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Artificial insemination of red deer (*Cervus elaphus*) hinds late in the breeding season

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ABSTRACT

Red deer hinds in New Zealand typically conceive around April, however they have spontaneous ovulatory activity extending until September. There is little experience with mating late in the potential breeding season and there is some evidence that they may not be able to achieve pregnancy at this time of the year. The present study determined whether red deer hinds are capable of becoming pregnant following an induced ovulation in August. Ten adult red deer hinds were treated with intravaginal progesterone-releasing devices (CIDRs) and equine chorionic gonadotrophin during May prior to set time artificial insemination or were untreated, non inseminated controls. Ovulation and pregnancy were monitored by measurement of plasma progesterone concentration and rectal ultrasonography, respectively. This procedure was repeated in August. The progesterone data showed that the control hinds experienced spontaneous ovulations on both occasions and that, following CIDR removal, 9/10 and 10/10 treated animals ovulated in May and August respectively. Pregnancy rate at 45 days post insemination was 5/9 and 3/10 animals ovulating in May and August, respectively. Although these results showed modest success of set time artificial insemination following induced ovulation, they clearly indicated that red deer hinds are capable of conceiving successfully late in the breeding season.

Keywords: *Cervus elaphus*; red deer; seasonal breeding; progesterone; pregnancy rate; ovulatory activity.

INTRODUCTION

Red deer exhibit a high degree of reproductive seasonality with mating occurring during autumn (Lincoln, 1992). However, in the absence of pregnancy red deer hinds are polyoestrous and have oestrous cycles of 14 to 22 days length that are repeated six to nine times during a potential breeding season of five to seven months (Duckworth & Barrell, 1992; Meikle & Fisher, 1996; Asher *et al.*, 1997; 2000). In spite of the potential for achieving pregnancy throughout this period, there is little or no information about the mating of red deer hinds late in the breeding season. Studies in New Zealand using *in vitro* fertilised oocytes, obtained from hinds either by ovum pick-up or from abattoir-derived deer ovaries, have shown a marked inability of the resulting embryos to develop to the blastocyst stage when this is attempted in August, but no such difficulty with those collected from April to June/July (D.K. Berg, Personal communication). This *in vitro* finding indicated that although red deer hinds might be capable of ovulating and even conceiving in August, they may not be able to establish a successful pregnancy at this time of the year. Therefore, the study described in this paper was designed to test the hypothesis that conception and a successful pregnancy would occur in red deer hinds inseminated in August. In addition to providing data on ovulatory activity of the hinds, blood sampling was carried out to determine the plasma concentration of progesterone achieved with a

prototype deer controlled internal drug releasing (CIDR) device.

MATERIALS AND METHODS

Animals and management

Animals were mixed-age, adult red deer hinds, ranging in live weight from 90 to 112 kg kept at the Deer Unit of the Lincoln University Research Farm (43° 39'S, 172° 28'E). They had unrestricted access to ryegrass-white clover pasture with dietary supplementation when required during winter. No males were included with the study animals, but mature stags were present on the property. All manipulation of animals was approved by the Lincoln University Animal Ethics Committee.

Procedure

In May, 10 hinds received a deer prototype CIDR (Deer Improvement, Hamilton, New Zealand. McMillan & Asher, 2007) that was withdrawn 12 days later. At CIDR withdrawal they received a single intramuscular injection of 200 I.U. equine chorionic gonadotrophin (Folligon; Intervet Ltd., Upper Hutt, New Zealand). Trans-cervical artificial insemination with a single straw of thawed semen (see below) was conducted by an experienced Deer Improvement technician 52 hours after CIDR withdrawal. Another 10 hinds were untreated controls that were not inseminated. The procedure was repeated in August using 10 treated and 8 control hinds.

FIGURE 1: Profile of plasma progesterone concentration recorded from two representative untreated (Control) red deer hinds in May indicative of a regressing (●) and a developing (○) corpus luteum.

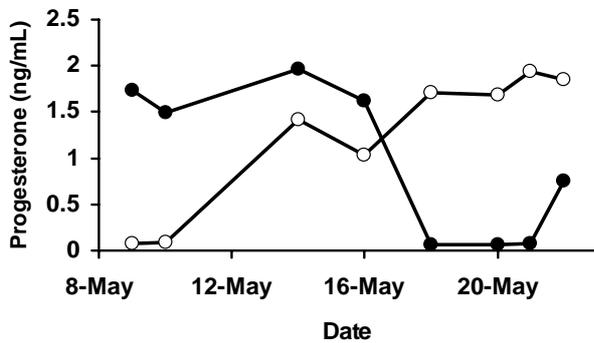
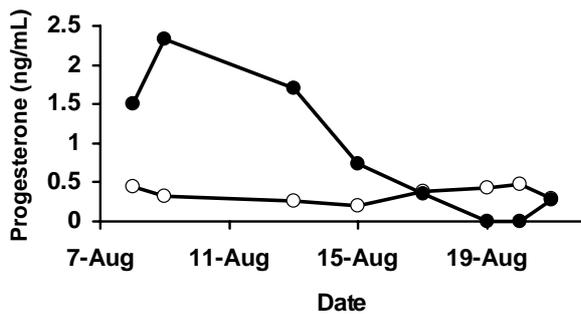


FIGURE 2: Profile of plasma progesterone concentration recorded from two representative untreated (Control) red deer hinds in August indicative of a regressing corpus luteum (●) and of no ovulatory activity (○).



Throughout each phase of the study, a single heparinised blood sample (10 mL) was obtained by jugular venipuncture on Day 0 (immediately prior to CIDR insertion), 1, 5, 7, 9, 11, 12, 13, and 26 from all hinds. Plasma obtained following centrifugation of the blood sample was stored at -20°C.

The semen used on both occasions was provided by Deer Improvement. It was collected once from a stag in early May by electro-ejaculation and was diluted in a proprietary diluent (Triladyl® containing Tris, citric acid, glycerol, sugar, buffers and antibiotics, Minitüb, Tiefenbach, Germany) mixed with 5% egg yolk so that each 0.25 mL straw contained approximately 35 million live spermatozoa prior to freezing. Semen straws were kept in liquid nitrogen until required, at which point they were thawed in warm water and used within a few minutes. The semen had been evaluated by visual appraisal under phase-contrast microscopy as having over 60% live spermatozoa post thaw and a motility score of 5 (1 = No motility; 5 = High motility).

Ovulatory activity

Based on previous literature, plasma progesterone concentrations greater than 1.0 ng/mL

were considered indicative of ovulation and presence of a functional corpus luteum (Kelly *et al.*, 1982; Adam *et al.*, 1985; Duckworth and Barrell, 1988; 1991; Jopson *et al.*, 1990).

Pregnancy scanning

Pregnancy was assessed by rectal ultrasonography (15 mm, Echo Camera, Aloka Company, Japan) at 45 days post-insemination (Revol and Wilson, 1991).

Progesterone assay

Progesterone concentration of plasma was measured by staff of TESTlink milk analysis laboratory in Hamilton, New Zealand using a commercial bovine plasma progesterone enzyme immunoassay kit (Ridgeway Research Limited, Park Farm Buildings, Park Lane, St. Briavels, Gloucestershire, United Kingdom). Samples were assayed singly. Assay standards were dissolved in cervid plasma and the within-assay coefficient of variation was 9.7% for a control serum containing 1.51 ng/mL progesterone.

Statistical analysis

Comparison of mean plasma progesterone concentration within control hinds between May and August was performed using a repeated measures ANOVA. The progesterone data for treated hinds during the CIDR treatment periods were examined by use of simple linear regression. Differences between the May and August pregnancy rates were analysed by a binomial test.

RESULTS

Progesterone concentration

In control hinds, plasma progesterone concentration was highly variable and ranged between 0 and 4.8 ng/mL in May (Figure 1) and between 0 and 2.3 ng/mL in August (Figure 2). The August values were on average lower than those in May ($F_{1, 128} = 7.97$; $P = 0.01$). All 10 control hinds in May and 6 out of 8 in August had at least one plasma progesterone concentration value that exceeded 1.0 ng/mL.

On the day of CIDR insertion 5/10 and 4/10 hinds in May and August, respectively, had a plasma progesterone concentration above 1.0 ng/mL. Insertion of CIDRs elevated mean plasma progesterone concentration to 7.6 ± 0.91 and 7.7 ± 0.55 ng/mL (May and August respectively) one day later. (Figure 3 shows data from the August study). Thereafter, they underwent a linear decrease of 0.33 and 0.25 ng/mL/day in May and August respectively (Figure 4), but this difference was not significant ($P = 0.303$). They fell to baseline values one day after CIDR withdrawal (0.8 ± 0.18 and 0.8 ± 0.25 ng/mL, May and August respectively), but 14 days later 9/10 hinds in May and all 10 in August had a plasma progesterone concentration that exceeded

FIGURE 3: Profile of mean plasma progesterone concentration recorded from treated red deer hinds in August (n = 10). The 1st arrow from left marks date of insertion of prototype intravaginal CIDR, the 2nd arrow marks CIDR withdrawal and intramuscular injection of 200 units equine chorionic gonadotrophin. Hinds were artificially inseminated at 52 hours following CIDR withdrawal. Vertical bars denote standard error of mean.

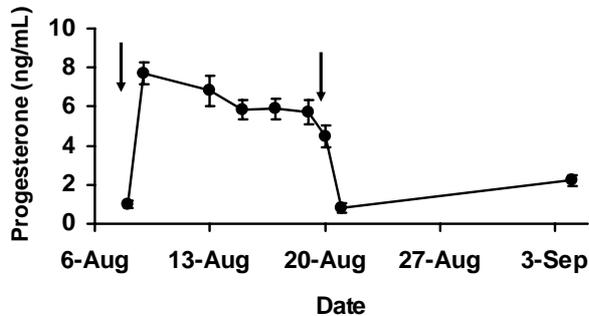
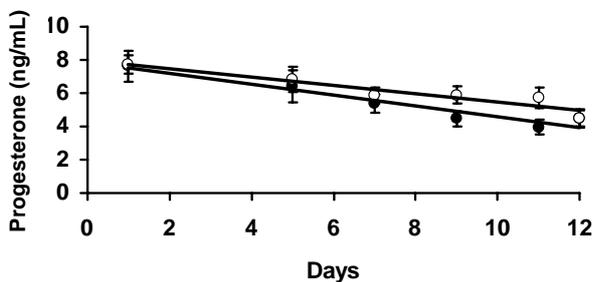


FIGURE 4: Changes in mean plasma progesterone concentration recorded from ten red deer hinds during the period (Days 1 to 12) of prototype CIDR administration in May (●) and August (○). The lines represent the linear regressions (slope = -0.33 and -0.25 ng/mL for May and August, respectively). Vertical bars denote standard error of mean.



1.0 ng/mL and represents the number of animals that had ovulated. The progesterone profile of the single hind that failed to ovulate in May did not appear to be different from that of other treated hinds.

Pregnancy rate

At 45 days post insemination, five hinds in May and three in August were pregnant. This difference was not significant ($P = 0.361$) and the numbers pregnant represent 5/9 and 3/10 animals in May and August, respectively, of those that had ovulated. All five hinds pregnant in May gave birth to calves in early January that were within the normal weight range of 8.5 to 10 kg. One of the three hinds diagnosed as newly pregnant in August failed to calve, but the other two delivered calves on 18 April that weighed 8.5 and 9 kg.

DISCUSSION

These results support the hypothesis that conception and successful pregnancy can occur in red deer hinds inseminated during August in New Zealand. This implies that the inferences based on *in vitro* culture studies (D.K. Berg, Personal communication) do not necessarily apply to the *in vivo* situation. It is possible this disparity indicates a seasonal impairment of oocyte competence that applies to artificially matured oocytes but not to those maturing naturally *in vivo*. Further evidence of a seasonal fall off in deer blastocyst formation rate with *in vitro* studies comes from a related species, sika deer, where the rates were 34% and 22% for the breeding and non-breeding seasons, respectively (Locatelli *et al.*, 2006). However, it should be noted that hinds in the present study received equine chorionic gonadotrophin during the procedure for induction of ovulation and this stimulus may have improved oocyte competence, even to the extent of overcoming any seasonal impairment.

In spite of the successful establishment of pregnancy in these hinds, the conception rates are low (i.e. 50% and 30%) compared with data from other studies that used trans-cervical AI (e.g. 74%, Rhodes *et al.*, 2003; 68%, McMillan & Asher, 2007), although Rhodes *et al.* (2003) quote a range of 10 – 100%. We cannot explain the apparently low success of trans-cervical timed AI in our hinds, but the sample size (n = 10) in both of the studies is smaller than would be required to provide a robust measure of conception rate. Also, we have not made any allowance for possible seasonal differences in optimal timing of insemination in relation to CIDR withdrawal or requirement for presence of stags. Nevertheless, the object of the study was to determine whether pregnancy could occur at all in August and this was achieved despite the low number of pregnancies that were recorded.

In contrast with the pregnancy results, induction of ovulation with the prototype CIDR and equine chorionic gonadotrophin procedure used here was almost 100% successful. This is similar to the results, based on heat detection in hinds, reported by McMillan and Asher (2007) who also used the prototype CIDRs. The present study extends their findings by showing that this procedure works equally well late in the breeding season (August). It adds to the study of early-season (February) AI, also using prototype CIDRs, where rising 2- and 3-year-old red deer hinds attained pregnancy rates of 49% and 38%, respectively (McMillan & Asher, 2007). Consistent with the induction of ovulation following the treatment in August is the occurrence of spontaneous ovulations in most (6/8) of the control hinds at the same time of the year. This confirms the findings of others, also based on circulating progesterone concentrations

(Duckworth & Barrell, 1992; Meikle & Fisher, 1996; Asher *et al.*, 1997; 2000), that August is within the ovulatory season for red deer in New Zealand and provides the *a priori* basis on which successful breeding could be anticipated.

A secondary objective of this study was to examine the performance of the prototype CIDR device as a means of delivering progesterone to red deer hinds. It is remarkable that the plasma progesterone concentrations achieved here were essentially identical to those reported by McMillan and Asher (2007) from hinds treated in March (i.e. about 7.5 ng/mL at 24 hours) and with virtually the same decline (0.34 ng/mL/day) as our hinds treated in May (0.33 ng/mL/day). We do not know why there was a trend for the decline in concentration to be slower in August, however this may be related to the slight loss in live weight (mean 4.7 kg) that the hinds incurred between May and August. It is possible that the lower live weight status of hinds in August could impair ovulatory activity (hence the trend seen in controls) and reduce metabolic (e.g. liver) and other functions that are involved in clearance of hormones from the circulation. The progesterone profiles of the hind that did not ovulate in response to the induction procedure and of the hinds that failed to conceive did not appear to differ from those of the other treated hinds, and thus have provided no insight into the underlying cause of these failures.

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