Plasma concentrations of progesterone and luteinizing hormone during CIDR device insertion and the oestrous cycle of fallow deer (*Dama dama*)

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ABSTRACT

This study investigated the effect of a progesterone containing intravaginal CIDR (Controlled Internal Drug Releasing) device on plasma progesterone and luteinizing hormone (LH) concentrations during 14-day device insertion and the subsequent oestrous cycle of fallow deer (*Dama dama*). Blood samples were collected from 4 entire does at 20 minute intervals for periods of 8-12h on three occasions following device insertion (i.e. insertion, mid-treatment and withdrawal) and on Days 0, 5, 12 and 19 of the subsequent oestrous cycle. Plasma was analysed for progesterone and LH concentrations by radioimmunoassay. Plasma progesterone values were elevated (>6 ng/ml) and LH values reduced (< 1 ng/ml) during device insertion relative to pre-insertion values. Plasma LH concentrations were highest on Day 0 of the oestrous cycle, with a marked increase in the pulse amplitude. One doe exhibited a pre-ovulatory LH surge. Plasma progesterone increased from < 0.5 ng/ml on Day 0 to 1.5-4.2 ng/ml on Day 12 (luteal phase) and high-amplitude progesterone pulses were exhibited by all does on Day 19 (onset of luteolysis). The study has demonstrated that CIDR devices deliver sufficient progesterone to inhibit ovulation and the declining plasma LH concentrations during device insertion support a hypothesis of negative feedback by progesterone on LH secretion.

Keywords: Fallow deer, *Dama dama*, oestrous cycle, CIDR devices, progesterone, luteinizing hormone.

INTRODUCTION

Administration of exogenous progestagens has been used to delay oestrus and ovulation in domestic livestock by preventing follicular maturation and blocking the pre-ovulatory LH surge after natural luteolysis. Intravaginal progestagen-releasing devices, such as the CIDR device, act essentially as an artificial corpus luteum that simulates the pattern of progesterone secretion during the luteal cycle. Ovarian steroids, such as progesterone (or synthetic progestagens), exert a regulatory effect on the hypothalamus via a negative feedback mechanism (Karsch *et al.*, 1984), manifest as a reduction in gonadotrophin releasing hormone (GnRH) secretion during the luteal cycle. This results in a reduction in release of pituitary gonadotrophins, particularly luteinizing hormone (LH), leading to inhibition of completion of follicular development. This further inhibits the occurrence of oestrus and ovulation. Destruction of the corpus luteum (i.e. luteolysis) or removal of the exogenous source of progestagen (eg. CIDR device), result in a rapid decline in circulating progestagen concentrations, reducing the steroidal inhibition of LH secretion. Subsequent increase in basal and/or tonic LH secretion leads to completion of follicular development and eventually the initiation of the pre-ovulatory LH surge, oestrus and ovulation.

To be effective in ovulation control, the CIDR device, like other forms of exogenous progestagen delivery, must exert sufficient control over pituitary function to prevent the LH surge and inhibit ovulation. While previous studies have shown the CIDR device to be effective in controlling oestrus and ovulation in fallow deer (Asher *et al.*, 1986, 1990; Asher and Thompson, 1989; Jabbour *et al.*, 1991; Morrow *et al.*, 1992), no studies have investigated the effect of the CIDR device on progesterone concentrations and LH secretion in fallow deer does.

The aim of this study was to determine the effect of an exogenous source of progesterone, in the form of the intravaginal CIDR device, on circulating plasma progesterone concentrations and endogenous secretion of LH in entire fallow deer does during the breeding season, and compare the endocrine events during device insertion with those occurring during the subsequent oestrous cycle.

METHODS AND MATERIALS

Animal Management: Four mature (> 6 years old) entire fallow deer does, with a mean (± s.e.m.) liveweight of 42.4 ± 1.0 kg, were run continuously with a vasectomised buck from 1 February for 13 months. They were maintained as a single group with four other does that were involved in a separate study to evaluate oestrous cyclicity. All deer were grazed on pasture and offered supplements of hay and whole-grain maize. They had unlimited access to fresh water for the duration of the study.

Synchronisation treatment and blood sampling: Each doe received a single intravaginal CIDR device (Type G, 0.365g progesterone per device; InterAg, Hamilton, NZ) on 7 June (mid-breeding season). The stage of the oestrous cycle when treatment was initiated was not determined. The device was removed 14 days later on 21 June. On three occasions during the period of CIDR device insertion and on four occasions during the subsequent oestrous cycle, the four does were subjected to intensive blood sampling. On the day of insertion (7 June) each doe was sampled every 20 minutes for

12 hours, with devices inserted at Hour 6. On the seventh day of insertion (14 June) each doe was sampled every 20 minutes for 8 hours, and on the final day of insertion (21 June), every 20 minutes for 10 hours, with device removal at Hour 5. Further blood sampling sessions, each of 8-hours duration, were conducted on the predicted day of oestrus: Day 0 (23 June: 48 hr after device withdrawal), Day 5 (28 June), Day 12 (5 July) and Day 19 (12 July) of the subsequent oestrous cycle. Blood (5ml) was withdrawn from the right jugular vein via an indwelling cannula (Intracath intravenous 16gauge 12inch catheter; Deseret Medical Inc. Becton-Dickinson and Co., Utah, USA). The cannula was flushed with 1-2ml of heparinised saline after each sample was withdrawn to maintain patency. Upon removal of the cannula at the end of the sampling session, each doe received an intramuscular injection of 5ml long-acting antibiotic (Propen LA; Glaxo New Zealand Ltd, Auckland, New Zealand). The blood samples were centrifuged within 30 minutes of collection and the plasma stored at -10°C until assayed.

Plasma hormone determinations: Plasma progesterone concentrations were measured in duplicate by a direct radioimmunoassay method previously validated for use in fallow deer by Asher *et al.*, (1988). All samples from an individual doe were included in a single assay. Multiple control samples of low (0.83ng/ml), medium (3.55ng/ml) and high (8.55ng/ml) progesterone concentrations were included in each assay. The inter-assay coefficients of variation were 16.0, 6.5 and 7.4% for the three controls respectively. Intra-assay coefficients of variation were 14.9, 10.5 and 5.5% respectively. Sensitivity of the assay, being the least amount statistically distinguishable from zero, was 0.16 ng/ml.

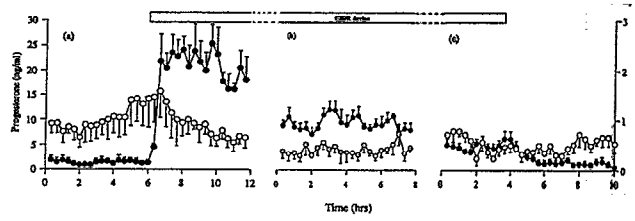
Plasma LH concentrations were measured using the heterologous ovine radioimmunoassay of Scaramuzzi *et al.*, (1970) and validated for fallow deer plasma by Asher *et al.*, (1986). Crossreactivity with other hormones was low (<0.5%) as described previously by Kelly *et al.*, (1982) and Asher *et al.*, (1986). Multiple control samples of low (0.97ng/ml), medium (5.12ng/ml) and high (8.75ng/ml) LH concentrations were included in each assay. The inter-assay coefficients of variation were 23.0, 7.4 and 8.1% for the three controls respectively. Intra-assay coefficients of variation were 16.5, 4.3, and 4.9% respectively. Sensitivity of the assay was 0.30ng/ml.

Statistical analysis: Plasma progesterone and LH profiles were analysed separately for each doe at each sampling session using the PULSAR pulse identification routine (Merriam & Wachter, 1982). Inputs into PULSAR included intra-assay standard deviation and the smoothing time was set for 180 minutes. A limiting value of 2.7 standard deviation units was used for peak splitting. Temporal trends in plasma LH and progesterone concentrations during insertion of CIDR devices were analysed by analysis of variance.

RESULTS

Mean (+ s.e.m.) plasma progesterone and LH concentration profiles over the three intensive sampling periods during device insertion are presented in Figure 1. Mean plasma progesterone concentrations showed a rapid increase from basal concentrations of 1-2 ng/ml to 22 ng/ml within 40 minutes of insertion of CIDR devices (Figure 1a). Concentrations declined slightly after four hours of insertion, but remained elevated between 15-20 ng/ml for the remainder of the first sampling period. The mean LH concentrations exhibited a steady decline and were significantly reduced after insertion of the device from 1.5 ng/ml at insertion to 0.5 ng/ml six hours later. On the seventh day of device insertion (i.e. second sampling period) the mean plasma progesterone concentrations had declined to approximately half of the immediate post-insertion concentrations and fluctuated, over the eight hours of sampling, between 7-12 ng/ml (Figure 1b). Mean plasma LH concentrations were low and fluctuated between 0.20 and 0.75 ng/ml throughout the 8-hour period, with no pattern evident for individual profiles. On the final day of device insertion (i.e. third sampling period), mean plasma progesterone concentrations had declined over the 7-day period to 4-7ng/ml. Withdrawal of the device was associated with a further decline from 6.3 ng/ml to 4.8 ng/ml 20 minutes after removal, 2.6 ng/ml one hour after removal and 1.4 ng/ml two hours after removal. Mean plasma LH concentrations fluctuated erratically between 0.40 and 0.75 ng/ml during the sampling period (Figure 1c) with no significant increase within six hours of removal of the device. LH pulse frequency was generally low (0-1 pulse/12 hr) throughout the insertion period.

FIGURE 1: Mean (+ s.e.m.) profiles of plasma progesterone (•) and LH (o) concentrations for entire fallow deer does during intravaginal insertion of CIDR devices. Samples were collected at 20 minute intervals for periods of 8-12 hours. (a) day of insertion (b) mid-treatment (c) day of removal.

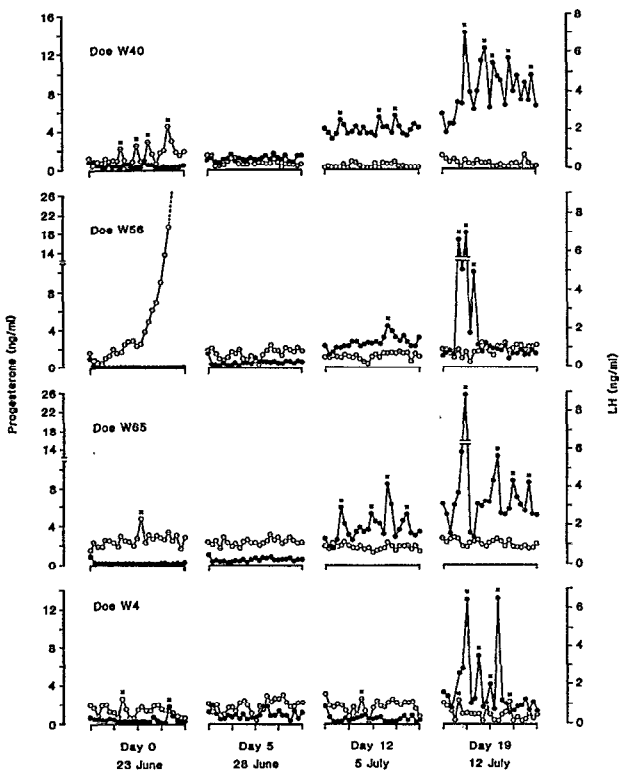


Individual plasma progesterone and LH concentration profiles over the four intensive sampling periods of the subsequent oestrous cycle are presented in Figure 2. During the oestrous cycle, plasma progesterone concentrations increased progressively with each sampling period. Mean concentrations on Day 0 were uniformly low (< 0.5 ng/ml) with no pulsatility, increasing to 0.5-1.2 ng/ml on Day 5 with occasional pulses, 1.5-4.2 ng/ml on Day 12 and 3-10 ng/ml on Day 19. There was a significant increase in pulse frequency on Days 12 (6 pulses/12 hr period) and Day 19 (4.5-7.5 pulses/12 hr period). Mean plasma LH concentrations were highest on Day 0, ranging from 0.5-10.0 ng/ml. This wide range in values reflected the dramatic influence of one doe (W56) which exhibited the initiation of a pre-ovulatory LH surge within the sampling period (Figure 2). In addition, LH pulse frequency was increased (4 pulses/8hr period) for one doe (W40) on Day 0 (Figure 2). During subsequent sampling sessions (Days 5, 12, 19) plasma LH concentrations fluctuated between 0.2-1.2 ng/ml, with no pattern being evi-

dent between or within sampling periods and LH frequency was low (0-1 pulses/8hr period). The plasma LH profile of Doe W40 showed a marked increase in pulse frequency (4 pulses/8hr) and amplitude on Day 0 of the oestrous cycle. In contrast, the profile of Doe W56 on Day 0 showed a successive increase in plasma LH concentrations from 0.8 ng/ml to 22.0 ng/ml over the final four hours of the sampling session, beginning approximately 48 hours after removal of the CIDR device. This was interpreted as the initiation of the pre-ovulatory LH surge.

Plasma progesterone profiles on Day 19 of the oestrous cycle (final sampling session) were similar for all does, with a marked tendency to exhibit high amplitude pulses, with peak values being approximately 2-4 times higher than basal values on the day (Figure 2).

FIGURE 2: Profiles of plasma progesterone (•) and LH (o) concentrations of entire fallow deer does on Days 0, 5, 12 and 19 of the subsequent oestrous cycle. Samples were collected at 20 minute intervals for periods of 8 hours. The asterisks denote discrete pulses.



DISCUSSION

The results of this study on fallow deer demonstrate the ability of the intravaginal CIDR device to deliver physiological levels of progesterone via the vagina, and to inhibit ovulation over a period of 14 days. This is consistent with previous studies in this species (Asher *et al.*, 1986, 1990; Asher and Thompson, 1989; Jabbour *et al.*, 1991; Morrow *et al.*, 1992). The initial rise in plasma progesterone concentration occurring within 40 minutes of device insertion (> 20 ng/ml) exceeded concentrations observed during the oestrous cycle (6-8 ng/ml) in this and previous studies (Asher, 1985; Mulley, 1989). However, this situation is probably of short duration, with concentrations declining close to normal physiological values (< 10 ng/ml) within 24 hours of device insertion (Asher *et al.*, 1986, 1990; Asher and Thompson, 1989; Morrow *et al.*, 1992). In the present study, plasma progesterone concentrations seven days after device insertion fluctuated between 6-12 ng/ml. By 14 days post-insertion, plasma concentrations were about 4-6 ng/ml, well within the normal physiological range observed during peak luteal development (Asher, 1985; Asher *et al.*, 1990; Mulley, 1989).

Device withdrawal 14 days after insertion was associated with a rather gradual decline in plasma progesterone concentrations (i.e. 1ng/ml/hr), such that basal values (< 0.5 ng/ml) were attained about six hours after device withdrawal. Although previous studies have reported a more rapid decline in plasma progesterone concentrations (Asher and Thompson, 1989; Asher *et al.*, 1990), these studies were based on wider blood sampling intervals (i.e. 2 hours) than in the present study (i.e. 20 minutes). It is evident that clearance of exogenous progesterone is not instantaneous, and may be influenced by residual exogenous progesterone residing in the vagina at withdrawal (presumably absorbed into the vaginal mucus secretions) or metabolic clearance rates. Nevertheless, the return to basal values following withdrawal of the CIDR device is more rapid than natural luteolysis (Asher *et al.*, 1988) or cloprostenol-induced luteolysis (Asher and Thompson, 1989; Asher *et al.*, 1990).

The decline in basal plasma LH concentrations concomitant with the increased plasma progesterone concentrations as CIDR devices were inserted supports the hypothesis of a negative feedback effect of progesterone on LH secretion, as described for sheep (Karsch *et al.*, 1979, 1984; Goodman and Karsch, 1980) and cattle (Ireland and Roche, 1982). Mean plasma LH concentrations remained depressed (< 1 ng/ml) 7 and 14 days after device insertion, indicating that the CIDR device effectively controlled pituitary function throughout the entire 14-day insertion period. Pulse frequency was generally low (0-1 pulses/12 hr period) during insertion. While device removal was not associated with an immediate increase in LH secretion, the subsequent sampling period, approximately 48 hours later, was associated with a marked increase in the amplitude of LH pulse episodes. A similar finding has been reported for Pere David's deer (Elaphurus davidianus; Loudon *et al.*, 1990) and further supports the regulatory role of progesterone on LH secretion, whereby progesterone withdrawal leads to an increase in LH secretion during the pre-ovulatory phase (Karsch *et al.*, 1979, 1984).

The plasma progesterone concentrations observed on Days 0, 5, 12 and 19 of the subsequent oestrous cycle are similar to previous reports for this species (Asher, 1985; Asher *et al.*, 1986, 1988; Mulley, 1989; Morrow *et al.*, 1992). However, this study presents the most intensive frequency of blood sampling reported for fallow deer does, and has indicated that the secretion of luteal progesterone involves both tonic and pulsatile patterns. There is little doubt that the tonic secretion increases with progressive luteal development up to, and beyond, Day 12. This is evidenced by a progressive elevation in basal concentrations in the plasma, with mean values increasing from < 0.5 ng/ml (Day 0) to approximately 2 ng/ml (Day 5) and 4 ng/ml (Day 12). However, individual

plasma progesterone profiles show a certain amount of variation, manifest as apparent pulsing of progesterone secretion at Day 5 and 12. This is in accord with other domestic species such as cattle (Schallenberger *et al.*, 1984). In the study on cattle, it was demonstrated that pulses in luteal progesterone secretion were associated temporally with pulses in LH and follicle stimulating hormone (FSH) secretion. However, this has been shown to be not the case in sheep (McNeilly *et al.*, 1992) and was also not apparent in the present study on fallow deer.

The pattern of progesterone secretion on Day 19 of the fallow deer oestrous cycle is even more indicative of pulsatility in this species. Day 19 represents the approximate period of luteolysis in fallow deer (Asher *et al.*, 1988; Mulley, 1989). High amplitude pulses of plasma progesterone were observed for all four does in this study, with some pulses being 4-5 times greater than the Day 19 basal values (eg. Doe W56, Figure 2). This has not been previously reported for fallow deer, probably because of the wider interval blood sampling schedule of previous studies (eg. 2 hourly; Asher *et al.*, 1988). The progesterone pulse episodes on Day 19 did not appear to be temporally related to any noticeable LH secretory events. It is probable that the corpus luteum, which is undergoing luteolysis, is exhibiting the erratic progesterone secretory pattern in response to other hormonal events. In particular, prostaglandin F2α (PGF2α) of uterine origin and oxytocin of luteal origin are secreted as intermittent pulses during luteolysis (Asher *et al.*, 1988). Either of these two hormones may be eliciting temporarily increased progesterone secretion from the regressing corpus luteum. This hypothesis is supported by studies on sheep (Peterson *et al.*, 1976) and cattle (Peterson *et al.*, 1975; Schallenberger *et al.*, 1984). The data from intensive blood sampling during luteolysis in fallow deer indicate that single point sampling during this period is likely to yield highly variable plasma progesterone concentrations, depending on the chance effect of sampling during or beyond a progesterone pulse episode. This may partly explain the wide level of variance observed in mean plasma progesterone concentration between Days 19-20 in other studies on this species (Asher, 1985).

Plasma LH concentrations during the oestrous cycle (Days 0-19) have not been previously recorded for fallow deer, although a number of studies have investigated changes in LH secretion during the pre-ovulatory period (i.e. Day 0 ± 24 hours)(Asher *et al.*, 1986, 1990). The LH secretory pattern on Day 0 is indicative of the release of steroidal negative feedback control following removal of the CIDR device. Two patterns of LH secretion were evident in the present study. First, as seen in the profile of Doe W40 (Figure 2), a general increase in both the basal plasma LH concentration and frequency of LH pulses was observed. Second, one doe (W56; Figure 2) also exhibited the onset of the pre-ovulatory LH surge, whereby plasma LH concentration increased progressively from approximately 1 ng/ml at Hour 2 to > 22 ng/ml by Hour 8. On the basis of other studies during the pre-ovulatory period of fallow deer (Asher and Thompson, 1989; Asher *et al.*, 1986, 1990), this event clearly represented the initiation of the pre-ovulatory LH surge. The onset occurred approximately 48 hours after the removal of the CIDR device. In other studies, the pre-ovulatory LH surge was initiated at a mean time of 41 hours after CIDR device withdrawal (or prostaglandin injection) and lasted for approximately 16-20 hours (Asher and Thompson, 1989; Asher *et al.*, 1990). It is not known whether Doe W56 exhibited oestrus during the sampling period, as no buck was present during blood sampling and oestrous observations were not made before or after the sampling session. However, in all previous studies, the onset of the pre-ovulatory LH surge was associated with the onset of overt oestrus (Asher and Thompson, 1989; Asher *et al.*, 1986, 1990).

Plasma LH concentrations occurring on Days 5, 12 and 19 of the oestrous cycle were generally lower than those occurring during the pre-ovulatory period (Day 0), and were similar to those occurring during the previous period of insertion of the CIDR device. While some apparent pulses are noticeable, the overall values are close to the lower limit of assay sensitivity (0.3 ng/ml), implicating a degree of assay variation rather than a true physiological event.

In conclusion, the sequential pattern of plasma progesterone concentration during insertion of CIDR devices clearly indicates a suitable level of hormonal release by the devices sufficient to control pituitary LH release and ovarian follicular patterns. This was manifest as a notable reduction in plasma LH concentrations immediately following insertion of the device, and a notable increase in LH concentrations 48 hours after removal of the device. The pattern of luteal progesterone secretion during the subsequent oestrous cycle has raised some interesting questions about tonic versus pulsatile secretion of the hormone, particularly during the period of luteolysis.

ACKNOWLEDGEMENTS

We thank staff of the Ruakura Deer Unit for assistance with animal management and during the blood sampling sessions. We also thank D. Duganzich for statistical analysis.

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