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Artificial insemination and synchronised natural breeding in Red deer

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ABSTRACT

Synchronised breeding systems involving 290 red deer hinds were investigated in 5 experiments with fixed-time laparoscopic artificial insemination (AI) following a synchronised oestrus and in 1 experiment with synchronised natural breeding. Experiment 1 involved an investigation of the potential for twinning; hinds were treated with PMSG and inseminated before (March 1) or during (April 12) the normal breeding season, with 29 and 40% respectively having multiple ovulations. There was no effect of AI date on pregnancy rate (0.80 and 0.71 respectively) but a greater proportion of hinds which had multiple ovulations and had not been lactating in the previous season were twin-pregnant than hinds which had been lactating (0.19 and 0.63, P<0.05). In other experiments AI was performed using red (R; n=70), Canadian wapiti (CW; n=59) or CW x R hybrid (n=11) frozen-thawed semen with pregnancy rates of 0.67, 0.66 and 0.82 respectively. Fresh Pére David's deer x R hybrid semen gave a pregnancy rate of 0.61 (n=31). A pregnancy rate of 0.84 was recorded for the synchronised natural mating of (CW x R) males with red females (n=49). Overall 3% of the R pregnancies and 9% of the hybrid pregnancies failed to reach term.

Keywords Red deer, *Cervus elaphus*, artificial insemination, oestrous synchronisation, breeding.

INTRODUCTION

There is considerable interest in artificial breeding techniques, particularly artificial insemination (AI) and synchronised natural breeding in deer, both to facilitate hybridisation between species/sub-species and to make better use of high-priced individual males (Asher *et al.*, 1988a, 1988b; Fennessy *et al.*, 1987). Previous studies with AI in red deer (*Cervus elaphus*) have been concerned with evaluation of synchronisation and insemination protocols (Fennessy *et al.*, 1987, 1990a) while studies with synchronised natural breeding have been concerned with attempts to advance the breeding season (Moore and Cowie, 1986) and to increase the number of conceptions in hinds mated to high-priced stags (Bringans and Lawrence, 1988). The present studies involved fixed-time AI or synchronised natural breeding in order to generate red deer or hybrids with red deer for research purposes at Invermay in 1990.

MATERIALS AND METHODS

Animals

Six experiments involving a total of 290 red deer hinds (rising 3 to 10 years of age) were conducted at the Invermay Agricultural Centre in 1990. Red deer (R) (Experiment 1 and 2) and Canadian wapiti (CW) semen (Experiment 3) was collected, processed and frozen at Invermay in the autumn/winter of 1988 and 1989, using electro-ejaculation while the males (all ≥5 years of age) were under general anaesthesia (Fennessy *et al.*, 1990a). Similar procedures were used off station to collect semen from 2 R males in the New Zealand national red deer sire referencing scheme and from a Pére David's deer x red (PD x R) hybrid male. In the latter case the semen was used fresh. CW x R hybrid males (2 aged 3 and 1 aged 4 years) were treated with melatonin implants to advance the breeding season (36 mg Regulin, Regulin Ltd, Melbourne; Fennessy *et al.*, 1990c) on December 4, 1989 and January 4, 1990 and some semen collected in March, 1990. These CW x R males were then used for the synchronised natural mating.

Experimental Design

The objectives of Experiment 1 were to induce multiple ovulations and twin pregnancies in red hinds and investigate whether there were any differences between hinds treated about 3 to 4 weeks prior to the normal breeding season and those treated in the normal breeding season, which at Invermay starts about March 27 (Moore and Cowie, 1986). Experiment 2 formed part of a sire referencing genetic evaluation programme for red deer, while Experiments 3 to 6 were designed to generate hybrids with red deer for comparative evaluation of growth and reproductive traits. In all experiments, hinds were ultrasonically scanned *per rectum* to assess pregnancy at days 35 to 40.

Experiment 1

The 70 rising 4 year old lactating hinds were weaned of their calves on February 14. In each part of the experiment, 35 hinds had 9% progesterone-CIDR-G devices (Carter Holt Harvey Ltd, Hamilton, New Zealand) inserted intravaginally for a total of 12 days with replacement after 8 days. At CIDR withdrawal (early group 1A, February 27; late group 1B, April 10) the hinds were given 420 iu pregnant mares serum gonadotrophin (PMSG; Folligon, Intervet, NSW, Australia). Group 1A hinds were run with two vasectomised melatonin-treated stags and Group 1B with untreated stags for 12 days from CIDR withdrawal. At 52-54 hours after CIDR withdrawal/PMSG treatment the hinds were inseminated with a minimum of 20×10^6 frozen-thawed motile sperm (15 to 40% motility) directly into the uterus using laparoscopy while under general anaesthesia (Fennessy *et al.*, 1990a). The hinds were again anaesthetised 8 days later and laparoscoped to record ovulation rate.

Experiment 2

The 70 mixed age red hinds were synchronised as for Experiment 1, except that 225 iu PMSG were used. The hinds were inseminated by laparoscopic AI using semen (minimum 20×10^6 motile sperm) from 4 males on April 5. The anaesthetic used was ketamine (Parnell Ketamine, Phoenix Ltd, Auckland; 17 mg/ml final solution)/ Rompun (xylazine hydrochloride, Bayer; 17 mg/ml final solution) at a dose of 3.5 to 4.0 mls/hind. The anaesthetic was reversed with 2.5 ml of 1% yohimbine hydrochloride (Sigma Chemicals, Mo., USA).

Experiment 3

The 59 mixed age hinds were treated as for Experiment 2, except that only 30 of the hinds had their CIDR replaced at day 8, in order to compare their pregnancy rates with hinds in which the CIDR was not replaced (Fennessy *et al.*, 1990a). The hinds were inseminated on March 23 using frozen-thawed semen from 3 CW males at a dose rate of at least 20×10^6 motile sperm (motility of 15 to 60%).

Experiment 4.

The 11 mixed age hinds were treated as for Experiment 3 and inseminated with frozen-thawed semen (minimum of 20×10^6 motile sperm) from 2 CW x R males on March 23.

Experiment 5

The 31 rising 3 year old hinds were treated as for Experiment 2 and inseminated with fresh PD x R semen on April 5; 2 ejaculate fractions were used, A (21 hinds) containing 5.5×10^6 motile (9.1×10^6 total) and B (10 hinds) containing 7.7×10^6 motile (9.6×10^6 total) sperm/dose.

Experiment 6

The 49 mixed age hinds were synchronised with progesterone-CIDR devices inserted on March 9 and withdrawn on March 21 (12 day CIDR, n = 25) or inserted on March 9, replaced on March 16 and withdrawn on March 23 (14 day CIDR, n = 24). The hinds were given 225 iu PMSG at CIDR withdrawal. On March 21, the 25 hinds were allocated at random to 3 CW x R males, and on March 23, the remaining 24 were similarly randomly allocated. The males were removed on March 30.

RESULTS AND DISCUSSION

Multiple Ovulation and Twinning

The results of Experiment 1 are presented in Table 1. There was no difference in pregnancy rate between the 2 insemination dates (0.80 for 1 March and 0.71 for 12 April). Of the 70 hinds, 49 had been lactating (wet) and 21 had failed to rear calves in the previous season (dry). While there was no effect of the time of breeding (1 March or 12 April) or previous lactational status on the proportion of hinds with multiple ovulations or on pregnancy rate, there was evidence of an effect of previous lactational status on the proportion of hinds with multiple ovulations which were twin-pregnant at

TABLE 1 (Experiment 1) Ovulation and pregnancy status of red hinds synchronised and artificially inseminated prior to (Expt 1A, 1 March) or during (Expt 1B, 12 April) the normal breeding season.

Expt.	Number (proportion) of hinds: Total	Ovulating	Ovulators with multiple ovulations	Pregnant at day 39	Ovulation rate	Twin pregnancies / Multiple ovulators
Wet hinds in previous season:						
1A	24	22	7 (0.32)	19 (0.79)	1.32	1(0.14)
1B	25	25	9 (0.36)	19 (0.76)	1.40	2(0.22)
	49	47	16 (0.34)	38 (0.78)	1.37	3 (0.19)
Dry hinds in previous season:						
1A	11	11	3 (0.33)	9 (0.81)	1.45	2 (0.67)
1B	10	10	5 (0.50)	6 (0.60)	1.90	3 (0.60)
	21	21	8 (0.38)	15 (0.71)	1.67	5 (0.63)

day 39 (3 of 16 wet and 5 of 8 dry hinds; P<0.05, Generalised linear model). While such a result could be related to the body condition of the hinds, there was no difference in the mean liveweight of wet and dry hinds (102.0 and 100.0, SED ± 4.0) at mating. Of the 8 twin-pregnant hinds, 2 produced single calves and 6 produced twins at term. The overall twinning rate was lower than that recorded in previous studies at Invermay using similar rates of PMSG (Moore, 1987; Fennessy *et al.*, 1990b), but since ovulation rates were not recorded in these studies, any comparisons are difficult.

Pregnancy and Calving Rates

The pregnancy rates for Experiment 1 to 4 recorded at day 35-40 after AI are summarised in Table 2. There were no significant differences between males within experiments in pregnancy rates with the overall rate of 0.70 (147/210) for intrauterine laparoscopic insemination with frozen semen being higher than that reported in previous studies with red females (56%, Fennessy *et al.*, 1990a; 64%, Bowen, 1990). There were also no relationships between pregnancy rates and semen dose or motility within experiments.

There was no significant difference between the single CIDR and CIDR replacement treatments in Experiment 3 (0.63 and 0.69 respectively), although the direction of the difference was the same as in previous work with a slightly higher pregnancy rate following CIDR replacement (Fennessy *et al.*, 1990a).

The conception rate of 0.65 with the fresh PD x R hybrid semen was very satisfactory, with conception rates of 0.71 to fraction A and 0.50 to fraction B. The overall rate contrasts with the rate of 0.17 recorded to AI with frozen PD x R hybrid semen in 18 red hinds in 1989 although this was due to the failure of semen from 1 male (3/6 and 0/12 pregnancies from the 2 males; P.F. Fennessy, C.G. Mackintosh and G.H. Shackell, *pers. comm.*). However, in our experience with 2 PD x R hybrids, the frozen semen has low post-thaw motility (10% or less, G.H. Shackell, I.C. Scott and G.W. Asher, *pers. comm.*) despite pre-freezing motilities of >80%. In such a situation, AI with fresh semen is strongly indicated.

The pregnancy rate for synchronised natural mating to the CW x R males at a ratio of 1 stag to 8 or 9 hinds on each occasion in Experiment 6 was 0.84, with rates of 0.88 and 0.79 for the 2 synchronisation treatments (ie. 12 and 14 day CIDR treatments) respectively. For the individual males, the rates over both CIDR treatments were 0.88, 0.82 and 0.81. These rates are similar to, or higher than, those reported previously for mating at a synchronised oestrus (64%, Moore and Cowie, 1986; 68% for 34 hinds, C. McMahon and M.W. Fisher, *pers.*

TABLE 2 Details and results of experiments with artificial insemination (AI) of synchronised red deer hinds with semen[1] from various stags.

Expt.	Species of stag	No. of hinds	No. of stags	Date of AI	Pregnancy rate	Calving rate	Individual stag pregnancy rate
1A	R	35	3	1 Mar	0.80	0.80	0.92, 0.73, 0.75
1B	R	35	3	12 Apr	0.71	0.71	0.85, 0.73, 0.55
2	R	70	4	5 Apr	0.67	0.63[2]	0.70, 0.74, 0.64, 0.55
3	CW	59	3	23 Mar	0.66	0.61	0.70, 0.71, 0.60
4	CW x R	11	2	23 Mar	0.82	0.64	1.00. 0.50
5	PD x R	31	1	5 Apr	0.65	0.58	0.65

[1] All frozen-thawed semen, except Experiment 5 (fresh semen).

[2] One hind slaughtered for behavioural reasons at c.180 days gestation; it is assumed she would have carried the calf to term.

comm.; 87%, Bringans and Lawrence, 1988) and similar to conception rates to a single natural oestrus (82% for 49 hinds, P.F. Fennessy and M.W. Fisher, *pers. comm.*).

Over all of the experiments, 13 of the 208 hinds pregnant on ultrasonic diagnosis, failed to calve. Of these, 3 were pregnant to a single red male in Experiment 2, 1 and 2 to CW males in Experiment 3, 2 to a CW x R hybrid male in Experiment 4, 1 and 2 to the 2 other CW x R hybrid males in Experiment 6 and 2 to the PD x R hybrid male in Experiment 5. The overall abortion rate from around day 40 to term was 6.3%, but was 3.0% (3/100) for R x R pregnancies, which is similar to previous work at Invermay (4 of 167 or 2.4%, Fennessy *et al.*, 1990a and P.F. Fennessy, unpublished data). The major contributors to the abortion rate were the hybrid pregnancies where 9.2% (10/109) failed to reach term. This is not unexpected, in that the greater the genetic distance between the parental strains/species, the more likely pregnancies are to fail. This is exemplified by the situation with PD x R pregnancies where only 6 of 11, recorded at day 40 in Invermay studies, have gone to term (P.F. Fennessy, C.G. Mackintosh and G.H. Shackell, *pers. comm.*).

Practical Implications

The high pregnancy rates to AI and to a synchronised natural mating in these studies are encouraging in terms of the potential for artificial breeding systems in the New Zealand red deer industry. This is especially so with AI in view of the increased availability of semen, albeit often at high prices. The success of synchronised natural breeding highlights the possibilities for more intensive usage of valuable stags, possibly over an extended mating period (Bringans and Lawrence, 1988). As a routine AI system, laparoscopic AI is expensive, but the success rates reported here and in previous papers (Fennessy *et al.*, 1990a; Bowen, 1990), particularly in comparison with cervical AI provide a basis for considering its use, although in field usage some poor conception rates have been reported (Hegarty, 1991). Interestingly as with previous studies at Invermay (Fennessy *et al.*, 1990a) there was no evidence of any difference in the pregnancy rate to AI before and during the normal breeding season. Although twinning can be induced successfully (Moore, 1987; Bringans and Lawrence, 1988; Fennessy *et al.*, 1990b), and reasonable rates of growth obtained, intensive management is required to achieve high weaning rates. For practical application a more reliable induction procedure would also be a considerable advantage.

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