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Seasonal effects on the efficacy of intravaginal CIDR devices for oestrous synchronization of farmed fallow deer (*Dama dama*)

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ABSTRACT

Eighty mature fallow deer does were allocated to four treatment groups (n=20) for which oestrous synchronization treatment consisted of intravaginal insertion of single CIDR devices for 14 days and prostaglandin administration on days 0 and 10 of device insertion. Treatment groups were staggered successively by 7-day intervals from early March to late May, the period spanning the natural mating season (rut). Observations to determine the onset of oestrus were performed 24-72 hours after CIDR device withdrawal and blood samples were collected four times during insertion and then every 2 days, for 14 days, from CIDR device withdrawal. Plasma samples were analysed for progesterone concentration to determine luteal development. A group of untreated contemporary control does were allowed to mate naturally to crayon-harnessed bucks to determine the period of the 1990 natural rut. Oestrus and ovulation were detected on 69% and 80% of occasions respectively following CIDR device withdrawal, with ovulation following oestrus on 98% of occasions. Ovulation not preceeded by observed oestrus, probably representing silent ovulations, occurred early in the breeding season. There was complete failure of response, in terms of does exhibiting overt oestrus, at the start of the trial. As the breeding season progressed, the number of does exhibiting oestrus increased from 0-10% to 89-100% with the greatest response coinciding with the onset of the natural rut. In addition, the mean interval from CIDR device withdrawal to onset of oestrus tended to decrease from between 71-88h to 47-63h and oestrous synchrony improved progressively during the trial. The efficacy of CIDR devices in synchronizing oestrus and ovulation in fallow deer does is clearly influenced by timing of treatment relative to the natural breeding season of the herd.

Keywords Fallow deer, *Dama dama*, oestrous synchronization, CIDR devices.

INTRODUCTION

In light of recent trends to establish artificial insemination of fallow deer (*Dama dama*) as a genetic tool for farmers (Asher *et al.*, 1988a; Asher *et al.*, 1990b; Mulley *et al.*, 1988) it has become necessary to establish suitable protocols for oestrous synchronization. The use of progesterone priming with intravaginal Controlled Internal Drug Releasing (CIDR) devices followed by administration of pregnant mare serum gonadotrophin (PMSG) treatment to successfully induce oestrus and ovulation prior to and during the breeding season has been documented for red deer (Fisher and Fennessy, 1987; Fisher *et al.*, 1989; Moore and Cowie, 1986) and fallow deer (Asher and Smith, 1987). However, the use of PMSG is contra-indicated in fallow deer due to an increased incidence of multiple ovulation, low fertility rates and increased embryonic mortality (Asher and Smith, 1987; Jabbour *et al.*, 1991). While PMSG administration may be necessary for the induction of oestrus and ovulation during anoestrus, recent studies in fallow deer have shown that a high level of oestrous/ovulation synchrony can be achieved within the breeding season by the use of intravaginal CIDR devices alone (Asher and Thompson, 1989). However, there is little information on precise changes in the response to CIDR device treatment throughout the natural breeding season. Any changes in reproductive parameters such as interval to onset of oestrus, oestrous synchrony and incidence of ovulation, could dramatically alter the efficacy of breeding programmes employing fixed-time artificial insemination. Limited data indicates that the incidence of oestrus and ovulation increases and the degree of oestrous synchrony improves as CIDR device treatment is performed towards the end of the natural rut period (G.W. Asher; unpublished data).

The aim of the present study was to determine the effect of season on the efficacy of the CIDR device to synchronize oestrus and ovulation in farmed fallow deer, with the overall objective being to define the optimum treatment protocol for the use of CIDR devices without resorting to the use of PMSG.

MATERIALS AND METHODS

Animal Management

Eighty mature (> 2 years old) fallow deer does, with a mean (± s.e.m.) liveweight of 42.0 ± 0.3 kg, were each allocated to one of four treatment groups (Groups A-D; n=20) balanced for liveweight on 19 March 1990. The does were either from the Ruakura herd (n=23) or on loan from a commercial deer farm (n=57). Because of time constraints, most of the Ruakura does were allocated to Group A. Each group was run continuously with single fertile bucks from 20 March to 30 May, the period spanning synchronization treatment. An additional group of 42 contemporary control does from the Ruakura herd was run continuously with a crayon-harnessed fertile buck during this period and allowed to mate naturally in order to precisely define the natural rut. All deer were pasture fed and offered supplements of hay and maize for the duration of the trial.

Synchronization Treatment

Oestrous synchronization treatment involved intravaginal insertion of single CIDR devices (type G, 0.365g progesterone per

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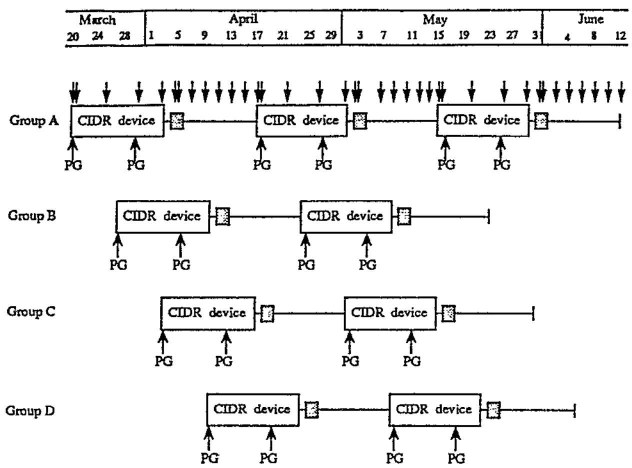
device; Agricultural Division, CHH Plastic Products Group Ltd, Hamilton, NZ) for 14 days. Each doe received an i.m. injection of prostaglandin analogue (2ml Estrumate; Imperial Chemical Industries PLC; Cheshire,UK) on Days 0 and 10 of CIDR device insertion to ensure that all does in successive treatment groups were of identical ovarian status at CIDR device withdrawal. Oestrous synchronization treatments were staggered by 7-day intervals for successive groups, with each group receiving a repeat treatment 4 weeks after the initial treatment and Group A being treated a third time. This gave a total of 9 weekly treatment observations spanning the 1990 rut (Figure 1).

Blood sampling

All does were blood sampled by jugular venepuncture (10ml heparinized Vacutainers) at CIDR device insertion (Day 0),

FIGURE 1 Schematic representation of the trial design illustrating the successive 7-day intervals in synchronization treatments across the four groups from March 20 1990 to June 12 1990; arrow indicates blood sampling; PG, prostaglandin analogue injection; shaded box represents continuous observation period.



three times during insertion (Days 1, 5, 10), at CIDR device withdrawal (Day 14), the following day (Day 15) and every second day from CIDR device withdrawal (Days 16, 18, 20, 22, 24, 26, 28), giving a total of 13 blood samples per treatment period. The blood samples were centrifuged within 30 minutes of collection and the plasma stored at -10°C until assayed.

Oestrous Observations

Does in each group were drafted into two equal sub-groups on Day 10 of CIDR device insertion. Each sub-group was joined with a buck and grazed in the observation paddocks. The bucks were fitted with ram mating harnesses (Asher, 1985) on the day of CIDR device withdrawal. Continuous behavioural observations were conducted between 24 and 72 h after CIDR device withdrawal. The observation period was extended for 4 - 10 hours on occasions if there was evidence of continuing oestrous activity. Observations to determine the onset of oestrus and to record subsequent mating activities were conducted from an enclosed observation platform overlooking the paddocks containing the treated does. All does were fitted with neck collars (Cross Range Group, Palmerston North, NZ) that allowed for rapid identification of individuals. The observation paddocks were floodlit during the hours of darkness. Observations to detect crayon mating marks were performed 24 hours after CIDR device withdrawal (i.e. at the start intensive behavioural observations) and at daily intervals for several days after the intensive observations had ceased, particularly for does not recorded as having exhibited oestrus. The sub-groups were rejoined at the blood sampling session at the end of the intensive observation period and one of the bucks removed. During the observation periods for the repeat treatments (i.e. weeks 6 - 9) the does were maintained as a single group of 20 and run with a single buck.

The group of untreated contemporary does was checked daily from 13 April to 10 May for crayon mating marks. They were frequently mustered into the handling facility to have mating marks recorded and removed. The buck's crayon was replaced at least every 3-4 days.

Progesterone Radioimmunoassay

Plasma progesterone concentrations were measured in duplicate by direct radioimmunoassay (Asher, 1990). The antiserum was raised in a rabbit against progesterone - 11-BSA conjugate and used at a final dilution of 1:13500. The second antibody was raised in a goat against rabbit IGG and used at a final dilution of 1:65. All samples from an individual doe were included in a single assay. Multiple control samples of low (5.1 ng/ml) and high (11.0 ng/ml) progesterone concentrations were included in each assay.The inter-assay coefficients of variation were 18.0% for the low control and 11.3% for the high control. The intra-assay coefficients of variation were 10.9% and 6.6% respectively. The sensitivity of the assay, being the least amount distinguishable from zero, was 0.16 ng/ml.

Determination of ovulatory response

Ovulation was judged to have occurred when post-CIDR device withdrawal plasma progesterone concentrations exhib-

TABLE 1 Incidence of oestrus and time to onset of oestrus for fallow deer does following CIDR device withdrawal at weekly intervals.

Group	Date of CIDR device withdrawal	Incidence of oestrus	Mean (±s.e.m.) time to onset of oestrus (h)	Range of time to onset of oestrus (h)	Synchrony (h)
A	3 April	0% (0/20)	-	-	-
B	10 April	10% (2/20)	80.3 ± 8.8	71.5 - 89.0	24.5
C	17 April	70% (14/20)	88.0 ± 6.1	51.0 - 133.0	93.0
D	24 April	94% (17/18)	71.0 + 3.9	37.0 - 97.0	60.0
A	1 May	100% (10/10)	47.5 ± 3.7	34.5 - 71.5	37.0
B	8 May	100% (20/20)	62.8 ± 2.4	39.8 - 76.0	36.3
C	15 May	90% (18/20)	54.3 ± 2.2	39.0 - 76.0	37.0
D	22 May	89% (16/18)	59.5 ± 2.7	44.3 - 75.5	31.3
A	29 May	90% (18/20)	50.5 ± 2.0	38.5 - 67.3	28.8

ited a steady increase in successive samples with the final sample (Day 28) being >2.0 ng/ml. A generally flat profile, with plasma progesterone concentrations < 1.0 ng/ml throughout, was deemed to represent failure of an ovulatory response. The doe responses to CIDR device treatment fell into 4 categories; (a) does not detected in oestrus and exhibiting no ovulatory response (anoestrous / anovulatory), (b) does not detected in oestrus but exhibiting an ovulatory response (anoestrous / ovulatory), (c) does detected in oestrus but exhibiting no ovulatory response (oestrous / anovulatory), (d) does detected in oestrus and exhibiting an ovulatory response (oestrous / ovulatory).

RESULTS

Incidence of oestrus

Data for 2 does in Group D (No's 70, 77; weeks 4 and 8) were excluded because they were in poor body condition due to pithemycotosis (facial eczema). The data for one sub-group of A (n=10 does) during week 5 were also excluded because the buck failed to respond to oestrous does.

Oestrus was detected on 115/166 (69%) occasions following CIDR device withdrawal and for 38/42 (90%) of control does. The incidence of oestrus, the mean (± s.e.m) time to onset of oestrus from CIDR device withdrawal and the degree of oestrous synchrony obtained over the successive weeks of treatment are presented in Table 1. The incidence of oestrus prior to the natural rut was 0-10% (weeks 1 and 2) but increased to 100% during the period after the rut (weeks 5 and 6). The time of onset to oestrus following CIDR device withdrawal decreased over this period, ranging from 88.0±6.1 hours (week 3) to 47.5±3.7 hours (week 5). The degree of oestrous synchrony improved progressively, as indicated by the range of time to onset of oestrus, which decreased from 93.0 hours (week 3) to 28.8 hours (week 9). The incidence of oestrus and time to onset for the respective weekly observations, plotted relative to the incidence of spontaneous oestrus observed for control does, is presented in Figure 2.

Plasma Progesterone Profiles

A total of 166 plasma progesterone profiles were obtained. Does generally exhibited low plasma progesterone concentrations (<1.0 ng/ml) on the day of CIDR device insertion (Day 0). This was followed by a marked elevation attributable to CIDR device insertion (Days 1-14). Withdrawal of the CIDR device caused a universal decline in progesterone concentrations to basal levels of 0-0.5 ng/ml before the subsequent sample (i.e. within 24 h) (Figure 3). Ovulation was assessed to have occurred on 133 occasions (80%). The individual progesterone profiles in each category were combined and the mean profiles for the respective categories are presented in Figure 3. The majority of the does failed to ovulate or ovulated in the absence of recorded

FIGURE 2 (a) Frequency histogram of the time to onset of oestrus from CIDR device withdrawal at 7 day intervals, plotted relative to (b) the incidence of first spontaneous oestrus observed for contemporary control does. The solid portion of the abscissa axes represents the period of continuous observations following CIDR device withdrawal, while the shaded bars indicate observations of crayon mating marks (+ time ranges for onset of oestrus). For each treatment group in (a), the proportion of does observed to exhibit oestrus is presented.

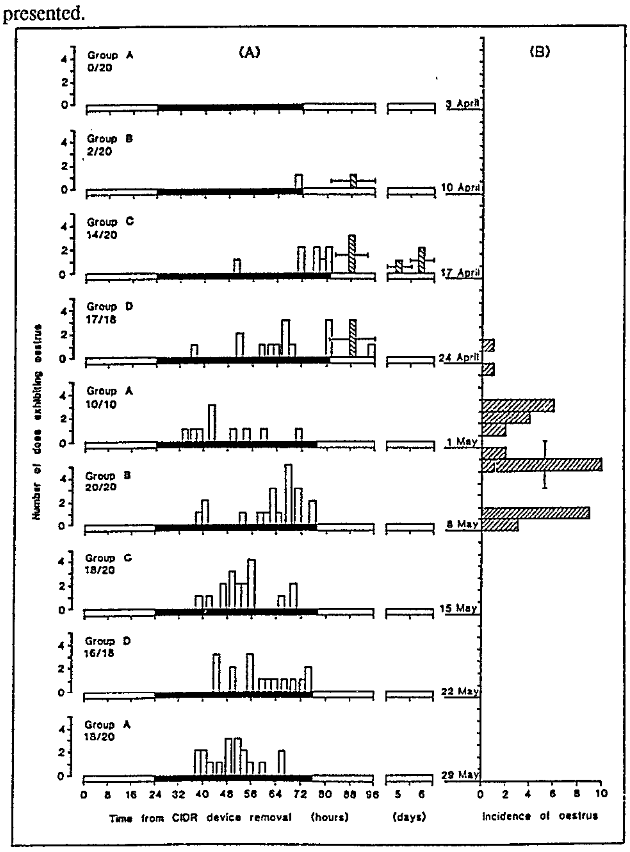


FIGURE 3 Profiles of mean (± s.e.m.) plasma progesterone concentrations of fallow deer does during and following intravaginal treatment with CIDR devices. (a) Oestrous does with ovulatory profiles (• ; N = 113) or anovulatory profiles (o ; N = 2); (b) Anoestrous does with ovulatory profiles (• ; N = 20) or anovulatory profiles (o ; N = 31).

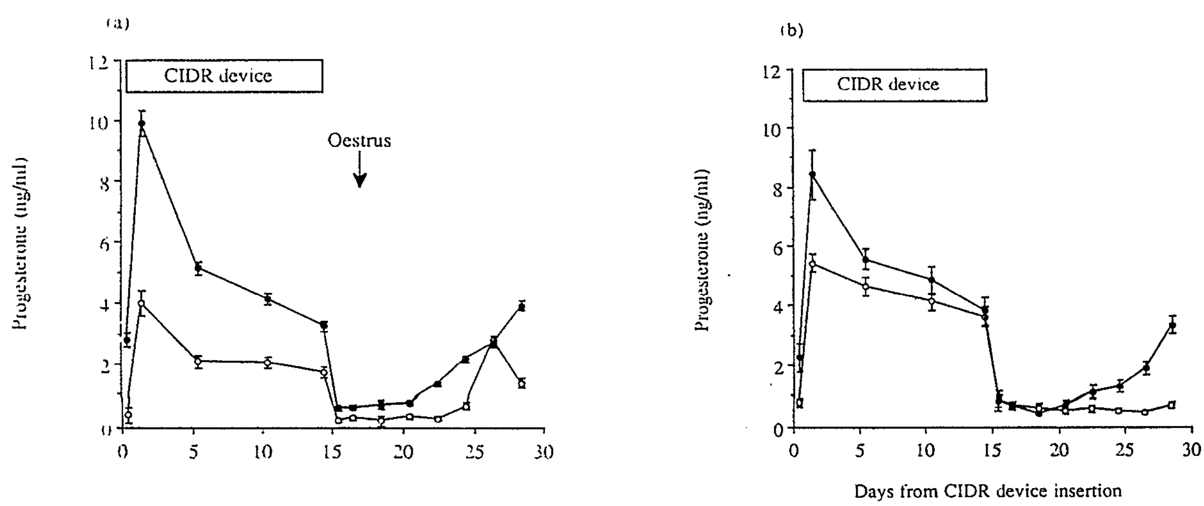


TABLE 2 Ovulatory response of fallow deer does following CIDR device withdrawal at weekly intervals.

Group	Date of CIDR device withdrawal	Anoestrous		Oestrous	
		Anovulatory	Ovulatory	Anovulatory	Ovulatory
A	3 April	75% (15/20)	25% (5/20)	-	-
B	10 April	75% (15/20)	15% (3/20)	-	10% (2/20)
C	17 April	-	30% (6/20)	-	70% (14/20)
D	24 April	6% (1/18)	-	11% (2/18)	83% (15/18)
A	1 May	-	-	-	100% (10/10)
B	8 May	-	-	-	100% (20/20)
C	15 May	-	10% (2/20)	-	90% (18/20)
D	22 May	-	11% (2/18)	-	89% (16/18)
A	29 May	-	10% (2/20)	-	90% (18/20)

oestrus prior to the onset of the natural rut (week 1 and 2). As the breeding season progressed a greater proportion of does showed an ovulatory response in conjunction with recorded oestrous behaviour. All does were oestrous and ovulatory during weeks 5 and 6. In weeks 7, 8 and 9 all does ovulated, however oestrus was not detected for two does per group (Table 2).

DISCUSSION

The results of this trial clearly demonstrate a pronounced seasonal effect on the efficacy of intravaginal CIDR devices in synchronizing oestrus and ovulation in fallow deer. This was manifest as almost complete failure of a response in early April, only 2-3 weeks before the commencement of the natural mating season (rut) in the southern hemisphere. Furthermore, the transition from this situation to one of maximum response, in terms of proportion of does exhibiting oestrus and oestrous synchrony, was abrupt and coincided with the period of first spontaneous oestrus of the contemporary control does. This supports the concept of marked seasonality of fallow deer reproduction, as observed in other studies (Asher, 1985; Asher and Macmillan, 1986; Asher *et al.*, 1988b) and has important implications for the development of successful artificial insemination programmes (Asher *et al.*,1990b).

The interval from CIDR device withdrawal to time to onset of oestrus tended to decrease as the season progressed. By week 5 (1 May) all does were recorded in oestrus within 72h from device withdrawal. The longer intervals recorded earlier in the trial (weeks 2-4) were similar to those reported for fallow does treated during anoestrum (February/ March) with CIDR devices and GnRH minipumps (Asher and Macmillan, 1986) or PMSG injections (Asher and Smith, 1987). This may reflect delayed follicular development in response to progesterone withdrawal due to reduced levels of LH secretion, as has been observed in fallow deer bucks (Asher et al., 1989a) and does (G.W. Asher, unpublished data) during the latter part of the non-breeding season.

Oestrous synchrony, defined as the interval between the onsets of oestrus of the first and last responding doe in each group, improved throughout the trial period. Initially greater than 60h in weeks 3 and 4 , it improved to 36-37h in weeks 5-7 and 29-31h in weeks 8 and 9. While the degree of synchrony in the latter weeks was generally greater than recorded in previous studies utilizing smaller numbers of fallow deer does (Asher *et al.*, 1986; Asher and Thompson, 1989), the data do confirm the suspicions of Asher *et al.*, (1990b) with regard to the seasonal efficacy of the CIDR device in synchronizing oestrus for fixed-time artifical insemination (AI). As fallow deer does treated after the onset of the rut are more responsive to treatment, it is reasonable to expect higher conception rates to AI from later, rather than earlier, treatment. This would be particularly so if using semen of limited duration viability.

The data of Jabbour *et al.*, (1991) clearly demonstrate that the additional treatment of fallow deer does with 50 i.u. PMSG at CIDR device withdrawal reduces the mean interval to onset of oestrus and tightens oestrous synchrony. However, there was a marked reduction in fertility, as assessed by AI, of does treated with PMSG, even though the dosage was considerably lower than used in previous studies (Asher and Smith, 1987) and that routinely used for oestrous synchronization in red deer (Fennessy *et al.*, 1990). This clearly mitigates against the use of PMSG in fallow deer and further highlights the need to define precisely the effects of season of CIDR device withdrawal alone on oestrous synchrony. However, the data from the present trial are not altogether consistent with those of previous trials in which CIDR devices alone were used to synchronize oestrus during the immediate post-rut period (i.e. when does are expected to be highly responsive to progesterone withdrawal). The mean (± s.e.m) interval from CIDR device removal to the onset of oestrus ranged from 47.5 ± 3.7h (week 5) to 62.8 ± 2.4h (week 6) during the rut/post-rut period of the present study. This compares to 53.3 ± 3.6h (Asher *et al.*, 1986), 43.5 ± 11.8h (Asher and Thompson, 1989), 34.1 ± 2.5h (Asher *et al.*, 1990a) and 43.0 ± 3.6h (Jabbour *et al.*, 1991) in other studies. This inconsistency may reflect factors other than seasonality and, as discussed by Asher *et al.*, (1990a), may include factors such as the duration of CIDR device insertion and the type of CIDR device used.

One such factor that may have partly accounted for the inconsistencies observed in the present trial was the source of animals. All but two of the does in Groups B, C and D were transported to the trial from a commercial deer farm prior to the start of treatment. It is notable that Group A does, consisting entirely of resident animals, exhibited the shortest mean interval to onset of oestrus during the rut/post-rut period. Therefore, there may well have been confounding effects of stress due to adaption to new surroundings among non-resident animals. It has been shown that acute stress can result in adrenal progesterone secretion in fallow deer (Asher *et al.*,1989b), occasionally leading to anomalies in preovulatory LH secretion (Asher *et al.*, 1986; Asher and Thompson, 1989). It is also possible that chronic forms of stress may also in some way affect the adrenal-pituitary axis and result in delayed follicular development or altered responses to follicular steroid secretion. This hypothesis remains to be tested.

On the basis of observed plasma progesterone profiles, ovulation followed oestrus on 98% of occasions, indicating a close association between the two events in fallow deer, as implied in previous studies (Asher *et al.*,1986; Asher and Thompson, 1989; Asher *et al.*, 1990a). On the 2 occasions when oestrus was apparently not followed by ovulation (week 4), plasma progesterone profiles during CIDR device insertion were notably lower than the mean profiles for the other does. The reasons for this are unclear but may indicate the necessity of adequate exogenous progesterone delivery to ensure a complete oestrous and ovulatory response.

Ovulation in the absence of observed oestrus was recorded on 20 occasions (39% of anoestrous does) and was particularly prevalent during the first three weeks of the trial. These observations probably represent silent ovulations, which occur naturally at the onset of the fallow deer breeding season (Asher, 1985). However, failure of observers to detect oestrus may account for some of these observations, particularly later in the breeding season. Interestingly, there was no clear evidence of short luteal cycles occurring at any stage during the trial, even though short cycles often occur at the onset of the breeding season (Asher, 1985; Mulley, 1989).

Further studies to refine oestrous synchronization in fallow deer, aimed at increasing the level of synchrony, may need to investigate the duration of CIDR device insertion and the use of various hormones (GnRH, prostaglandins, oestradiol) to attain follicular control.

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