Fertility of fallow deer (*Dama dama*) does following synchronisation of oestrus with CIDR devices or prostaglandin

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ABSTRACT

Two trials were conducted to determine the effect of different oestrous synchronisation regimens on oestrous behaviour, timing of ovulation and fertility of fallow deer. In Trial 1, 59 mature does were allocated to 6 treatment groups (n=9-10 per group). Does in Groups 1, 2 and 3 each received an i.m. injection of an analogue of prostaglandin $F_{2\alpha}$ (PG; 500 μg cloprostenol) on Day 13 of a synchronised oestrous cycle. Animals in Groups 2 and 3 also received 50 or 100 IU PMSG respectively at the time of PG administration. Does assigned to Groups 4, 5 and 6 each received an intravaginal CIDR device for 14 days with animals in Groups 5 and 6 also receiving 50 or 100 IU PMSG respectively, at the time of CIDR device withdrawal. Incidence of oestrus was higher following treatment with CIDR devices than with PG (29/30 vs 12/29, $P<0.001$). PMSG induced earlier onset of oestrus (34.6 ± 0.9 h vs 44.7 ± 2.4 h, $P<0.01$) and reduced the range in the time of onset of oestrus (14 h for PG-treated does and 22 h for progesterone-treated does). The overall median time to ovulation was 26 h after the onset of oestrus. The administration of 100 IU PMSG increased the incidence of luteinised follicles and twin *corpora lutea*.

In Trial 2, 105 does were allocated to 4 groups (n=26-27 per group) and treated with PG, PG + 50 IU PMSG, CIDR devices or CIDR devices + 50 IU PMSG. The does were inseminated cervically with 200×10^6 frozen-thawed spermatozoa at 12 h before the median time of ovulation as calculated for each group from Trial 1. Pregnancy rates were determined by rectal ultrasonography. The proportion of pregnant does was higher following treatment with CIDR devices than with PG (38/52 vs 28/53, $P<0.05$) and there was an interaction effect ($P<0.01$), with PMSG being beneficial only for does treated with PG.

Keywords Fallow deer, *Dama dama*, oestrus, ovulation, CIDR devices, prostaglandin, cervical insemination.

INTRODUCTION

Recent studies have optimised the timing of intrauterine insemination of fallow (Asher *et al.*, 1988a; 1990b; Mulley *et al.*, 1988) and red (Fennessy *et al.*, 1990) deer, with conception rates in the range of 60-70% commonly achieved. However, fertility following cervical insemination has not been very satisfactory (Asher *et al.*, 1988a; Fennessy *et al.*, 1990; Mylrea *et al.*, 1990). This may be related to the method of oestrous synchronisation employed, the timing of cervical insemination in relation to ovulation and the inseminated dose of spermatozoa.

Oestrous synchronisation can be achieved in deer by timed withdrawal of an exogenous source of progesterone (fallow: Asher and Thompson, 1989; red: Fisher *et al.*, 1986; chital; Mylrea *et al.*, 1990) or a progestagen (fallow: Mulley *et al.*, 1988; red: Kelly *et al.*, 1982) or by the administration of a prostaglandin $F_{2\alpha}$ (PG) analogue (wapiti: Glover, 1985; fallow: Asher and Thompson, 1989). However, irrespective of the type of oestrous synchronisation treatment, there seems to be less variation in the interval from the onset of oestrus to ovulation than from the end of oestrous synchronisation treatment to ovulation (Asher *et al.*, 1990a). Therefore, reducing the spread in the time to onset of oestrus and consequently ovulation, and ensuring appropriate timing of insemination in relation to ovulation may be key determinants in the success of fixed-time cervical insemination programmes.

The present study compared the effect of 6 oestrous synchronisation regimens on the temporal relationship between oestrus and ovulation in mature fallow deer during the early phase of the breeding season. Moreover, the effect of 4 regimens on conception rates following cervical insemination with frozen-thawed spermatozoa at a fixed interval from the median time of ovulation was investigated.

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MATERIALS AND METHODS

Trial 1

A total of 59 mature fallow deer does and 5 entire bucks on the Winchmore Irrigation Research Station (43° 48'S, 171° 48'E) were used between April and May 1990. The animals were held in 5 separate paddocks (n=11-12) from the time of commencement of treatment. Each group had 1 or 2 animals representative of each treatment regimen and which were balanced for liveweight. Treatment schedules were staggered one day between consecutive groups to restrict the number of does undergoing laparoscopy on any one day. Each doe assigned to treatments 1, 2 and 3 initially received a single intravaginal CIDR (Controlled Internal Drug Release) device (type-G, 0.3 g progesterone; Carter Holt Harvey Agricultural Division, Hamilton, NZ) inserted for a 14-day period on April 11-15 inclusive, and then followed by an i.m. injection of 500 μg cloprostenol (Estrumate; Imperial Chemical Industries, PLC, Cheshire, UK) administered on Day 13 of the luteal phase of the oestrous cycle. The does in groups 2 and 3 also received 50 or 100 IU PMSG (Folligon, Chemavet, Auckland, NZ) respectively, at the time of PG administration. Does assigned to Groups 4, 5 and 6 each had an intravaginal CIDR device for 14 days, inserted on April 24-28 inclusive. Does in Groups 5 and 6 received 50 or 100 IU PMSG respectively, at the time of CIDR device withdrawal.

Incidence and time to onset of oestrus were checked every 2 h for 72 h from the time of PG administration or CIDR device withdrawal by running the does with bucks fitted with mating harnesses and crayons. The incidence of ovulation, time to onset of ovulation, and ovulation rate were determined by ovarian examination at laparoscopy (Asher *et al.*, 1990a), performed 16 or 20 hours after the onset of oestrus and repeated every 6 h until ovulation was recorded. The ovaries of those does which failed to exhibit oestrus were examined 72 hours after PG administration or CIDR device withdrawal.

Trial 2

A total of 105 mature fallow deer does and 4 vasectomised bucks on the Winchmore Irrigation Research Station were used between April and June 1990. The animals were held in 4 separate groups and treated with PG (n=27), PG + 50 IU PMSG (n=26), CIDR devices (n=26) or CIDR devices + 50 IU PMSG (n=26) as described in Trial 1. The does were individually restrained in a cradle and inseminated cervically with $200x10^6$ frozen-thawed spermatozoa. Inseminations were conducted by one operator at about 12 h before the median time to ovulation estimated for each group from Trial 1 (i.e. at 61, 49, 55 or 49 h after PG administration or CIDR device withdrawal for does treated with PG, PG + 50 IU PMSG, CIDR devices or CIDR devices + 50 IU PMSG respectively). The semen was collected from 7 cross-bred fallow bucks (*Dama dama dama* x *Dama dama mesopotamica*) by electroejaculation (Asher *et al.*, 1987). The semen was assessed for quality, extended to a total concentration of $200x10^6$ spermatozoa and frozen as reported by Asher *et al.* (1988a). Only ejaculates with a post-thaw motility rate >70% were used for insemination. Pregnancy was determined by rectal ultrasonography on Day 42-43 after insemination (Asher *et al.*, 1990b).

Statistical Analyses

The data were analysed by χ^2 analysis or analysis of variance using the Genstat package.

RESULTS

Oestrous Behaviour

A total of 41 does exhibited oestrous behaviour, within a 38 h period, with an overall mean (± s.e.m.) time to onset of oestrus of 38.8 ± 1.4 h after PG administration or CIDR device withdrawal (Table 1). Treatment with CIDR devices resulted in a higher proportion of does exhibiting oestrus than treatment with PG (29/30 vs 12/29, χ^2=27.1, P<0.001). The does treated with PMSG exhibited oestrus at a mean (± s.e.m.) time of 34.6 ± 0.9 h as compared to 44.7 ± 2.4 h for does treated with PG or CIDR devices alone (P<0.01). Treatment with PMSG also reduced the range of the time to onset of oestrus from 22 to 8 h for PG-treated does and from 36 to 14 h for CIDR device-treated does.

TABLE 1 The effect of PG and CIDR devices with and without 50 or 100 IU PMSG on oestrus and ovulation.

Treatment	Incidence of oestrus	Mean (±s.e.m.) time to onset of oestrus (h)	Incidence of ovulation	Median time of ovulation (h)
PG	7/10	47.1±2.9	9/10	26
PG + 50 IU PMSG	4/9	33.5±1.7	5/9	28
PG + 100 IU PMSG	1/10	44.0	5/10	28
CIDR device	10/10	43.0±3.6	8/10	24
CIDR device + 50 IU PMSG	9/10	35.1±1.3	10/10	26
CIDR device + 100 IU PMSG	10/10	33.6±1.7	7/10	26
Total	41/59	38.8±1.4	44/59	26

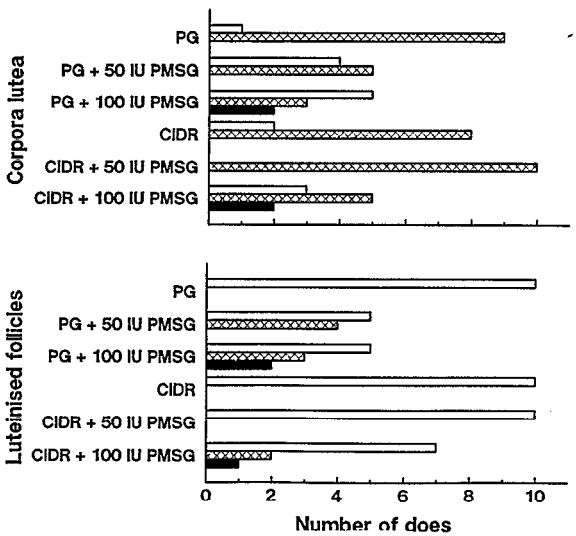

FIG 1 The effect of PG and CIDR devices with and without 50 or 100 IU PMSG on the number of fallow deer with 0 (open bar), 1 (hatched bar) or 2 (closed bar) *corpora lutea* and luteinised follicles.

Ovulatory Response

Of the 41 does that exhibited oestrous behaviour, 35 had ovulated during the repeated laparoscopy schedule. The other 6 does had either 1 (n=3) or 2 (n=1) luteinised follicles or 1 (n=2) small atretic follicle at the final laparoscopic inspection.

There was a tendency for a higher incidence of ovulation following treatment with CIDR devices than with PG (25/30 vs 19/29, χ^2=3.50, P<0.07). The overall median time to ovulation was 26 h after the onset of oestrus (Table 1), with no differences detected between treatments. The does within each group ovulated within 6-10 h of each other.

Of the does treated with PG or CIDR devices alone, 8/10 and 9/10 had a *corpus luteum* respectively (Fig. 1). Each of the remaining 3 does had one small atretic follicle. The administration of 50 or 100 IU PMSG to PG-treated does and 100 IU PMSG to CIDR device-treated does increased the incidence of luteinised follicles and twin *corpora lutea* (Fig. 1).

Conception to Synchronised Oestrus

The numbers of does in each treatment group which conceived after insemination during the synchronised oestrus were 11/27, 17/26, 22/26 and 16/26 for does treated with PG, PG and 50 IU PMSG, CIDR devices or CIDR devices and 50 IU PMSG respectively. The overall conception rate was 62.9% (Table 2). The proportion of pregnant does was higher following treatment with CIDR devices than with PG (38/52 vs 28/53, χ^2=4.7, P<0.05). There was a significant interaction effect between the method of oestrus synchronisation and PMSG treatment (χ^2=6.86, P<0.01). The administration of 50 IU PMSG proved beneficial for does treated with PG and reduced the conception rate for does treated with CIDR devices (Table 2).

TABLE 2 The effect of PG and CIDR devices with and without 50 IU PMSG on the conception rates of fallow deer following cervical insemination with 200 x 10^6 frozen-thawed spermatozoa.

Treatment	No. of treated does	No. of pregnant does	Conception rate (%)
PG	27	11	40.7
PG + 50 IU PMSG	26	17	65.4
CIDR device	26	22	84.6
CIDR device + 50 IU PMSG	26	16	61.5
Total	105	66	62.9

DISCUSSION

CIDR devices are more reliable than PG for the synchronisation of oestrus early in the breeding season of fallow deer. In the present study, the initial oestrous synchronisation of PG-treated does was performed with CIDR devices that may have been withdrawn relatively early in relation to the onset of the rut. CIDR-device withdrawal just prior to the onset of the natural rut results in low incidence of oestrus (C.J. Morrow, *pers. comm.*). The use of CIDR devices need not be a pre-requisite for the success of a PG-regimen. The limited data available show that the *corpus luteum* is sensitive to the luteolytic effect of PG on Day 13 of the oestrous cycle (Asher and Thompson, 1989); this necessitates pre-treatment with CIDR devices to ensure that the luteolytic agent is administered at an appropriate time. Further work is warranted to determine the sensitivity of the *corpus luteum* to PG at all stages of the oestrous cycle. This may help establish a suitable interval for a double-injection PG regimen that can be applied after the onset of the natural rut. This could reduce the duration of the oestrous synchronisation regimen and may improve the oestrous and ovulatory responses of fallow deer to PG.

The administration of 50 or 100 IU PMSG advanced the time to onset of oestrus and improved the synchrony of oestrus. This may be attributed to rapid follicular maturation and enhanced oestrogen secretion by follicles following stimulation with PMSG. Enhanced oestrogen secretion in a greater proportion of large antral follicles has been demonstrated in sheep following the administration of 500 IU PMSG at the time of PG administration (McNatty *et al.*, 1982). The better synchrony of oestrus observed following the administration of PMSG ultimately improved the synchrony of ovulation. Although, treatment with PMSG advanced the time of ovulation from the end of the synchronisation treatment, the interval between the onset of oestrus and the time of ovulation remained constant. PMSG at doses of 500 IU (Asher and Smith, 1987) or 200 IU (G.W. Asher, unpublished data) have been used previously in fallow deer. However, multiple ovulations or complete failure of ovulation was observed in a proportion of treated animals. This ultimately reduced conception rates and increased the incidence of embryonic mortality. In the present study, the administration of 100 IU PMSG increased the incidence of twin ovulations and the administration of 100 or 50 IU PMSG increased the incidence of luteinised follicles. This attests to the high sensitivity of the ovarian follicles of fallow deer to this gonadotrophin and mitigates against its general use in oestrous synchronisation programmes. The administration of 200 IU PMSG is recommended in red deer to improve the synchrony of oestrus/ovulation and to increase the incidence of ovulation prior to the onset of the natural rut (Fisher *et al.*, 1986; Fennessy *et al.*, 1989). Although such doses have been shown to increase the incidence of multiple ovulations (G.W. Asher, unpublished data) and to induce conception and births of twins in red deer artificial insemination programmes (Asher *et al.*, 1988b), there is little evidence of production losses through reduced fertility and increased embryonic loss.

Previous attempts at cervical insemination have resulted in moderate fawning rates following the deposition of 85x10^6 motile fresh or frozen-thawed spermatozoa at 48 hours after CIDR device withdrawal (Asher *et al.*, 1988a). The reasons for the improved fertility observed in this study are not clear. This is possibly a consequence of the higher concentration of deposited spermatozoa and/or insemination closer to the time of ovulation. In red deer, conception rates after vaginal inseminations, over a range of times from CIDR device withdrawal, were invariably < 50%. This may be related to a suboptimal dosage of spermatozoa (Fennessy *et al.*, 1990).

Treatment with CIDR devices resulted in higher

conception rates than treatment with PG. This can be attributed partly to the lower efficiency of PG to synchronise oestrus, particularly when treatment is applied early in the breeding season. It may also be due to a reduced efficiency in sperm transport in the reproductive tract, possibly due to the effect of PG on uterine motility (Hawk, 1973). The administration of 50 IU PMSG reduced conception rates following oestrous synchronisation with CIDR devices. The reasons for this are not clear; as oestrous and ovulation synchrony is improved following the administration of the gonadotrophin, it would be expected that fertility following fixed-time insemination would also improve. It is possible that PMSG, even at such low doses, altered the rate of gamete transport through the reproductive tract. Ova arriving prematurely in the uterus may degenerate and be promptly expelled from the uterus (Whyman and Moore, 1980).

CONCLUSION

This study has demonstrated that CIDR devices are more suitable than PG for oestrous synchronisation early in the breeding season. Moreover, high conception rates can be achieved following cervical insemination of fallow does with frozen-thawed semen 12 h before the median time of ovulation. The inclusion of PMSG is contra-indicated following oestrous synchronisation with CIDR devices, although the gonadotrophin improved the synchrony of oestrus and ovulation.

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