Anim. Prod. 1988, **47:** 487-492

0003-3561/88/82

ARTIFICIAL INSEMINATION OF FARMED FALLOW DEER (*Dama dama*): FIXED-TIME INSEMINATION AT A SYNCHRONIZED OESTRUS

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ABSTRACT

Two trials were conducted in 1986 on artificial insemination of female fallow deer at fixed intervals from the cessation of oestrous synchronization treatment. Semen had been collected previously from mature bucks by electroejaculation and extended in sodium citrate/egg yolk diluent.

In the first trial involving a comparison of the fertilization rates of fresh and frozen-thawed semen delivered intravaginally, 57 does each received a single intravaginal progesterone-releasing device (CIDR-type S, Carter Holt Harvey Plastic Products Group Ltd, Hamilton, NZ) for a 14-day period early in the 1986 breeding season. All does were inseminated intravaginally with either fresh (no. = 26) or frozen-thawed (no. = 31) semen (85×10^6 motile spermatozoa per inseminate) at 48 h after CIDR removal. The apparent conception rates for the two types of semen were 65·4% and 64·5% respectively ($P > 0{\cdot}1$) and the actual fawning rates were 50·0% and 48·4% respectively ($P > 0{\cdot}1$).

In the second trial involving an investigation of the feasibility of laparoscopic intrauterine insemination, 55 does were synchronized as for the first trial. At 56 to 58 h from CIDR removal, the does were anaesthetized and laparoscopically inseminated with frozen-thawed semen (85×10^6 motile spermatozoa per animal) by direct injection into both uterine horns. Anaesthesia was reversed immediately following artificial insemination. The apparent conception rate was 47·3% and the actual fawning rate was 41·8%.

Data from both trials indicate that reasonable fawning rates can be obtained for artificially inseminated fallow deer. Between 11 and 25% of does expected to fawn did not and this may represent embryonic mortality attributable to the method of oestrus/ovulation synchronization.

INTRODUCTION

Farming of fallow deer (*Dama dama*) for venison production has gained widespread acceptance around the world and, in New Zealand, the species has been integrated successfully into traditional low-cost pastoral grazing systems. However, only in recent years have there been significant advances in handling and restraint techniques for this species, which has a reputation for intractability and volatility when yarded. It is not surprising, therefore, that little attention has been given previously to manipulative control of fallow deer reproduction. However, the recent establishment in New Zealand of genetic improvement programmes for farmed fallow deer has highlighted the need for establishing suitable protocols for artificial insemination as a means of disseminating genes of high genetic-merit bucks more widely and more rapidly than through natural mating.

Fallow deer are highly seasonal breeders. In New Zealand, does exhibit their first oestrus of the breeding season in late April to early May (autumn). Most conceptions occur during this period, which corresponds to the rutting period of bucks (Asher, 1986 and 1987). In the absence of conception, does can exhibit regular 21- to 22-day oestrous cycles for a 5 to 6 month period (Asher, 1985). Recent studies have shown that artificial synchronization of oestrus can be achieved within the breeding season by the use of intravaginal progesterone-releasing devices (Asher, Barrell and Peterson, 1986). As spermatogenesis in fallow bucks is not continuous throughout the year (Chaplin and

White, 1972; Chapman and Chapman, 1975) semen collection is also under seasonal constraints. However, recent studies have shown that ejaculates containing high concentrations of motile spermatozoa ($> 4 \times 10^9$ spermatozoa per ml) can be obtained by electroejaculation during and immediately after the rut (Asher, Day and Barrell, 1987).

The present studies investigated the conception and fawning rates for fallow does which were artificially inseminated at a synchronized oestrus within the natural breeding season.

MATERIAL AND METHODS

Animals and management

The fallow deer used in these trials consisted of farm-reared, parous does (no = 112) and mature (> 3 years old) bucks maintained at pasture on the Ruakura Animal Research Station (37° 46′ S, 175° 20′ E). The deer were offered supplementary rations of meadow hay over the winter period.

Semen collection and processing

Semen was collected from bucks by electroejaculation as described by Asher *et al.* (1987). Ejaculates processed for freezing were collected from 11 bucks in July 1985 (i.e. post rut). Each ejaculate was evaluated for volume, density and motility (Asher *et al.*, 1987), extended in sodium citrate/egg yolk diluent with 8% glycerol as cryoprotectant (Krzywinski and Jaczewski, 1978) to a concentration of 200×10^6 motile spermatozoa per ml, equilibrated for 4 h at 4°C and subsequently loaded into 0·5 ml straws (100×10^6 spermatozoa per straw). The semen was then frozen in a programmable freezer. It was immersed into N_2 vapour and further reduced from −75°C to −125°C at a rate of 6°C/min, at which point it was immersed into liquid N_2 until required for insemination.

Ejaculates processed for fresh inseminations (no. = 4) were collected 2 h prior to artificial insemination (i.e. 7 May 1986), evaluated for volume, density and motility, and extended in the same diluent (+ cryoprotectant) to a live spermatozoa concentration equivalent to that observed for frozen-thawed semen for use on the same day (i.e. 85×10^6 motile spermatozoa per 0·5 ml inseminate). The extended semen was maintained at 37°C until required for insemination.

Trial 1: intravaginal insemination of thawed and fresh semen

A total of 57 does had their first oestrus of 1986 synchronized with intravaginal silastic elastomer devices containing 0·5 g progesterone (CIDR-Type S, 12% progesterone; Carter Holt Harvey Plastic Products Group Ltd, Hamilton). Each doe received a single CIDR for a 14-day period from 21 April. At CIDR removal (10.00 h) the does were joined with crayon-harnessed vasectomized bucks (Asher, 1985) at a buck:doe ratio of about 1:28. Each doe received a single intravaginal insemination (i.e. one straw) of either fresh (no. = 26) or frozen-thawed (no. = 31) semen (85×10^6 motile spermatozoa per insemination), 48 h following CIDR removal. This timing was estimated as the mean interval to onset of oestrus (Asher *et al.*, 1986). At each insemination each doe was restrained in a specially designed cradle and the semen deposited near the os cervix without the aid of a speculum. The entire procedure lasted about 20 s per doe and mating marks were recorded at this time.

Trial 2 : intrauterine inseminations

A total of 55 does each received a single intravaginal CIDR for a 14-day period from 22 April (no. = 27) or 23 April (no. = 28). At CIDR removal (06.00 h) the does were joined with crayon-harnessed vasectomized bucks at a buck:doe ratio of about 1:27. Each doe received a laparoscopic insemination (Asher, Adam, Otway, Bowmar, Van Reenan, Mackintosh and Dratch, 1988) of frozen-thawed semen (85×10^6 motile spermatozoa per doe) into both uterine horns 56 to 58 h following CIDR removal, based on studies on intrauterine insemination in sheep (Maxwell, 1984). At insemination each doe was anaesthetized with 5 mg xylazine hydrochloride (Rompun; Bayer Leverkusen, Germany) and 5 mg ketamine hydrochloride (Ketalar; Parke-Davis Pty Ltd, USA) per kg

live weight. The abdomen was inflated with CO_2 gas and the semen injected into the lumen of the uterus. Following insemination, anaesthesia was reversed with 0·25 mg yohimbine hydrochloride (Recervyl; Aspiring Animal Services Ltd, Wanaka, NZ) per kg live weight. The entire procedure lasted about 20 min per doe and mating marks were recorded at this time.

Assessment of conception rate

Vasectomized bucks were removed from the doe groups following artificial insemination. Crayon-harnessed fertile bucks were joined with the does (one buck : 30 does) from about 14 days following artificial insemination to 10 June. Twice daily observations were performed to detect crayon mating marks (i.e. returns-to-service) while bucks were present with does. Observations were performed with a telescope from an enclosed observation tower.

All does were blood sampled by jugular venipuncture on day 21 following artificial insemination (i.e. 28, 29 or 30 May). The plasma samples were analysed for concentrations of progesterone (Asher *et al.*, 1986) to identify cycling does (i.e. failure to conceive to artificial insemination). Further blood samples were collected on 10 October (late gestation or anoestrum) and plasma progesterone concentrations determined to identify pregnant and non-pregnant does (Asher, 1987).

Fawning data

All fawns born during the subsequent fawning season in December 1986 and January 1987 were tagged, weighed and had their sex recorded within 12 h of birth. They were identified to their respective dams either by direct observation of parturition or by multiple observations of suckling activity (Asher and Adam, 1985).

Fawns were assessed as having been conceived to artificial insemination if born within 234 ± 8 days of the insemination (Asher, 1987).

Statistical analyses

The data were subjected to χ^2 analyses where appropriate. Individual buck variation was investigated by logit analysis and found to be negligible.

RESULTS

Evaluation of frozen-thawed semen

Evaluation of frozen-thawed semen from straws representing all processed ejaculates revealed post-thaw motility rates of about 85%, with no apparent differences between ejaculates. Therefore, each straw (inseminate) was estimated to contain 85×10^6 live spermatozoa and the concentration of motile spermatozoa in fresh semen was adjusted accordingly.

Trial 1

Of the 57 does receiving intravaginal inseminations, 31 (54%) were recorded as having crayon mating marks at the time of insemination (48 h from CIDR removal). The proportion of does apparently conceiving to artificial insemination, based on the non-return data and the occurrence of plasma progesterone concentrations in excess of 2·0 µg/l (6·4 nmol/l) on day 21, was very similar for does receiving fresh or frozen-thawed semen (65·4% *v.* 64·5%, $P > 0·05$; Table 1). Similarly, the actual proportion of does fawning to artificial insemination was not significantly different between the two groups (50·5% *v.* 48·4%, $P > 0·05$; Table 1).

TABLE 1

Conception rates and fawning rates of intravaginal insemination with fresh and frozen-thawed semen (trial 1)

	Does inseminated	Does conceiving to insemination	Does fawning to insemination	Embryonic mortality
Fresh semen	26	17 (65·4%)	13 (50·0%)	4 (23·5%)
Frozen-thawed semen	31	20 (64·5%)	15 (48·4%)	5 (25·0%)
Total	57	37 (64·9%)	28 (49·1%)	9 (24·3%)

Differences between the apparent conception rates and the actual fawning rates (Table 1) provide a possible indication of the incidence of embryonic mortality (23·5 to 25·0%). The does involved failed to fawn.

Trial 2

Of the 55 does receiving intrauterine inseminations via laparoscopy, 24 (44%) were recorded as having crayon mating marks at the time of insemination (56 to 58 h from CIDR removal). A total of 26 does (47·3%) apparently conceived to artificial insemination but, of these, three does (11·5%) failed to fawn. The actual fawning rate to artificial insemination was 41·8% (Table 2).

Relationship between detected occurrence of induced oestrus and subsequent fawning rates to insemination

There was a tendency in both trials for does marked by the buck at the time of artificial insemination to have a higher fawning rate to the insemination than unmarked does (Table 3) although the difference in proportions was significant for trial 1 only (64·5% *v.* 30·8%, $P < 0·05$).

DISCUSSION

The present studies have demonstrated that artificial insemination of farmed fallow deer can result in acceptable fawning rates, although there is considerable room for improvement. Previous studies on other cervids have produced variable and often low (< 30%) fawning rates following various methods of oestrous synchronization/detection and insemination (e.g. red deer: Krzywinski and Jaczewski, 1978; Asher *et al.*, 1988; wapiti: Haigh, Shadbolt and Glover, 1984; white-tailed deer: Haigh, 1984; reindeer: Dott and Utsi, 1973).

The approximate 50% fawning rate obtained from intravaginal insemination of fallow does indicates that this technique may have widespread acceptance amongst fallow deer farmers, especially considering the high apparent viability of frozen-thawed semen. This method of insemination is of low cost and simple to perform compared with laparoscopic intrauterine inseminations. The timing of intravaginal inseminations relative to the oestrous synchronization treatment was based on the average interval between CIDR removal and the onset of oestrus observed in previous studies on fallow deer (Asher *et al.*, 1986). In experiment 1, 54% of does were recorded as having mating marks by the time of insemination (i.e. 48 h after CIDR removal), which is in accordance with the average interval from the previous studies. However, only 44% of does in experiment 2 had mating marks 56 to 58 h after CIDR removal. It seems likely that this reflects variation in the ability of bucks adequately to

TABLE 2

Conception rate and fawning rate to intrauterine insemination of frozen-thawed semen (trial 2)

Does inseminated	Does conceiving to insemination	Does fawning to insemination	Embryonic mortality
55	26 (47·3%)	23 (41·8%)	3 (11·5%)

TABLE 3

Relationship between fawning and detected mating marks at the time of insemination in trials 1 and 2

	No. of does	Does marked by buck (%)	Fawns born to: marked does (% of marked does)	Fawns born to: unmarked does (% of unmarked does)
Trial 1	57	31 (54·4%)	20 (64·5%)	8 (30·8%)**
Trial 2	55	24 (43·6%)	13 (54·2%)	10 (32·3%)
Total	112	55 (49·1%)	33 (60·0%)	18 (31·6%)*

serve does at the synchronized oestrus. Previous studies have resulted in 95 to 100% of does being detected in oestrus following CIDR removal (Asher *et al.*, 1986; G. W. Asher, unpublished data) but at much lower buck : doe ratios (1:10) than used in the present study (1:27 to 28). It is interesting to note, however, that the success rate to artificial insemination was apparently higher for does mated by the time of insemination than for unmated does. This suggests that some does may have failed to respond to CIDR removal within the critical time period from insemination or that perhaps mating (copulation) is an important stimulus to ovulation in fallow deer. The latter point is highly speculative as there are no data pertaining to reflex ovulation in cervids and earlier studies on fallow deer have shown that the pre-ovulatory LH surge is initiated just prior to the onset of oestrus (Asher *et al.*, 1986).

The data from the present study indicate the possibility of high embryonic mortality (up to 25% in these trials) following artificial insemination at the CIDR-induced oestrus, although the possibility of some mis-diagnosis of conception cannot be discounted. As the does involved all failed to fawn, it seems likely that if embryonic loss occurred it did so sometime after buck removal on 10 June (i.e. at least 31 days after artificial insemination). Similarly, in a 1985 trial in which 20 fallow does were naturally mated following CIDR removal in early May, all does apparently conceived to the induced oestrus, but 4 (20%) failed to fawn (G. W. Asher, unpublished data). Similar cases of apparent embryonic mortality have not been observed in fallow does conceiving to spontaneous (natural) oestrus (Asher, 1987) and it therefore is possible that conceptions following CIDR-induced ovulations have lower viability than those from spontaneous ovulations. It is, of course, possible that some does in the present trial exhibited undetected short (8 to 10 day) oestrous/luteal cycles following the induction of oestrus, resulting in high plasma progesterone concentration on day 21 from insemination. This would result in mis-diagnosis of conception in some does. However, it would be expected that at least some of these does would have been mated by the fertile bucks and become pregnant sometime following artificial insemination. Plasma progesterone profiles (3-day samples) of the 20 does in the 1985 study did not lend support to the occurrence of short cycles following CIDR removal (G. W. Asher, unpublished data).

In the present study, intrauterine inseminations produced lower conception/fawning rates than intravaginal inseminations. The timing of intrauterine inseminations (56 to 58 h from CIDR removal) may have been suboptimal for this species. However, there are no data pertaining to sperm transit rates and the timing of ovulation relative to the onset of oestrus (or CIDR removal) in fallow deer from which to establish the optimum timing for intrauterine inseminations. In addition, the use of general anaesthesia for laparoscopic insemination, coupled with the additional stresses of handling imposed on the does, may have influenced the fertility rate.

ACKNOWLEDGEMENTS

Thanks are due to T. Braggins for performing the progesterone analyses and A. M. Day for veterinary assistance during intrauterine inseminations.

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(*Received* 12 *April* 1988—*Accepted* 7 *June* 1988)