Induction of oestrus and ovulation in farmed fallow deer (*Dama dama*) by using progesterone and PMSG treatment

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Summary. Twenty non-lactating fallow does were each treated with an intravaginal progesterone device (CIDR) for 14 days followed by an i.m. injection of 500 i.u. PMSG, 3–4 weeks before the expected start of the natural rut. An additional 7 does received no treatments and were used as controls. Induced oestrus was observed for 19 (95%) of the treated does, the onset occurring between 48 and 76 h after CIDR removal/PMSG administration. Laparoscopic examination 12 days after CIDR removal revealed ovulation rates ranging from 1 to 4 in treated does. Non-ovulating luteinized follicles were also a feature of PMSG treatment and there was a significant inverse relationship between ovulation rate and numbers of luteinized follicles. Only 3 (14.3%) of the treated does conceived at or near the induced oestrus, the remainder returning to oestrus 21–27 days later. Mid-cycle progesterone concentrations were positively related to ovulation rate. All control does showed evidence of a recent single ovulation at laparoscopy, although first oestrus did not occur until 7–15 days later, indicating the occurrence of silent ovulations before the natural breeding season.

Introduction

Fallow deer (*Dama dama*) are highly seasonal breeders with first oestrus of the breeding season in autumn occurring within a 12-day period in late April–early May in New Zealand (Asher, 1985). Most does conceive to first oestrus and, after a 234-day gestation, give birth to single fawns in December (Asher & Adam, 1985; Asher, 1987). Recent moves to farm the species under intensive pastoral grazing regimens have highlighted the desirability of more closely aligning peak pasture production in spring (September–November) with the high energy demands of lactation that normally occur in summer (December–March).

Oestrus and ovulation in various livestock species are commonly controlled using progestagen and subsequent pregnant mare serum gonadotrophin (PMSG) treatment. While this technique has been used for the induction of fertile oestrus in red deer (Kelly *et al.*, 1982; Adam *et al.*, 1985), it has not been reported previously for fallow deer.

Materials and Methods

Animals and management. Twenty-seven mature (>4 years old) fallow does had their fawns weaned on 12 March 1984. They were grazed as one group, rotationally in 0.25 ha paddocks within 100 m of an enclosed observation tower. A buck was introduced on 25 March and removed on 30 May. Between early November and late December 1984 the fawning paddocks were monitored twice daily and all fawns were tagged within 12 h of birth, and later identified to dams by observing suckling.

Induction of oestrus and ovulation. On 15 March, 20 does were each given intravaginally a silicone elastomer device impregnated with 0.5 g progesterone (CIDR-Type S; NZ Dairy Board, Hamilton). The devices were removed after 14 days and the does each received an i.m. injection of 500 i.u. PMSG (Folligon; Intervet, Arturmon, Australia). The remaining 7 does received no hormonal treatments. One control doe died from accidental causes on 19 April.

Detection of oestrus. The buck was fitted with a ram mating harness (after Asher, 1985). Observation of the does, to detect oestrous behaviour and crayon mating marks, was conducted from the observation tower throughout daylight hours from 30 March to 2 April inclusive. Thereafter, observations were conducted at least twice daily while the buck was present.

Assessment of ovulation rate. All 27 does had their ovaries examined by laparoscopy on 10 April, 12 days after CIDR removal from treated does. Anaesthesia was induced by an i.m. injection of 5 mg ketamine hydrochloride (Ketalar; Parke-Davis Pty Ltd, USA) and 5 mg xylazine hydrochloride (Rompun; Bayer Leverkusen, Germany) per kg liveweight. The total numbers of corpora lutea and non-ovulating luteinized follicles were recorded, with the two structures being differentiated primarily on size and presence or absence of rupture sites.

Blood sampling. Blood samples were collected by jugular venepuncture every 3 days from 12 March to 3 May and the separated serum was stored at -10°C before analysis.

Analysis of serum progesterone concentrations. Serum samples were assayed for progesterone by radioimmunoassay (Asher *et al.*, 1986). The progesterone antiserum was raised in a rabbit against progesterone-11-BSA conjugate and used at a final dilution of 1:3000. The only detectable cross-reaction with other steroids was with cholesterol (1.5%). The sensitivity of the standard curve was 0.03 ng/tube (0.3 ng/ml serum) and the intra- and inter-assay coefficients of variation were 6.7% and 10.6–11.5% respectively.

Statistical procedures. Differences between mean dates of oestrus and between mean serum progesterone concentrations on any one day were tested with Student's *t* test. The relationship between the number of corpora lutea and the number of luteinized follicles or mid-cycle serum progesterone concentrations were investigated by Pearson's correlation coefficient.

Results

Ovulation rate

The ovulation rate of treated does 12 days after CIDR removal/PMSG administration was variable (1–4 corpora lutea; Table 1). Furthermore, a variable number of non-ovulating luteinized follicles was observed (Table 1). There was a significant inverse relationship between ovulation rate and the number of luteinized follicles ($r = -0.47$; $P < 0.05$).

All 7 control does each had a single corpus luteum although none had exhibited oestrus by the day of laparoscopy (10 April). No luteinized follicles were observed in these does (Table 1).

Table 1. Numbers of fallow does with variable numbers of corpora lutea and luteinized follicles following progesterone + PMSG treatment before the breeding season

	No. of corpora lutea (ovulation rate)						Mean ovulation rate
	0	1	2	3	4	5	
Treated does (N = 20)	0	8	9	1	2	0	1.85
Control does (N = 7)	0	7*	0	0	0	0	1.00
	No. of luteinized follicles						
	0	1	2	3	4	5+	
Treated does (N = 20)	9	0	1	5	3	2	
Control does (N = 7)	7*	0	0	0	0	0	

*One control doe died 9 days after these data were collected.

Occurrence of oestrus

Induced oestrus was observed for 19 (95%) of the treated does and occurred between 48 and 76 h after CIDR removal/PMSG administration (mean $\pm$ s.d. = 58 ± 11 h) with no significant difference ($P > 0.1$) between does having single (59 ± 11 h; $N = 8$) and multiple (57 ± 11 h; $N = 11$) ovulations. The mean ($\pm$ s.d.) date of induced oestrus (31 March ± 0.45 days) was about 3 weeks earlier than first natural oestrus of 6 surviving control does (20 April ± 3.0 days).

Sixteen treated does that had exhibited induced oestrus were observed to exhibit second oestrus 21–27 days later (mean $\pm$ s.d. = 23.6 ± 1.8 days). The mean length of this cycle was longer for does with multiple corpora lutea (24.2 ± 1.9 days; $N = 9$) than for does with a single corpus luteum (22.7 ± 1.2 days; $N = 7$), although the difference was not significant ($P > 0.1$).

One treated doe exhibited an unusually short (8-day) oestrous cycle. Return to oestrus occurred on 9 April and was associated with a double ovulation. The additional presence of 2 regressing corpora lutea on 10 April suggested that an earlier double ovulation had occurred in response to PMSG treatment.

The single treated doe that failed to exhibit oestrus immediately after CIDR removal/PMSG administration was later observed to exhibit oestrus on 23 April, coincidental with return to oestrus of most other treated does.

Conception rate to induced oestrus and return oestrus

Of the 19 treated does that exhibited induced oestrus on 31 March or 1 April, only 2 apparently conceived to that mating. Another doe apparently conceived to a return oestrus 8 days after induced oestrus. All 3 does conceived to multiple ovulations (2–4 corpora lutea). One doe gave birth to a single viable (3.1 kg) fawn on 28 November (242 days gestation), another gave birth to non-viable (< 1.4 kg each) twin fawns on 9 November (223 days) and the third aborted twin fetuses on 10 September (155 days).

In all, 14 of the 17 (82.4%) treated does that did not conceive to an induced oestrus, conceived to the return oestrus, having a mean ($\pm$ s.d.) gestation length of 235.2 ± 2.9 days. Two of the remaining 3 does exhibited a second return oestrus 23 days after first return oestrus, both does conceiving to that mating and having gestation lengths of 232 and 236 days respectively. The remaining doe failed to fawn and was not observed to exhibit a second return oestrus.

The 6 surviving control does all conceived to their first oestrus, having a mean ($\pm$ s.d.) gestation length of 234.7 ± 2.0 days.

Progesterone profiles

Mean serum progesterone profiles of single and multiple ovulating treated does that failed to conceive to induced oestrus are presented in Fig. 1(a). Mean concentrations during the oestrous cycle were higher for does having ≥ 2 corpora lutea than for does with a single corpus luteum. The difference between mean values on any sampling date was not significant ($P > 0.1$) even though there was a 2-fold mid-cycle difference. However, there was a significant correlation between the mean mid-cycle serum progesterone concentration of an individual (i.e. mean levels between 9 and 19 April inclusive) and previous ovulation rate ($r = 0.57$; $P < 0.05$).

The mean serum progesterone profile of does that conceived at, or close to, the induced oestrus is presented in Fig. 1(b). All 3 does had multiple corpora lutea and peak mean serum progesterone concentrations were of similar magnitude to mean mid-cycle values of cycling does that had multiple corpora lutea. High concentrations (~ 20 ng/ml) were maintained at the end of the blood sampling period. One of these does appeared to have conceived to a return oestrus 8 days after induced oestrus. The serum progesterone concentration on the day of return oestrus (9 April) was > 15 ng/ml. Such high concentrations were not recorded close to any other oestrus observed during the study.

Fig. 1. Profiles of mean ($\pm$ s.e.m.) serum progesterone concentrations of fallow does, recorded before and during the early phase of the breeding season, and the occurrence of oestrus (bar graph). The date of laparoscopy is indicated by arrows. (a) Does with 1 ($\circ$; $N = 8$) or ≥ 2 ($\bullet$; $N = 9$) corpora lutea and failing to conceive to an induced oestrus (profile includes 1 doe that failed to exhibit induced oestrus). (b) Does with ≥ 2 corpora lutea and conceiving at or near induced oestrus ($N = 3$). (c) Control does ($N = 6$).

The mean serum progesterone profile of the 6 surviving control does (Fig. 1c) shows a low level of progesterone secretion until after the period of first oestrus in mid-late April. However, all 6 does exhibited a transient elevation of serum progesterone concentrations (1.30–2.45 ng/ml) on the sampling day preceding that closest to oestrus.

Discussion

This trial has demonstrated that oestrus and ovulation can be induced in fallow does 3 weeks before the natural rut by using exogenous progesterone and PMSG treatment. Although CIDR insertion occurred before there was any evidence of luteal activity in treated does, as indicated by low (< 1.0 ng/ml) serum progesterone concentrations on 15 March, they were removed 14 days later at about the time that spontaneous silent ovulations would have been starting to occur in untreated does (Asher, 1985). Therefore, the artificial oestrus was induced during the period of transition from anoestrus to oestrus rather than during deep anoestrus.

Serum concentrations of progesterone during CIDR insertion were of similar magnitude to values recorded during the late luteal phase of the normal oestrous cycle of fallow does (Asher, 1985). As effective concentrations were maintained for the whole 14-day period, these intravaginal devices appear well suited for simulating or prolonging an oestrous cycle in fallow does.

The variable ovulation rate of fallow does in response to PMSG treatment has been observed following administration of similar or higher PMSG dose levels to red deer (Kelly *et al.*, 1982; Adam *et al.*, 1985). Non-ovulating follicles, which appeared ultimately to become luteinized structures, were also a feature of PMSG treatment in fallow deer, possibly to the detriment of ovulation rate. A similar phenomenon has been observed following administration of superovulatory doses of PMSG to goats (Armstrong *et al.*, 1982).

Both fallow does and red deer hinds (Kelly *et al.*, 1982) with multiple corpora lutea have higher serum progesterone concentrations than do females with a single corpus luteum, providing further evidence that corpus luteum tissue is the major source of progesterone in deer during the breeding season. However, it is likely that luteinized follicles are also contributing to luteal progesterone secretion in PMSG-treated fallow does, as indicated by the relatively high mid-cycle mean serum progesterone concentration of single ovulating treated does (~ 8 ng/ml) compared to mean values observed during natural oestrous cycles (~ 5 ng/ml; Asher, 1985).

The doe that exhibited an 8-day oestrous cycle after induced oestrus showed evidence of premature luteal regression, a phenomenon frequently observed in other domestic species induced to ovulate during anoestrus. However, this doe had a double ovulation around the time of return oestrus, 10 days after PMSG administration. As spontaneous multiple ovulations are rare events in fallow deer (Harrison & Hyett, 1954; Armstrong *et al.*, 1969; Chapman & Chapman, 1969, 1975; Baker, 1973) it is probable that there were residual effects of PMSG treatment. Furthermore, a high serum progesterone concentration (15.5 ng/ml) was evident on the day of return oestrus. Short-lived (1–3 h) surges of serum progesterone concentrations at oestrus have been observed occasionally for fallow deer (Asher *et al.*, 1986). However, such high concentrations in the present study may represent secretion from regressing corpora lutea and luteinized follicles. The physiological stimulus for the expression of oestrus and for multiple ovulations under such conditions remains unclear.

Low pregnancy rates observed in the present study have been a common outcome of the early induction of oestrus and ovulation in fallow (Asher & Macmillan, 1986) and red (Kelly *et al.*, 1982; Adam *et al.*, 1985) deer. At 20 induced oestrous periods recorded for does in the present study, 12 copulations were observed. Therefore, poor copulation responses by the buck to highly synchronized oestrus could not account completely for the low pregnancy rate. It is possible that buck fertility was suboptimal 3 weeks before the natural rut, given that fallow bucks exhibit highly seasonal fertility (Chaplin & White, 1972; Asher *et al.*, 1987). However, low pregnancy rates may also relate to progesterone/PMSG treatment effects on the does, as frequently postulated for sheep (e.g. Allison & Robinson, 1970; Croker *et al.*, 1975).

The non-viability of multiple fetuses in the present study, confirmed in a subsequent study in which all 9 sets of twins produced after PMSG treatment in 1985 died (G. W. Asher, unpublished data), suggests that fallow does have a poor ability to produce viable twins. The reasons remain unclear and future studies may need to investigate such factors as uterine capacity. Induction of multiple ovulations in fallow does induced to breed earlier may be counter-productive and the use of lower PMSG dosage rates should be investigated.

The 6 control does in this study ovulated at least 7–15 days before their first spontaneous oestrus indicating the occurrence of silent ovulations. This agrees with an earlier study in which transient elevations of serum progesterone concentrations preceded the first oestrus of the season (Asher, 1985). However, the mean date of the first oestrus of the control does was significantly earlier (although only by ~6 days) than for untreated does in a concurrent trial (Asher, 1986). This suggests that the incidence of first oestrus within a group of fallow does may be influenced by some of its members, as has been shown for sheep (Lindsay & Signoret, 1978) and goats (Pilsworth, 1982).

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