Anim. Prod. 1990, **51**: 613-621

0003-3561/

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ARTIFICIAL INSEMINATION OF FARMED RED DEER (*CERVUS ELAPHUS*)

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ABSTRACT

Six experiments involving the artificial insemination (AI) of a total of 300 female red deer (hinds) with frozen-thawed red deer semen (collected by electro-ejaculation) were conducted over 3 years. Insemination took place at fixed times following various oestrous synchronization procedures using progesterone withdrawal and treatment with pregnant mare's serum gonadotropin (PMSG). In the 1st year, the experiments evaluated basic AI techniques in which pregnancy rates were 45% in 20 hinds receiving two inseminations *per vaginam* (PV) and 56% in 27 hinds inseminated by the laparoscopic intrauterine method (IU). In the 2nd year, the experiments involved comparisons of the progesterone regime, one or two PV inseminations, and the timing of a single PV insemination. There was no effect of replacement of the progesterone device after 9 days and withdrawal 3 days later compared with the use of one device for the whole period in two experiments. The pregnancy rate for a double PV insemination was significantly higher than for a single PV insemination (58 and 34%; $P < 0{\cdot}05$) and there was also a small effect of timing of insemination relative to the synchronization treatment. In the 3rd year all hinds were inseminated by the IU method. The experiments involved a comparison of various times of AI following progesterone withdrawal and a comparison of the progesterone regime. The overall pregnancy rate for 63 hinds inseminated was 56% with no difference between three times of insemination (48, 52 and 55 h). In the second experiment, the difference in pregnancy rate between treatment with progesterone for 15 days and 12 days (44 and 72% for 18 hinds per group) was not significant, but the interaction between the length of progesterone treatment and insemination time was significant ($P < 0{\cdot}05$), with the 12-day progesterone/55 h insemination giving a much higher pregnancy rate than the 15-day/55 h insemination (89 and 20% respectively). Although no experiments involved direct comparisons of the routes of insemination, overall pregnancy rates were 56% for IU, 53% for double PV and 35% for single PV inseminations.

KEYWORDS: artificial insemination, conception, red deer, semen preservation.

INTRODUCTION

THERE is considerable interest in artificial insemination (AI) within the developing deer farming industry of New Zealand, particularly with red deer and wapiti (*Cervus elaphus* sp.) (Haigh, Shadbolt and Glover, 1984; Fennessy, Beatson and Mackintosh, 1987; Asher, Adam, James and Barnes, 1988a). There is also interest in the application of AI for the preservation of preferred breeding stocks and in the breeding of captive wild deer (Dott and Utsi, 1973; Krzywinski and Jaczewski, 1978; Haigh, 1984; Jacobson, Bearden and Whitehouse, 1989; Magyar, Biediger, Hodges, Kraemer and Seager, 1989).

There are difficulties with heat detection in female red deer and consequently, insemination around the time of synchronized oestrus is considered the most practical method. Therefore, the objectives of the work reported here were to evaluate insemination *per vaginam* and direct intrauterine insemination using a laparoscope in red deer and to compare different timings of insemination and oestrous synchronization procedures.

MATERIAL AND METHODS

General experimental details

Six experiments involving a total of 300 adult red hinds (> 3 years of age), were conducted over 3 years (mean live weights of

the groups of hinds range from 100 to 108 kg). All experiments were conducted at the Invermay Agricultural Centre with insemination carried out during late March/early April, the normal breeding season for adult red deer at Invermay (Moore and Cowie, 1986). The semen was collected at the Invermay Agricultural Centre (12 semen batches from seven stags) or was provided by the Monarch Stag Group, Canterbury (three batches from two stags), or by the New Zealand national sire referencing scheme (SRS; one batch). Details of semen batches and stags are given in Table 1.

Semen collection and processing

The semen at Invermay was collected from anaesthetized adult red males (>5 years of age) by electro-ejaculation (Electro-ejaculator : Pulsator III with a 240 mm long × 50 mm diameter probe (Lane Manufacturing Inc., 5560 E Pacific Place, Denver, Colorado 80222, USA)) in April 1987, March, June and July 1988. The animals were anaesthetized with 1·2 ml fentanyl citrate/azaperone/xylazine hydrochloride mixture given intramuscularly; the mixture contained 2 mg fentanyl citrate plus 16 mg azaperone (Fentaz, Roche) and 100 mg xylazine (Rompun, Bayer) per ml; the anaesthetic was reversed with an intravenous injection of about 4 ml nalorphine/yohimbine mixture containing about 1 mg nalorphine (Lethidrone, Roche) and 10 mg of yohimbine hydrochloride (Sigma Chemicals, MO, USA) per ml. Similar procedures were used for semen collection from both the Monarch stags and the stag from the SRS. Semen from these stags was collected on the owner's property in March 1987 (Monarch) and July 1988 (SRS).

Immediately following collection, the semen was held at 30°C and diluted with an equal part by volume of primary extender (84 ml of 2·9% sodium citrate, 3·0 g D-glucose, 15 ml fresh egg yolk, 1 ml antibiotic solution containing 0·1 g streptomycin sulphate). The semen was then placed in a 30°C water jacket and transported to the laboratory. Semen density was measured using a haemocytometer and diluted to contain 200 to 280 × 10^6 sperm per ml using a cryoprotectant extender (69 ml 2·9% sodium citrate, 16 ml Analar glycerol, 15 ml fresh egg yolk). Following cooling to 4°C over 2 to 4 h, the semen was drawn into 0·25 ml straws (IMV, France) (*ca.* 50 to 70 × 10^6 sperm per straw) and frozen slowly in liquid nitrogen

TABLE 1
Sources of semen batches used in the experiments

Batch	Stag	Location	Collection date	Experiment	No. of hinds inseminated
1	818	Invermay†	1 April 1987	2	27
2	850	Invermay	16 March 1988	3	8
3	259	Invermay	23 March 1988	3	8
4	149	Invermay	16 March 1988	3	8
5	106	Invermay	23 March 1988	3	9
6	745	Invermay	23 March 1988	4	30
7	818	Invermay	23 March 1988	4	30
8	850	Invermay	8 March 1988	4	30
9	850	Invermay	23 March 1988	4	30
10	899	Invermay	15 July 1988	5	21
11	850	Invermay	15 July 1988	5	21
12	850	Invermay	15 July 1988	6	19
13	745	Invermay	10 June 1988	6	18
14	A	Monarch‡	11 March 1987	1	6
15	A	Monarch	17 March 1987	1	5
16	B	Monarch	12 March 1987	1	9
17	C	SRS§	July 1988	5	21

† Collected from stags at the Invermay Agricultural Centre.
‡ Collected from two stags owned by the Monarch Stag Group in Canterbury.
§ Collected from one stag by the National sire referencing scheme (SRS).

vapour reaching −80°C in 8 to 9 min. A straw from each batch of semen used for insemination was thawed and subjectively assessed by an experienced technician at ×200 magnification for the proportion of sperm showing forward motility. Full details of the effects of time of the year on the semen collection and on semen quality of the Invermay stags will be published elsewhere (G. H. Shackell and C. G. Mackintosh, in preparation). The Monarch semen was diluted in the field and then flown to Palmerston North for processing by the New Zealand Dairy Board at Awahuri. The SRS semen was also diluted in the field and then taken to the Ruakura Agricultural Centre for processing on the same day.

Experimental design

Experiments 1 and 2 (1987). These two experiments were designed to evaluate basic AI techniques in red deer. A total of 47 hinds (20 in experiment 1 and 27 in experiment 2) were synchronized for oestrus as follows. One (experiment 1) or two (experiment 2) controlled internal drug-releasing devices (CIDR, containing 9% (340 mg) progesterone, Carter Holt Harvey Plastic Products Group Ltd, Hamilton, NZ) were inserted intravaginally for 15 (experiment 1) or 12 (experiment 2) days. At CIDR withdrawal (7 April) the hinds received 225 i.u. pregnant mare's serum gonadotropin (PMSG; Folligon, Intervet (Aust.) Pty Ltd, Lane Cove, NSW, Australia) intramuscularly. The hinds were run in groups (within experiments) with a vasectomized mature red stag from the time of CIDR withdrawal/PMSG administration until 10 to 12 days after insemination. Thereafter intact red stags were run with the hinds for 4 to 5 weeks.

In experiment 1, the hinds were inseminated *per vaginam* (PV) twice on consecutive days at around 44 and 68 h after CIDR withdrawal/PMSG treatment by one insemination technician (operator A). All hinds received at least 20×10^6 motile sperm of frozen-thawed semen from one of three semen batches (provided by the Monarch Stag Group) at each insemination. For insemination, the hinds were held securely in a hydraulically operated deer crush, and the inseminations carried out using a speculum, a light source and a lengthened goat inseminating pipette (Capristol, IMV, France). The degree of penetration of the pipette into the cervix and hence the site of semen deposition was assessed by the insemination technician and recorded as follows: at the os, or in the mid-cervical region or within the uterus itself.

In experiment 2, the hinds were inseminated directly into the uterus (IU) at around 54 h after CIDR withdrawal/PMSG treatment by operator B using laparoscopy. The IU inseminations were performed on anaesthetized hinds held in dorsal recumbency in a specially designed cradle. A laparoscope was passed through the abdominal wall just anterior to the mammary gland. Following location of the uterus, about half of the semen was deposited at about the middle of each uterine horn using needle-tipped Cassou pipettes (IMV, France). All hinds received at least 20×10^6 motile sperm from the one red stag. The anaesthetic was 3·5 ml fentanyl citrate / azaperone / xylazine hydrochloride mixture given intravenously; the mixture contained 0·4 mg fentanyl plus 3·1 mg azaperone and 20 mg xylazine per ml mixture. Following insemination, the hinds received an injection of antibiotic and the anaesthetic was reversed with an intravenous injection of 2·5 to 3·0 ml nalorphine/yohimbine mixture as used for the males.

Pregnancy status to the AI was assessed in both experiments using a real-time ultrasonic scanner (Aloka SSD-210 DXII) per rectum at about day 40 following insemination. Ultrasound diagnosis at this stage of pregnancy permits clear differentiation between a hind pregnant to the AI or to subsequent natural service (P. F. Fennessy, unpublished data). Fertility to the AI was subsequently confirmed by calving records of individual hinds.

Experiments 3 and 4 (1988). These two experiments involved comparisons of oestrous synchronization procedures with either one (experiment 3) or two inseminations (experiment 4A) PV, and a study of the effect of timing of a single PV insemination

relative to progesterone withdrawal/PMSG treatment (experiment 4B).

In experiment 3, two synchronization procedures were compared. Of the 33 hinds, 17 received a single CIDR for 12 days (A), while 16 had the original CIDR replaced by a fresh CIDR on day 9 (B). The CIDRs were then removed from all 33 hinds on 28 March, and the hinds given 225 i.u. PMSG intramuscularly as in experiment 1. The CIDR replacement treatment (B) was intended to ensure a high concentration of plasma progesterone right up to the time of CIDR withdrawal. The hinds were then inseminated once PV at around 48 h after CIDR withdrawal/PMSG treatment by operator C using the same techniques as in experiment 1. Each hind received at least 20 $\times$ 10^6 motile sperm as frozen-thawed semen from one of four red stags. The degree of penetration of the pipette through the cervix and the site of semen deposition was assessed by the operator as for experiment 1.

In experiment 4A, 40 hinds were subject to the same synchronization procedures as in experiment 3, with CIDR withdrawal/PMSG treatment on 5 April. The hinds were inseminated twice on consecutive days at around 44 and 68 h after CIDR withdrawal/PMSG treatment by operator A using the same techniques as in experiment 3. Each hind received at least 25 $\times$ 10^6 motile sperm as frozen-thawed semen from one of four red stags (five inseminations per sire per treatment group).

In experiment 4B, 80 hinds received a single CIDR for 12 days with CIDR withdrawal/PMSG treatment on 4, 5 or 6 April. The experiment was designed to compare five times of PV insemination (36, 44, 52, 60 and 68 h after CIDR withdrawal/PMSG treatment) using the same batches of semen as in experiment 4A. Each treatment group was divided into two replicates, each of eight hinds so that treatment and insemination times were not confounded. Operator A carried out the inseminations (two inseminations per semen batch per replicate).

Pregnancy status and subsequent fertility in both experiments 3 and 4 were assessed as for experiments 1 and 2.

Experiments 5 and 6 (1989). These two experiments involved comparisons of the timing of insemination by the IU route, while experiment 6 also included a comparison of two synchronization procedures.

In experiment 5, 63 hinds received a 12 day CIDR/PMSG treatment with CIDR replacement at day 9 as in experiment 3. The IU inseminations were carried out by operator B as for experiment 2, at either 48, 52 or 55 h after progesterone withdrawal/PMSG treatment. At each time, 21 hinds were inseminated with at least 20 $\times$ 10^6 motile sperm as frozen-thawed semen from one of three stags (seven inseminations per sire per treatment group).

Experiment 6 was a 2 $\times$ 2 factorial design with 37 (three groups of nine and one of 10) hinds. The hinds received either a 12 day CIDR/PMSG treatment with CIDR replacement at day 9 (as for experiment 5) or a 15 day CIDR/PMSG treatment, also with CIDR replacement at day 9. The hinds were then inseminated at either 48 or 55 h after progesterone withdrawal/PMSG treatment. The hinds were inseminated by operator B, as for experiment 2, with at least 20 $\times$ 10^6 motile sperm as frozen-thawed semen from one of two stags.

Pregnancy status for both experiments was assessed as for experiments 1 and 2.

Statistical procedures

The pregnancy rate data for the different variables in the experiments were analysed using generalized linear models (McCullagh and Nelder, 1983).

RESULTS

Semen collection

The response of the stags to electro-ejaculation was variable, although some semen was collected from most stags. Some samples were contaminated with either urine or seminal vesicular fluid rendering them unsuitable for freezing due to poor motility. Most of the good quality semen actually used in these experiments came from collections of 1 to 2·5 ml milky to thick milky semen.

TABLE 2

Pregnancy rates for the different semen batches (experiments 1 and 3)

Experiment	Semen batches used	Hinds	Semen batch†	Stag	Pregnancy rate (%)
1	3	20	14	A	0
			15	B	60
			16	B	67
3	4	33	2	850	0
			3	259	25
			4	149	63
			5	106	67

† See Table 1 for further details.

Experiments 1 and 2

The pregnancy rate in experiment 1 for the double PV insemination at 44 and 68 h was 45% (9/20 hinds), with evidence of considerable variability between semen batches ($P < 0{\cdot}05$; Table 2). There were no differences in the pregnancy rate according to the site of semen deposition with 2/4, 4/6, 3/4 for the os, mid-cervix and uterus respectively (data only for the two semen batches which resulted in pregnancies). The pregnancy rate in experiment 2 for the single IU insemination at 56 h was 56% (15/27 hinds).

Experiment 3

In experiment 3, in which each hind received a single PV insemination at 48 h, the overall pregnancy rate was 39% (13/33 hinds), with rates of 29% (5/17) following the single CIDR treatment (A) and 50% (8/16) following the CIDR replacement treatment (B); this difference was not significant. The ultrasonic pregnancy diagnoses were confirmed by the calving data. There was also evidence of variability in pregnancy rates between semen batches (Table 2; $P < 0{\cdot}05$).

Experiment 4

The pregnancy rate data for experiment 4 are summarized in Table 3. There was no significant difference between the two synchronization treatments in experiment 4A, but the double PV insemination treatment resulted in a significantly higher pregnancy rate than the single PV inseminations (58% compared with 34% overall; $P < 0{\cdot}05$). The only significant effect of the time of insemination on pregnancy rate in experiment 4B was the markedly lower rate (6%) to the 68 h PV insemination compared with the other four insemination times (41%).

There was variability in the pregnancy rate to the different semen batches in experiment 4, with a significant difference between the two batches of semen from stag 850, although there was little difference in the post-thaw motility of these two batches. The pregnancy rates and motility data for the four batches are presented in Table 4. There was no obvious relationship between the semen dose

TABLE 3

Pregnancy rate according to synchronization treatment (A or B) with the double PV insemination (experiment 4A) and the timing of the insemination with the single PV inseminations (experiment 4B)

Experiment	Synchronization/insemination treatmen: CIDR (days)	Insemination time (h)	Pregnancy rate (%)‡ Mean	s.e.	
4A	A 12	44 + 68	55	10·7	58 ± 7·5
	B 9 + 3†	44 + 68	60	10·5	
4B	A 12	36	38	11·8	34 ± 4·8
	A 12	44	44	12·0	
	A 12	52	44	12·0	
	A 12	60	38	11·7	
	A 12	68	6	5·9	

† Synchronization treatment B: original controlled internal drug-releasing devices (CIDR) replaced by a fresh one after 9 days for a further 3 days.

‡ Four batches of red deer semen were used (see Table 4).

TABLE 4

Pregnancy rates to and proportional motility post-thaw of the four batches of red deer semen used in experiment 4 (values predicted from the regression model)

Semen batch species/stag		Date of collection	Sperm per straw	Motility post-thaw	Pregnancy rate (%)	
					Mean	s.e.
Red	745	23 March	50×10^6	0·50	42	8·4
Red	818	23 March	58×10^6	0·65	49	8·5
Red	850	8 March	50×10^6	0·60	22	7·3
Red	850	23 March	50×10^6	0·65	49	8·5

TABLE 5

Pregnancy rate according to the site of deposition of semen for the single PV inseminations (experiments 3 and 4)

Site of semen deposition	Pregnancy rate			
			Overall (%)	
	Experiment 3	Experiment 4	Mean	s.e.
Os	3/4	4/20	29	9·3
Mid-cervical region	4/11	16/40	39	6·8
Intrauterine	6/18	7/20	34	7·7

used or the motility and the pregnancy rate achieved. There were also no significant interactions between the synchronization/AI procedure and the batch of semen used.

The site of semen deposition was recorded in experiments 3 and 4B, involving single PV inseminations and the data are summarized in Table 5. There was no significant effect of the site of semen deposition on pregnancy rate.

Experiment 5

The overall pregnancy rate in experiment 5 with the IU inseminations was 56% (35/63 hinds) with no significant difference between the 48, 52 and 55 h insemination times (62%, 43%, 62%) or between the three semen batches (62%, 67%, 38%) although the pregnancy rate to the third batch was markedly lower than the other two. There were no interactions between insemination time and semen batch.

Experiment 6

The results of experiment 6 are summarized in Table 6. Although the mean conception rate for the 15-day progesterone treatment was lower than for the 12-day treatment (44 and 72% respectively), the difference was not significant. However, the interaction between the length of progesterone treatment and insemination time was significant ($t = 2{\cdot}45$; $P < 0{\cdot}05$).

General

All hinds recovered uneventfully after the IU insemination while none of the hinds appeared to suffer adversely from any of the AI procedures. Two hinds diagnosed as pregnant on ultrasonic scanning at 40 days failed to calve, but both have been recorded as pregnant to AI.

No experiment involved a direct comparison of routes of insemination, although the pregnancy rates to AI by the same route across experiments were very consistent. The overall rates for the six experiments were 56% (71/127) for IU, 53% (32/60) for double PV and 35% (40/113) for single PV inseminations.

DISCUSSION

Rather than discussing the results of the individual experiments reported here, the

TABLE 6

Pregnancy rates (%) to semen for a single intrauterine insemination at 48 or 55 h (after progesterone withdrawal/PMSG treatment) following either a 12- or 15-day progesterone treatment (experiment 6)

	Insemination time (h)			
	48		55	
Progesterone (days)	Mean	s.e.	Mean	s.e.
12	56	17	89	10
15	68	16	20	13

discussion will be concerned with the overall results. The present studies indicate that timed AI with frozen-thawed red deer semen following oestrous synchronization can result in acceptable pregnancy rates in red deer hinds. This was the case for both the laparoscopic insemination into the uterus (56% overall) and for the double inseminations *per vaginam* (53%). These rates are comparable to a 60% pregnancy rate for 40 red hinds recorded following IU insemination using similar synchronization procedures in early March (about 1 month prior to the normal breeding season — P. F. Fennessy, C. G. Mackintosh and G. H. Shackell, unpublished data), and to the 49% pregnancy rate reported by Fennessy *et al.* (1987) for 152 hinds inseminated for frozen-thawed semen using a double PV procedure similar to that used in the present study. They are also similar to the fawning rates reported by Asher *et al.* (1988a) to single PV inseminations with fresh or frozen-thawed semen or a single PV insemination with frozen-thawed semen in a total of 112 fallow does. However, they are lower than the 75% rate for 53 white-tailed does inseminated with frozen-thawed semen *per vaginam* following an observed oestrus (Jacobson *et al.*, 1989). Most previously published work relating to AI in deer has involved only very small numbers of females (red, Krzywinski and Jaczewski, 1978; Asher, Adam, Otway, Bowmar, van Reenen, Mackintosh and Dratch, 1988b; wapiti, Haigh *et al.*, 1984; reindeer, Dott and Utsi, 1973; fallow, Mulley Moore and English, 1988; white-tailed deer, Haigh, 1984; and Magyar *et al.*, 1989) with variable, though often low conception rates.

It is often stated that AI at an observed oestrus results in a higher conception rate than timed AI following a progesterone/progestagen-synchronized oestrus (e.g. in cattle — Chenoweth, 1989) although in fact published data reveal little if any difference in cattle (Roche, 1974; Wishart and Young, 1974; Drew, 1978) or in sheep (Maxwell, 1976 a and b; Harvey, Johnson, Baker, Trust and Thomson, 1986; Harvey, Baker and Johnson, 1987). However, frequent handling is contraindicated in red deer, and therefore AI at an observed oestrus is not practicable on a large scale. In fact the conception rates of over 50% to the laparoscopic and double intravaginal AI in the present study are not far short of those recorded for natural mating at a synchronized oestrus (e.g. 64% for 92 red hinds synchronized with progesterone CIDR and PMSG, Moore and Cowie, 1986; 68% for 34 hinds synchronized with a progesterone CIDR alone, C. McMahon and M. W. Fisher, unpublished data). These latter values, however, are well below the conception rates resulting from natural mating at a single unsynchronized oestrus during the normal breeding season (40/49, or 82%), P. F. Fennessy and M. W. Fisher, unpublished data). In this respect, the low conception rate to a synchronized oestrus with natural mating may be due to a failure of a proportion of the hinds to exhibit oestrus and/or a failure to ovulate. In a timed AI programme, these hinds would be inseminated and although their fertility could be expected to be lower, a proportion would likely become pregnant. For example in fallow deer, Asher *et al.* (1988a) reported a 60% conception rate in those does marked by bucks compared with 30% in those not marked while Smith and Tervit (1980) reported 52 to 68% conception rate in beef cows exhibiting oestrus compared with 14 to 38% in those not exhibiting oestrus. Taken overall, these data would suggest that there is considerable potential for improving fertility at a synchronized oestrus and hence the potential conception rates to AI.

The lower fertility of the single compared with double intravaginal AI and the absence

of any effect of the timing of AI (i.e. apart from the 68 h insemination in experiment 4B) on fertility strongly suggest that there is considerable spread in the timing of ovulation in the synchronized hinds. Therefore, reducing the spread of ovulation following synchronization and ensuring that the timing of AI is more appropriate in relation to ovulation is another facet deserving further research. In this respect a higher progesterone concentration over the last few days of progesterone priming brought about by CIDR replacement could be expected to inhibit premature follicular development and perhaps result in a better synchronization. However, there was no apparent effect on conception rate in the present studies although the group sizes were too small to show small effects. This lack of a direct effect notwithstanding, there was an interaction between the duration of progesterone priming and the time of insemination in experiment 6. An improved synchronization technique could be expected to give a higher conception rate at a particular time and therefore a more marked pattern of conception rate with time of AI relative to synchronization. In fact premature follicular development in the 15-day progesterone treatment and a spread of ovulation is one possible reason for the interaction recorded in experiment 6. The actual timing of AI relative to ovulation is likely to be particularly important; for example Hunter (1985) suggests that frozen-thawed semen is probably best inseminated 12 to 18 h before ovulation.

In red hinds, the LH peak occurs about 48 h after progesterone (CIDR) withdrawal (M. W. Fisher, I. D. Corson and C. McMahon, unpublished data). Assuming the same interval between the LH peak and ovulation in red hinds as in ewes (22 to 26 h; Cumming, Buckmaster, Blockey, Goding, Winfield and Baxter, 1973), would mean that ovulation occurred about 72 h after withdrawal in the red hinds. According to the recommendation of Hunter (1985), insemination should take place about 54 to 60 h after CIDR withdrawal, which is slightly later than the timing of the IU inseminations in the present studies. However, the effect of the PMSG given at CIDR withdrawal on the synchronization or timing of ovulation is not known although Boshoff, van Niekerk and Morgenthal (1973) have shown that PMSG treatment markedly reduces the period from progesterone withdrawal to ovulation in ewes.

The lack of any obvious relationship between the post-thaw motility of semen and the conception rate suggests that this measurement is not an accurate predictor of fertility at AI, reflecting the need for further study of this aspect. However, there was considerable variability between semen batches, a common finding with AI in other species (e.g. cattle — Macmillan and Shannon, 1982). The variability was evident both between stags and for the same stag collected at different times (e.g. stag 850 in experiment 4). This variability highlights the importance of defining appropriate quality criteria and developing suitable techniques for assessment of semen quality without the expense of using the semen in AI trials. Surprisingly, there was no apparent effect of the site of semen deposition in the PV inseminations on the conception rate although the numbers of hinds were small; this lack of effect may also indicate an abundant supply of semen. In this respect, investigations of the effect of semen dosage would be worthwhile.

While the conception rates to the laparoscopic and double intravaginal AI were encouraging, there is potential for improvement. From a practical point of view, a single insemination *per vaginam* must be the objective for any large-scale AI scheme. In this respect, the New Zealand dairy cattle industry with its use of low doses of fresh semen (72 h shelf life, 2×10^6 sperm and a 60 to 65% conception rate — Macmillan and Shannon, 1982; Visser, Shannon and Wickham, 1988) provides a target for the deer industry.

ACKNOWLEDGEMENTS

We thank P. Johnstone for biometrical advice and the staff of the Invermay deer research programme, particularly M. W. Fisher, A. J. Pearse, R. W. Tibbotts, A. K. Searle, R. J. Young and A. J. Whaanga for their willing assistance with these studies. We also thank N. S. Beatson and the Monarch Stag Group for their gift of the semen used for experiment 1 and M. P. Shadbolt for the inseminations in experiments and 1 and 4.

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(*Received* 2 *January* 1990—*Accepted* 24 *April* 1990)