Induction of ovulation in anoestrous red deer hinds with a GnRH analogue

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ABSTRACT

Two trials were carried out in separate years to examine the effectiveness of buserelin, an analogue of gonadotrophin releasing hormone, for inducing ovulation in anoestrous red deer hinds. Anoestrous hinds were pre-treated with intravaginal progesterone controlled internal drug releasers (CIDR) for 14 d prior to CIDR withdrawal on 4 March (15 hinds with 1 CIDR each in Trial 1; 16 hinds with 2 CIDRs each in Trial 2) and injected intramuscularly with buserelin (7 hinds received; 4μg and 10μg on days 14 and 16 respectively in Trial 1: 8 hinds received; 2-4 μg per day, days 12-15 and 10 μg on day 16 in Trial 2). Two weeks after CIDR withdrawal 6 buserelin-treated hinds in both trials and 1 control hind in Trial 1 had a corpus luteum present and most had an elevated plasma progesterone level for at least 10 d commencing from 2 to 6 d after withdrawal of the CIDRs. Calving date was not affected by treatment with buserelin and there was no evidence that any of the induced ovulations resulted in pregnancy.

Although buserelin induced ovulation in progesterone-primed anoestrous red deer hinds, further work is required to develop a treatment regime which will produce successful establishment of pregnancy.

Keywords: ovulation; anoestrus; deer; GnRH analogue.

INTRODUCTION

Various treatments have been used to advance the breeding season of red deer hinds. These involve the administration of melatonin or intravaginal progestagen followed by gonadotrophins or gonadotrophinreleasing hormone (GnRH) (Barrell, 1985; Fisher and Fennessy, 1985; Fennessy *et al.*, 1986). One such treatment, which requires several days of continuous infusion of synthetic GnRH, has been used to induce ovulation prior to the breeding season in yearling (Fisher *et al.*, 1986) and adult red deer hinds (Fennessy *et al.*, 1986). However the requirement for continuous infusion of GnRH precludes this method for on-farm use.

A single injection of an analogue of GnRH, which is more potent and longer lasting than synthetic GnRH, induces ovulation in anoestrous ewes (Siddall and Crighton, 1977; Swift and Crighton, 1979; 1980). The present study examined the effectiveness of one such analogue, buserelin (Receptal®, Hoechst NZ Ltd), for inducing ovulation in progesterone-primed anoestrous red deer hinds.

MATERIALS AND METHODS

Trials were conducted at the Research Farm, Lincoln College (Trial 1 in 1986 and Trial 2 in 1987) using adult red deer hinds with an average live weight of 95.2 ± 1.9 kg.

On 18 February 1986 a controlled internal drug release device (CIDR-Type S, AHI Plastic Moulding Co.) containing 0.3 g progesterone was inserted intravaginally into each of 15 anoestrous hinds (Trial 1). At CIDR withdrawal, 14 d later (4 March), 7 hinds were injected with 4 μg buserelin intramuscularly followed by a second injection (10 μg) 48 h later. Controls (n=8) received progesterone treatment only. In 1987 (Trial 2) 2 CIDRs (Type-S), tied together, were inserted into each of 16 hinds on 19 February and withdrawn on 4 March. From 2 March 8 hinds were injected daily with buserelin according to the protocol in Table 1. Controls (n=8) received progesterone treatment only.

TABLE 1 Injection regime for GnRH-analogue (buserelin) used with red deer hinds in Trial 2.

Date	Dose of buserelin
2 March	4 μg intramuscular
3 March	3 μg intramuscular
4 March	2 μg intramuscular (CIDR withdrawal)
5 March	2 μg intramuscular
6 March	10 μg intramuscular

In both trials an entire red deer stag was run with the hind mob from the time of CIDR withdrawal. The stag in Trial 1 was fitted with a ram harness and crayon. Crayon marks on the hinds were recorded daily. Blood samples were collected from the hinds at 1 to 3 d intervals throughout the mating period and on 1 occasion (18 and 19 March for Trials 1 and 2,

respectively) ovaries of all hinds were examined by laparoscopy. Towards the end of each year hinds were monitored daily to record calving date.

Plasma samples were assayed for progesterone using an enzyme-linked immunosorbent assay (ELISA) (Elder *et al.*, 1987). The sensitivity of the assay, calculated as 2 standard deviations from zero, was 0.2 ng/ml. Plasma samples with mean progesterone levels of 0.33, 1.13 and 6.25 ng/ml had within-assay coefficients of variation of 14.2, 8.3 and 11.4% respectively. Between-assay coefficients of variation for these samples were 15.9, 12.2 and 8.5% respectively. Differences in concentration of plasma progesterone were tested using Student's and paired-sample *t*-tests after transformation of the data to their logarithms.

RESULTS

In Trial 1, 6 analogue-treated hinds and 1 control hind each had a single corpus luteum (C.L.) present at laparoscopy (Table 2). The mean plasma progesterone concentration before CIDRs were removed was 0.74 ± 0.22 ng/ml. After CIDR withdrawal plasma progesterone levels fell significantly ($P<0.01$) to less than 0.5 ng/ml in most hinds (Fig. 1). Four to 6 d later plasma progesterone levels increased and then remained elevated (>0.5 ng/ml) for at least 10 d in treated hinds which had a C.L. but remained low (<0.5 ng/ml) in all the other hinds (including the control hind which had a C.L. (Fig. 3a) until early April. The mean peak plasma progesterone concentration was 2.14 ± 0.18 ng/ml for treated hinds during the induced cycle. Crayon marks on the hinds were generally difficult to detect and many were produced by accidental contact with the stag during yarding. All hinds delivered live calves at term. The mean calving date of treated hinds was 27 November ± 2.3 d and that of the controls 26 November ± 2.0 d.

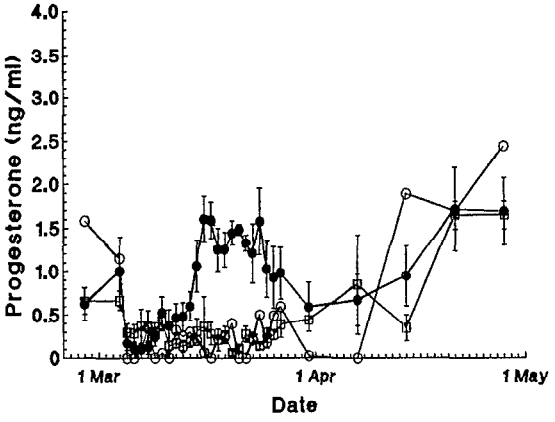

FIG. 1 Mean plasma progesterone concentrations of red deer hinds following intravaginal progesterone (CIDR) treatment in Trial 1. Hinds either received GnRH analogue and had a C.L. present on 18 March (n=6, ●) or did not have a C.L. present (n=1, ○), or did not receive GnRH analogue (n=8, □). Vertical bars denote S.E.M.

In Trial 2, 6 analogue-treated hinds had a single C.L. present on 19 March (Table 3). Mean plasma progesterone level was 1.65 ± 0.52 ng/ml prior to withdrawal of the doubled-up CIDRs on 4 March, after which concentrations decreased ($P<0.001$)

TABLE 2 Ovulatory response and calving dates of red deer hinds in Trial 1.

Group	No. in group	No. with C.L. visible on 18 March	Calving date (mean ± S.E.M.)
Treated (CIDR/GnRH analogue)	7	6	27 Nov ± 2.3 d
Control (CIDR only)	8	1	26 Nov ± 2.0 d

TABLE 3 Ovulatory response and calving dates of red deer hinds in Trial 2.

Group	No. in group	No. with C.L. visible on 19 March	Calving date (mean ± S.E.M.)
Treated (CIDR/GnRH analogue)	8	6	27 Nov ± 2.9 d
Control (CIDR only)	8	0	23 Nov ± 1.1 d

(Figure 2). Two to 6 d later plasma progesterone levels in 5 of the responding hinds (with C.L. present at laparoscopy) increased and remained high (>1.0 ng/ml) for 14 to 17 d before falling again. The mean peak progesterone concentration during this period was 3.02 ± 0.43 ng/ml. Only transient increases in plasma progesterone concentrations were recorded for the remaining hinds (including 1 treated hind which had a C.L. at laparoscopy (Fig. 3b)) until April, after which levels were elevated in most hinds (Fig. 2). Treatment with buserelin had no effect on calving date (26 Nov ± 2.0 d *v* 23 Nov ± 1.1 d) and, except for a treated hind that died prior to calving, all hinds produced live calves.

From calving dates and an estimated gestation length of 233 d (Kelly and Moore, 1977) it is possible to calculate that no pregnancies resulted from the ovulation recorded at laparoscopy in either trial. There was no relationship between the marking of hinds in Trial 1 and the estimated time of conception.

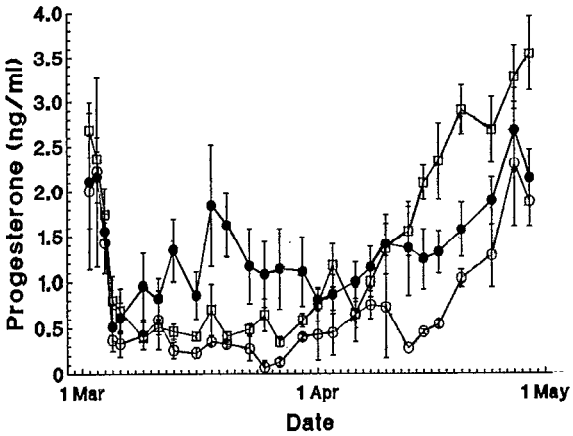

FIG. 2 Mean plasma progesterone concentrations of red deer hinds following intravaginal progesterone (CIDR) treatment in Trial 2. Hinds either received GnRH analogue and had a C.L. present on 18 March (n=6, ●) or did not have a C.L. present (n=1, ○), or did not receive GnRH analogue (n=8, □). Vertical bars denote S.E.M.

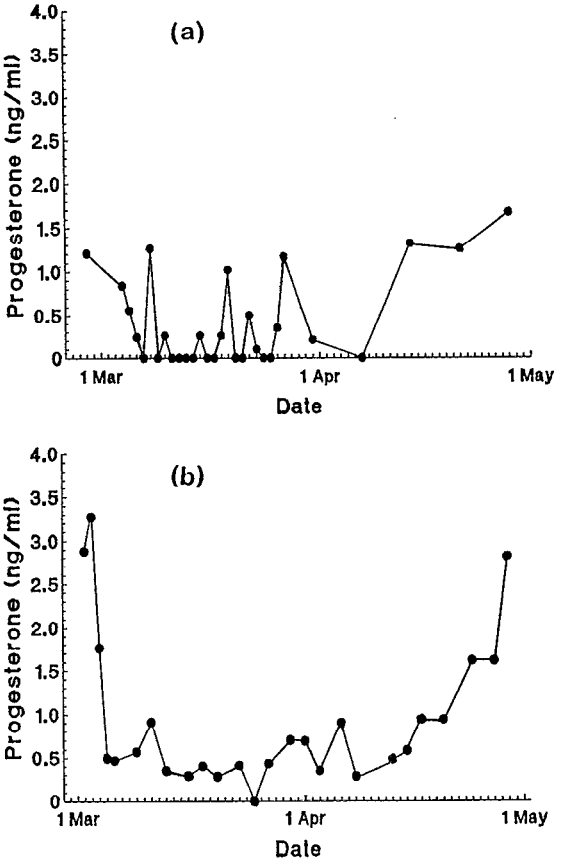

FIG. 3 Anomalous plasma progesterone profiles of red deer hinds following intravaginal progesterone (CIDR) treatment. (a) Hind not subsequently treated but which had a C.L. present on 18 March, Trial 1. (b) Hind which received GnRH analogue and had a C.L. present on 19 March, Trial 2.

DISCUSSION

In both trials single ovulations were induced in red deer hinds treated with the GnRH analogue, buserelin. The functional competence of the C.L. observed at laparoscopy was confirmed by the elevated plasma progesterone concentrations in the majority of analogue-treated animals 2 to 6 d after CIDR withdrawal. Plasma progesterone levels remained elevated for up to 17 d, which is similar to the duration of the luteal phase of the natural oestrous cycle (Adam *et al.*, 1985). Maximum plasma progesterone levels during the induced cycle ranged from 1.6 to 5.4 ng/ml, similar to the range of mid-cycle levels of 2.5 to 5.8 ng/ml described by Kelly *et al.* (1985) but generally lower than the peak levels reported elsewhere (Adam and Atkinson, 1984; Adam *et al.*, 1985). Although a control hind (CIDR treatment only) was recorded as having a C.L. at laparoscopy, the structure was described as pale in colour and there was no elevation of plasma progesteone. This indicates that the structure observed was not a C.L. arising from the progesterone treatment.

No early calvings resulted from the induced ovulations. This is similar to results of other studies where pregnant mare serum gonadotrophin (PMSG) and synthetic GnRH have been used to induce ovulation in progesterone-primed anoestrous hinds but where few of the hinds calved early

(*Fennessy et al.*, 1986; Fisher *et al.*, 1986). It is not known why induced ovulations should have such low fertility in red deer. One possibility is that the induced ovulations were *silent* and not accompanied by oestrus. For instance, in sheep Rodway and Swift (1985) found that progesterone-primed ewes treated with buserelin did not exhibit oestrus. In the present study an attempt to use a crayon harness for oestrous detection was unsuccessful as there was no relationship between the marking of hinds and the estimated date of conception. Consequently it is not possible to ascertain whether any of the hinds displayed oestrous behaviour after the induced ovulation or whether any were actually mated. It is also possible that the rutting behaviour and fertility of the stags used in this trial were inadequate. Although the stags did not receive any treatment there is some evidence that the reproductive competence of individual stags is highly variable prior to the onset of the breeding season (Haigh *et al.*, 1984; Moore and Cowie, 1986).

A single injection of buserelin will induce ovulation in ewes but some of the resulting C.L. secrete progesterone only for a short period and at subnormal levels (Swift and Crighton, 1980; Rodway and Swift, 1985). The response of anoestrous ewes to GnRH or its analogues appears to depend on the timing of the treatment during the non-breeding season (Shareha *et al.*, 1976; Swift and Crighton, 1980). In deep anoestrus, release of FSH after an injection of buserelin is minimal and may result in the ovulation of immature follicles. However, in this study most of the hinds which responded to the GnRH analogue appeared to have normally functioning C.L. so, in the case of deer, treatment with buserelin does appear to produce a competent C.L. It is therefore possible that the hinds which ovulated were in fact mated and the only reason for the failure of pregnancy to occur may have been an inappropriate endocrine environment arising from the exogenous hormones which were used in this study. Treated and control hinds had 100% calving and a similar range of calving dates which indicates that the treatment regime had no subsequent detrimental effects on reproductive performance.

It is concluded that use of GnRH analogues in progesterone-primed red deer hinds has potential for controlling ovulation prior to the normal breeding season, but further studies are required to develop a satisfactory protocol which will lead to successful establishment of pregnancy.

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