate scale of operations.

Synchronisation of oestrus

Successful artificial insemination depends either on a highly accurate system for detection of oestrus or a highly repeatable synchronisation procedure. The latter would allow successful insemination at prescribed times following the synchronisaiton treatment.

No completely satisfactory technique for detection of oestrus has been developed for red deer. However, the best technique so far is greasing a vasectomised stag using a thick automotive grease/coloured crayon mixture smeared liberally over the underside (avoiding the penis and scrotum) and down the inside of the legs. This technique is really only satisfactory over a short period (as the grease dries) and where hinds and stags are prevented from wallowing. Great care is also necessary during yarding to prevent stags randomly marking hinds as they are forced into close contact. Greasing usually requires anaesthetising the stag, which adversely affects his mating behaviour over the following 24 hours.

Techniques

For synchronisation, 3 techniques have been used with red deer at Invermay:

(i) progesterone (CIDR) alone
(ii) progesterone + gonadotrophic stimualtion (PMSG)
(iii) prostaglandin alone

Since much of the work has been concentrated on advancing the breeding season, few data are available from studies during the normal breeding season.

Progesterone - CIDR

The standard sheep CIDR used for red deer contains 320 mg progesterone (9% w/w). The retention rate in hinds has been very high (n=500, > 98%) with 100% during 1986 (n = 210). However, it is essential that the strings be cut short (6-8 cm in length) to prevent hinds removing the CIDRs themselves.

The aim in supplying exogenous progesterone is to mimic the luteal phase of the oestrous cycle with oestrus occurring following progesterone withdrawal. Fig 3 presents the plasma progesterone profile during a normal cycle for a red hind and Fig 4 presents that in a hind with a CIDR during seasonal anoestrus.

While the profiles differ, the basic pattern is satisfactory in that the CIDR has been used successfully to synchronise oestrus in red hinds at Invermay during the normal breeding season. Of 32 unmanipulated hinds, 27 (84%) were detected as having been mated by a vasectomised or entire stag on days 2-4 following CIDR withdrawal.

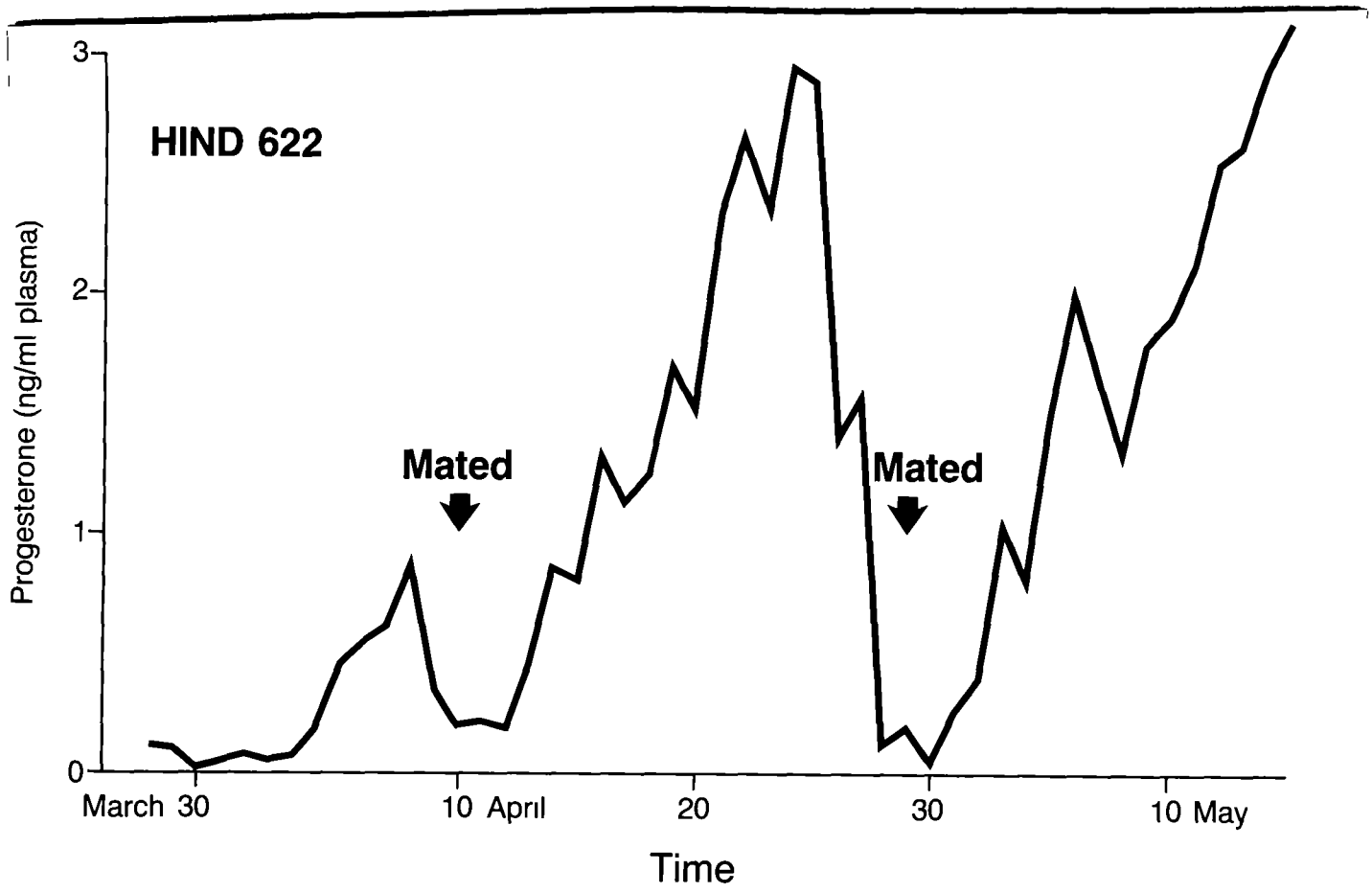

FIG 3: Plasma progesterone profile for a hind during the oestrous cycle.

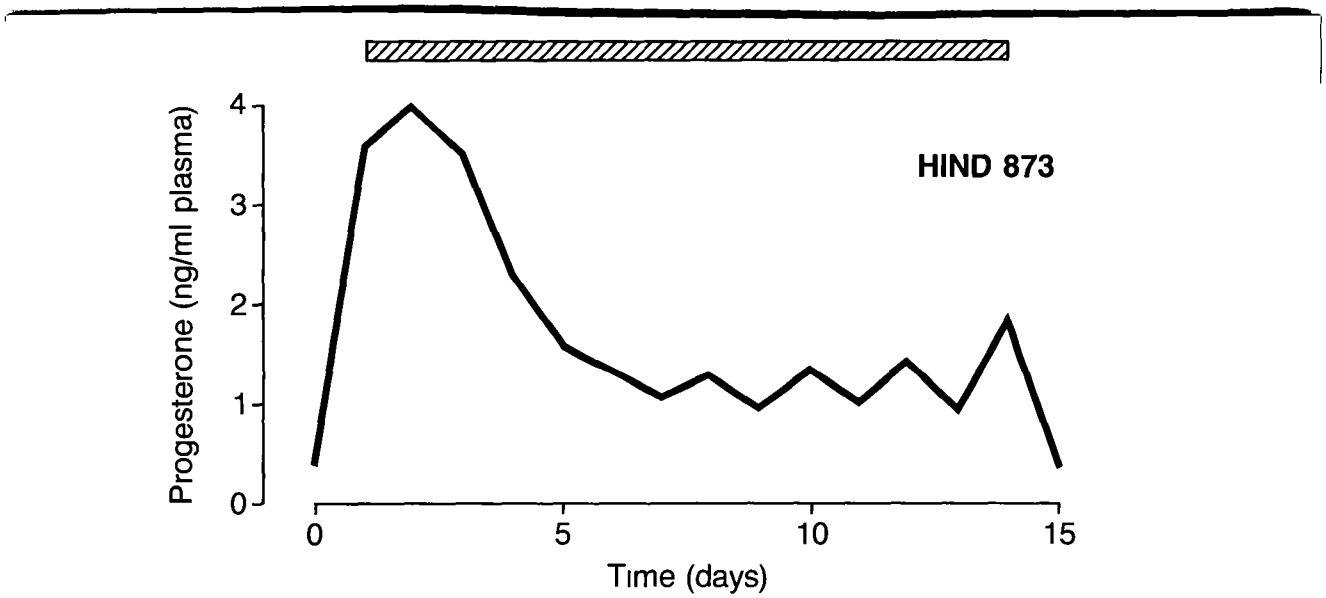

FIG 4: Plasma progesterone profile for a hind carrying a 9% CIDR (hatched area) during seasonal anoestrus.

Progesterone/gonadotrophin

Although the data are very limited, there is evidence that a higher proprotion of hinds are induced to ovulate after stimulation with 200-300iu PMSG. This may help to offset any stress which would inhibit ovulation.

In 2 experiments, treatment with PMSG advanced oestrus compared with a CIDR alone: treated hinds were marked by the stag within 24 hours of CIDR withdrawal whereas the first untreated hinds (CIDR only) were not marked until the second day (24-48 hours post CIDR).

Prostaglandins

Prostaglandin treatment has been used to induce ovulation in both wapiti females (Glover 1985) and in red deer (see Table 3). Glover (1985) found that a prostaglandin F2α analogue was luteolytic when administered 11 or more days after ovulation (cycle length of c.21 days in wapiti compared with about 18 in red deer). Treatment prior to day 9 was unsuccessful, suggesting that the early *corpus luteum* is refractory to prostaglandin induced luteolysis. Practically this would necessitate a double prostaglandin treatment with injections given 10 days apart to induce luteolysis followed by ovulation (see Table 3). Its success is absolutely dependent on the presence of a responsive *corpus luteum* at the time of treatment.

Other procedures including the combination of progesterone with prostaglandin treatment, a very effective procedure in cattle (Roche 1976; McPhee *et al.*, 1986) will be evaluated in the future.

Insemination

There are two alternative methods for insemination in red deer, namely intracervical (or intravaginal) and intrauterine, using the laparoscope. Both methods have been used at Invermay this year in attempting to produce Pere David x red hybrids. Since detection of oestrus, using a vasectomised stag is not very successful and also involves frequent yarding of hinds, a timed insemination procedure following oestrous synchronisation has been used. Although results are not yet available the procedures are outlined here.

Intrauterine insemination

Hinds were usually synchronised using a standard CIDR/PMSG regime. At 64 hours after PMSG (given at CIDR withdrawal) the hinds were anaesthetised using Immobilon (etorphine/acepromazine). When recumbent, a hind was placed in the laparoscopy cradle and given intravenous thiopentone. The hinds were inseminated with frozen Pere David or red deer semen (10-20 x 10^6 sperm per uterine horn) via the laparoscope. After allowing about 10 minutes for the thiopentone to wear off, the etorphine was reversed with Revivon (diprenorphine).

Intracervical insemination

The hinds were synchronised using the standard CIDR/PMSG regime. In one experiment the hinds were inseminated at 44 and 68 hours post CIDR withdrawal - with frozen red deer semen (25 x 10^6 per insemination) or Pere David semen (40 x 10^6 per insemination). In the second experiment, yearling hinds were inseminated at 44 and 68 hours or at 56 and 80 hours with 15 or 45 x 10^6 sperm per insemination.

In an effort to optimise the timing of insemination post-synchronisation, samples of cervical mucus have been collected and evaluated for spinbarkeit, ferning pattern, colour, etc along the lines

described in the human literature (see Elstein *et al.*, 1973). The data are promising and indicate that receptivity to the stag occurs at a time of high spinbarkeit and very marked ferning, both probably consequences of the high level of oestrogen at the time of oestrus.

PREGNANCY DIAGNOSIS

Two methods for pregnancy testing are available, based either on the concentration of a pregnancy-related factor in blood or ultrasound scanning.

Pregnancy-related factors

Ideally, diagnosis of pregnancy should be based on unequivocal evidence of the finding of a pregnancy-specific factor in body fluids (preferably blood). Unfortunately, such tests are not readily available, although recent developments with the identification of a pregnancy-specific protein in cattle mean that kits may well be available soon.

A pregnancy-specific protein (PSP-B), has been isolated from bovine placental membranes by Sasser and his group at the University of Idaho (Ivani *et al.*, 1984) and a double antibody radioimmunoassay (RIA) developed for laboratory use. PSP-B is accurate for pregnancy diagnosis in several ruminant species (cattle, goats, sheep).

In red deer hinds, PSP-B was first detected as early as 24 days after mating (Fisher *et al.*, 1986) but generally it was not detected until 30-32 days post-mating. The levels increased throughout pregnancy (Figs 4 and 5). The test is specific to pregnancy as PSP-B has not been detected in any non-pregnant hind nor in any hind prior to mating. The accuracy has been proven by real-time ultrasound scanning and by observations at calving. Although it is likely that the bovine protein, on which the assay is based has a different composition to the red deer protein, the degree of cross-reactivity in the assay makes it likely that a commercial pregnancy test kit developed for cattle will be suitable for red deer.

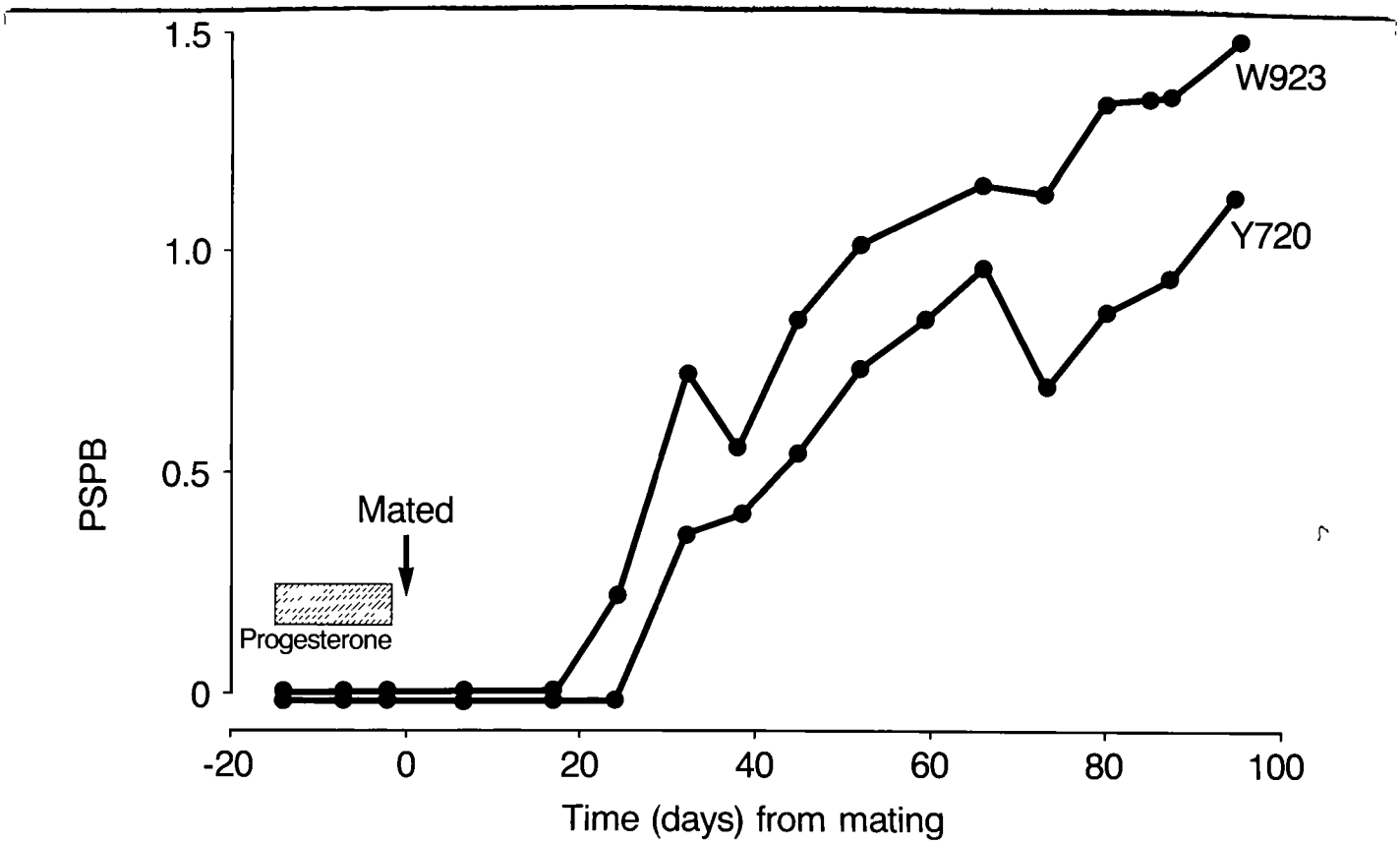

FIG 5: Pattern of PSP-B in plasma of 2 red deer hinds from the time of progesterone synchronisation through to day 95 of pregnancy.

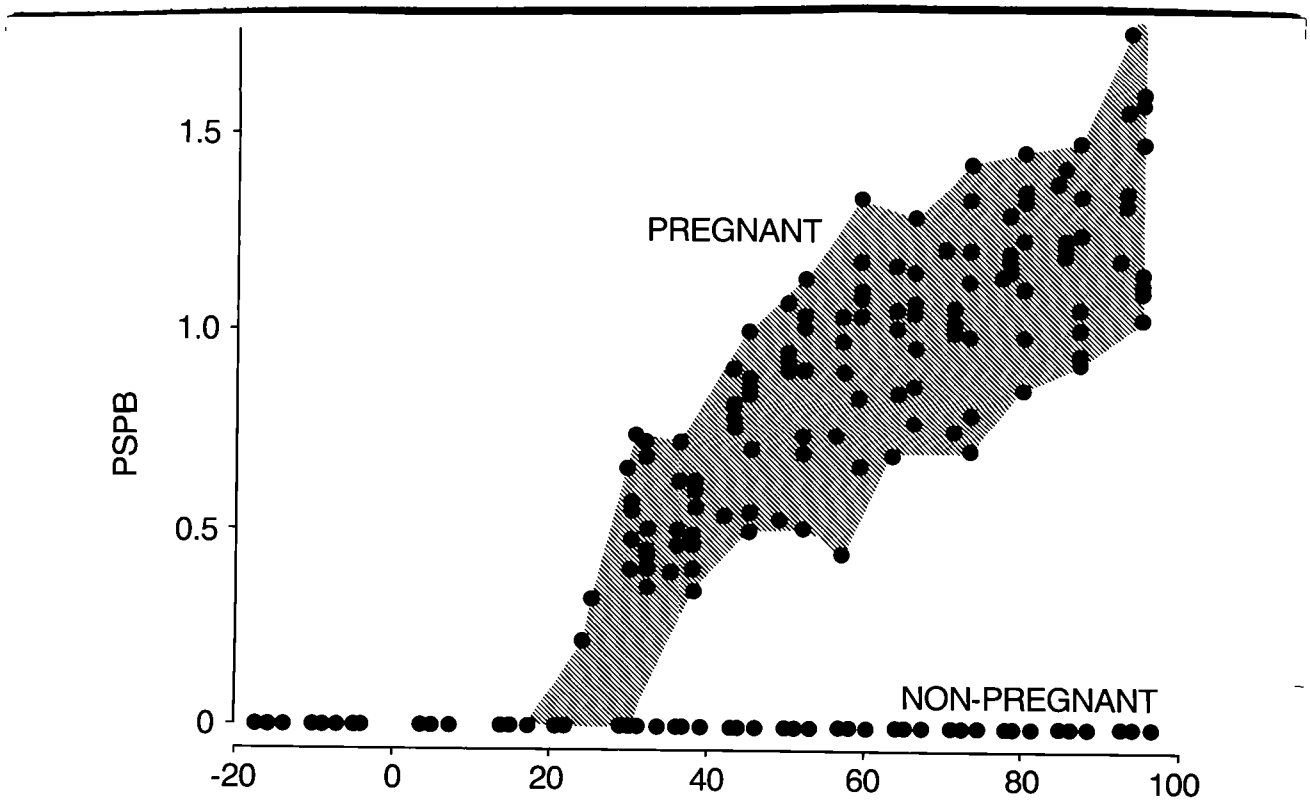

FIG 6: Plasma PSP-B concentrations in pregnant and non pregnant hinds.

Progesterone is often used for the diagnosis of pregnancy in cattle. However, since progesterone is not specific to pregnancy, accurate testing

requires sampling at times when levels would be expected to be low in non-pregnant animals and high in pregnant animals. The times when progesterone is very low are at the time of oestrus and after the end of the breeding season. The fact that the breeding season in red deer can be as long as 5 months (Guiness *et al.*, 1971) means that repeated sampling would be necessary to accurately diagnose pregnancy in hinds. However, even with repeated sampling false positives could be diagnosed due to the presence of luteal cysts or persistent *corpora lutea.* Alternatively a quantitiative difference between pregnant and non-pregnant hinds may give some improved accuracy with pregnancy testing, although the actual levels of progesterone during pregnancy have not yet been defined. However, it is likely that such a test would improve in accuracy beyond about the first trimester.

Other possibilities for pregnancy diagnosis from maternal blood samples include oestrone sulphate, which is not pregnancy-specific but is higher in some species during pregnancy than at other times. The levels have been used to diagnose pregnancy in the sow, ewe and cow 1/6 to 1/3 the way through pregnancy. Placental lactogen levels can provide a diagnosis at about 60 days in the ewe, although any red deer hormone apparently does not cross-react in the ovine assay, rendering the assay unsuitable for use in deer (P.D. Gluckman, pers. comm).

Ultrasound

Ultrasound does offer a moderately simple method for pregnancy diagnosis. However, to have any degree of certainty with diagnosis, it is essential to use a real-time scanner where the foetus and/or cotyledons can actually be visualised on a screen. With experience, the size of the foetus can give a very rough indication of the stage of pregnancy. Rectal ultrasound scanning can be simply performed with the hind well restrained in a suitable crush with positive diagnosis of pregnancy from about 45 days. However as pregnancy progresses beyond about day 80, the uterus drops away into the abdomen and it is often not possible to observe the foetus although cotyledons may still be seen. External scanning requires that the skin be well clipped and large quantities of oil applied to obtain good ultrasound coupling. The hind must be anaesthetised.

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