Induction of abortion in red deer hinds with prostaglandin analogue

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Summary: Effects of administration of a single i.m. injection of prostaglandin analogue (500 μg cloprostenol) to pregnant red deer hinds on the viability of the corpus luteum and post-implantation embryo (Days 30, 40 and 60) was studied using plasma progesterone profiling and ultrasonography. Fifty six mature hinds were joined with fertile stags following oestrous synchronisation with CIDR devices and PMSG. An initial rectal ultrasound scan was performed 31 days after CIDR device removal (ie. Day 29 from oestrus), whereafter 36 pregnant hinds were allocated randomly to 3 treatment groups (n = 12 per group). Six hinds in each of Groups 1, 2 and 3 received a single i.m. injection of cloprostenol on Day 30, 40 or 60 of pregnancy, respectively. The remaining 6 hinds in each group served as controls. Hinds were blood sampled twice weekly from 2 April - 30 June, with further blood samples taken every 6 hours for 72 hours following cloprostenol administration to treated hinds. Plasma samples were analysed for concentrations of progesterone to assess luteal function and luteinizing hormone (LH) to indicate ovulatory activity. All hinds were scanned every 10 days from Day 30 to Day 90 inclusive. For each group further scanning was performed 0, 24, 48, 72 and 120 hours after cloprostenol administration to treated hinds.

All control hinds maintained pregnancy throughout the trial, this being associated with a constant elevation in plasma progesterone concentrations of ~4-8 ng ml^{-1} from Day 8. For the 18 hinds receiving cloprostenol, abortion occurred in 3 hinds on Day 30, 5 hinds on Day 40 and 1 hind on Day 60; all other treated hinds maintained pregnancy throughout the trial. Abortion was associated with embryonic death and mummification between 48 and 72 hours, and fetal expulsion between 72 and 120 hours. Both aborting and non-aborting treated hinds exhibited initial reductions in plasma progesterone concentrations following cloprostenol administration, with recovery of initial levels of progesterone secretion occurring within 72 hours in non-aborting hinds. Progesterone concentrations in aborting hinds generally remained below 1.0 ng ml^{-1} by 72 hours from cloprostenol injection, indicating complete luteolysis, with 3 aborting hinds exhibiting a pre-ovulatory surge of LH within 72 hours. It is concluded that a single injection of 500 μg cloprostenol has a low level of efficacy in inducing abortion of the post-implantation embryo in red deer.

INTRODUCTION: Prostaglandin F2α (PGF) and its synthetic analogues are powerful luteolytic agents in placental mammals. Episodic release of endogenous PGF from the uterine endometrium is responsible for the luteolytic demise of the corpus luteum in the non-pregnant female, effectively terminating the progesterone secretory capacity of luteal tissue to induce a return to oestrus (1, 2). Recent studies on fallow deer (*Dama dama*) indicate a similar mechanism operating during the luteolytic phase of the cervid oestrous cycle (3). Furthermore, a number of studies on both fallow deer and red deer (*Cervus elaphus scoticus*) have shown that the corpus luteum in the non-pregnant female is sensitive to the luteolytic effects of exogenous prostaglandin analogues (4, 5, 6, 7).

While natural luteolysis in mammals is inhibited by the presence of the pre-implantation embryo (8, 9), artificial destruction of the corpus luteum during various stages of gestation (ie. by injection of PGF or analogues) can lead to embryonic death and fetal abortion in a number of species due to withdrawal of endogenous progesterone support of pregnancy (10, 11). This has been used to terminate unwanted pregnancy in domestic livestock species. However, the efficacy of exogenous PGF (or analogues) to induce embryonic wastage (pre-implantation; <20 days) or fetal abortion (post-implantation; >20 days) varies with species, particularly in relation to the stage of gestation. Induction of embryonic wastage prior to implantation (eg. following superovulation/embryo recovery programmes) is generally successful due to the embryo's absolute dependance on the presence of luteal tissue. However, placental contribution to progesterone synthesis in some mammalian species (eg. sheep and horse; 12, 13) often denigrates the role of the corpus luteum in later pregnancy maintenance, whereby luteal destruction or corpus luteum removal (lutectomy) after 60-80 days of pregnancy do not generally disrupt pregnancy (14). By contrast, species in which the placenta does not contribute to progesterone synthesis (eg. goat, cow, pig; 15, 16, 17) are reliant on the continual presence of luteal tissue for maintenance of pregnancy, which can be disrupted at any stage by luteal destruction or removal (15). The relative contributory roles of luteal tissue and placental tissue to maintanence of pregnancy in cervids have not been established. Furthermore, the efficacy of induction of abortion by corpus luteum destruction has not been evaluated.

Induction of abortion in farmed red deer has received little attention until recently. However, the requirement to place only non-pregnant hinds into live export quarantine, and a possible introduction of schedule penalties for presenting pregnant hinds for slaughter at DSPs, have created interest in the application of prostaglandin analogues to induce abortion in this species. The present study was a preliminary investigation into the effects of a single injection of prostaglandin analogue to pregnant red deer hinds on the viability of the corpus luteum and fetus.

MATERIALS AND METHODS: A total of 56 mature (2-10 years old) red deer hinds on the Ruakura Agricultural Centre (37° 46' S, 175° 20' E) each received a single intravaginal CIDR device (type G, 0.3 g progesterone per device; Agricultural Division, CHH Plastic Products Group Ltd, Hamilton) for 12 days from 2 April. Devices were replaced on the eighth day and an i.m. injection of 250 i.u. PMSG (Folligon; Intervet, Lane Cove, NSW, Australia) was administered at final device withdrawal. The hinds were drafted into six mobs, each joined with a single fertile stag (1 male: 9-10 females) for 96 hours. The mobs were then grouped together and run continuously with vasectomised stags for the remainder of the trial.

Hinds were blood sampled by jugular venepuncture every fourth day from 2 April to 30 June. Plasma were analysed for concentrations of progesterone to assess luteal (and/or placental) development. Ultrasound β-made imaging of the reproductive tracts was performed with a 5 MHz probe (Aloka SSD-210 DX II; Aloka Co. Ltd, Japan) inserted rectally while the hinds were restrained manually in a pneumatic crush. Fetal/pregnancy age was assessed by the criteria of White *et al.* (18) and Wilson and Bingham (19). An initial ultrasound scan was performed on all hinds 31 days after removal of CIDR devices (ie. Day 29 of pregnancy). Twelve hinds that were assessed as pregnant were drafted into a separate group. On the following day (Day 30) 6 of these hinds, chosen at random, received an intramuscular injection of 500 μg cloprostenol (2.0 ml Estrumate; Imperial Chemical Industries PLC, Cheshire, UK), while the remaining 6 hinds served as controls. All 12 hinds were blood sampled every 6 hours for 72 hours

and scanned 0, 24, 48, 72 and 120 hours after administration of cloprostenol. Plasma were analysed by radioimmunoassay (20, 21); for concentrations of progesterone to assess changes in luteal status, and for concentrations of luteinizing hormone (LH) to determine the incidence of the pre-ovulatory LH surge (21).

The procedure was repeated for Days 40 and 60 of pregnancy, using hinds not subjected previously to cloprostenol treatment or the intensive blood sampling protocol. Ultrasonography from Day 40 included observation of fetal heartbeat to validate fetal viability. Hinds that had failed to become pregnant at the synchronised oestrus were removed from the trial after scanning on Day 40. All remaining hinds, including those that aborted, were subjected to rectal ultrasonography every 10 days thereafter until Day 90.

RESULTS: Pregnancy was maintained to term (234±10 days) in all control hinds (n = 18) in this study, indicating no detrimental effects of repeat blood sampling and ultrasonography on pregnancy. Of 18 hinds receiving a single i.m. injection of 500 μg cloprostenol, nine (50%) aborted between 48 and 120 hours later. The remaining treated hinds all maintained pregnancy to term. The incidence of abortion was not uniform for the three stages of pregnancy investigated (Table 1), being 3 hinds on Day 30, 5 hinds on Day 40 and only 1 hind on Day 60.

Table 1 Incidence of abortion and ovulation in treated and control red deer hinds.

Treatment	No of hinds per group	No. of hinds aborting	No of hinds ovulating (LH surge)
Day 30	6	3	1*
Controls	6	0	0
Day 40	6	5	2*
Controls	6	0	0
Day 60	6	1	0
Controls	6	0	0

* aborting hinds only.

Abortion was preceded by fetal death (ie. cessation of visible heartbeat) and fluid resorption (mummification) 48-72 hours after injection of cloprostenol. Fetal expulsion, although not observed, was complete by 120 hours after injection. In one case (Day 40), residual placental tissue was observed following fetal expulsion at 120 hours, but had been expelled or resorbed 10 days after injection of cloprostenol.

Control hinds exhibited profiles of plasma progesterone concentrations typical of pregnant cervids, noteably on elevation in mean concentrations from < 1 ng ml^{-1} at Day 0 to 4-6 ng ml^{-1} by Day 15-20, followed by a plateau of 5-8 ng ml^{-1} through to termination of blood sampling on Day 90 (Fig. 1). Administration of cloprostenol resulted in a dramatic decline in mean plasma progesterone concentrations of treated hinds, such that values were generally below 1 ng ml^{-1} 24-28 hours after injection (Fig 1). In both aborting and non-aborting treated hinds there was a gradual increase in mean plasma progesterone concentrations between 5 and 15 days after administration of cloprosterol, with peak values being similar to those of control hinds on the same day.

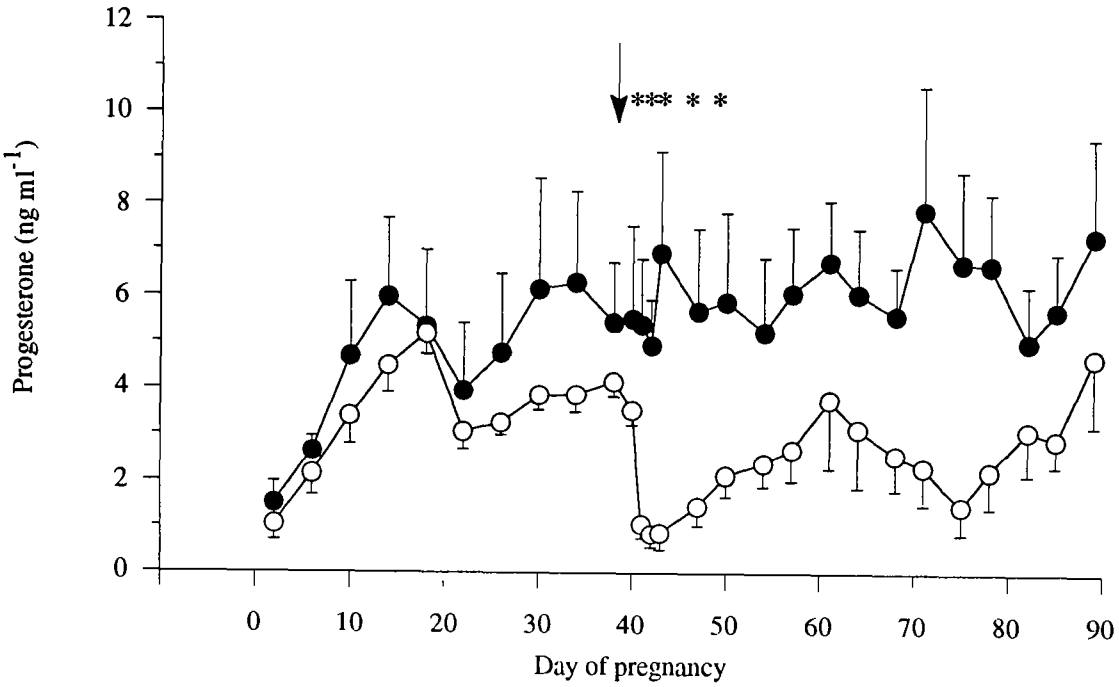

Fig 1 Profiles of mean (± sem) plasma progesterone concentrations for control hinds (●; n=6) and treated hinds (o, n=6) receiving 500 μg cloprostenol (arrow) on Day 40 of pregnancy. Asterisks indicate significant differences between means (P<0.005)

During the 72-hour period from injection of cloprostenol to treated hinds, mean plasma progesterone concentrations for control hinds remained stable between 5 and 7 ng ml^{-1}. However, mean concentrations declined rapidly for both aborting and non-aborting treated hinds, with aborting hinds exhibiting mean concentrations below 1 ng ml^{-1} by 54 hours after injection. For non-aborting treated hinds, mean values troughed at 1.5 ng ml^{-1} 42 hours after injection but then increased to 3 ng ml^{-1} 28 hours later (Fig. 2).

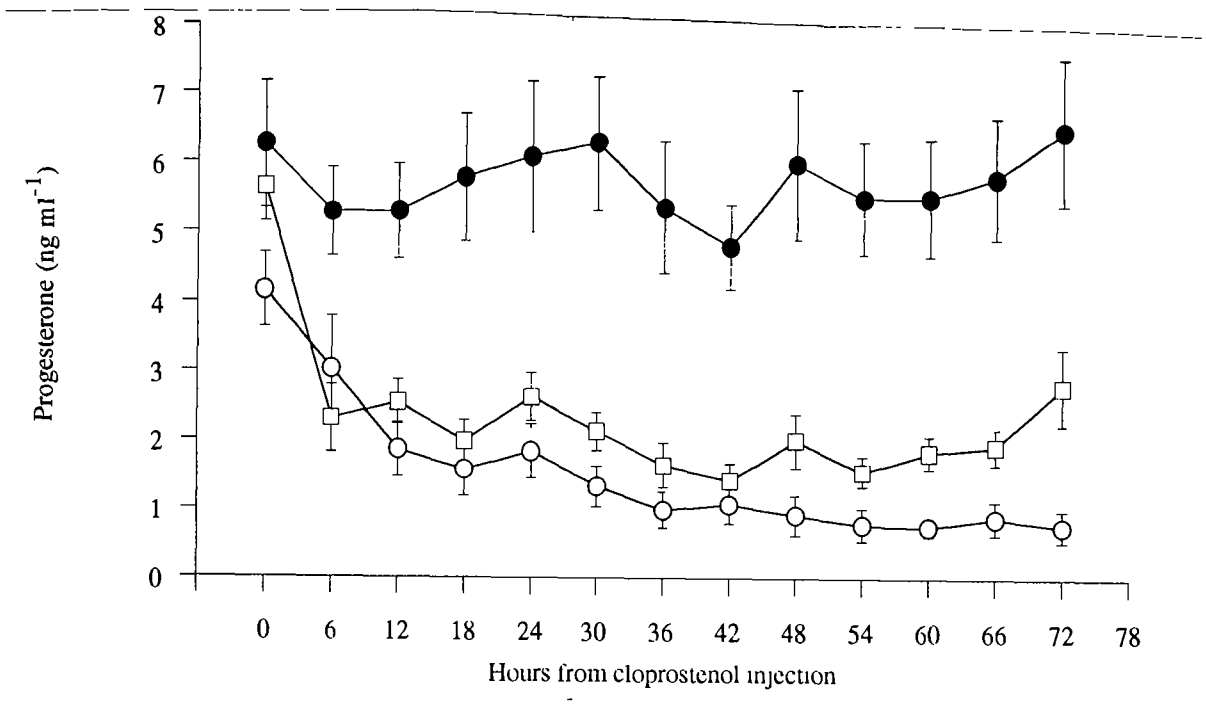

Fig. 2 Profiles of mean (± sem) plasma progesterone concentrations for control hinds (●, n=18), aborting treated hinds (o; n=9) and non-aborting treated hinds (□, n=9) for the 72 hour period after injection of cloprostenol to treated hinds.

The incidence of pre-ovulatory LH surges within 72 hours of cloprostenol injection was low for treated hinds, being $^{3}/_{9}$ for aborting hinds and $^{0}/_{9}$ for non-aborting hinds (Table 1). It is not known whether increasing progesterone secretion in the remaining treated hinds represented ovulations initiated after the intensive blood sampling period or regeneration of existing luteal tissue.

DISCUSSION: The use of synthetic prostaglandins to induce abortion and/or early labour in domestic livestock is largely restricted to those species which have complete dependance on the corpus luteum to maintain pregnancy. As such, reliable induction of abortion/labour can be obtained in this way in cattle (11, 22), goats (10, 23) and pigs (24). By contrast, the efficacy of induction of abortion/labour in sheep by exogenous prostaglandins is low, particularly beyond Day 100 of pregnancy (25), indicating the importance of the non-luteal sources of progesterone for maintenance of pregnancy in this species (14). It is concluded from the present study that a single intramuscular injection of 500 μg cloprostenol to red deer hinds has a low level of efficacy of inducing abortion between Days 30 and 60 of pregnancy. At no stage during the study was cloprostenol 100% effective in inducing abortion, and it appeared that the efficacy may have been lowest during the later stages of pregnancy (Day 60). There are a number of considerations with respect to these preliminary observations.

First, the data provide some support for a possible contributory role of the developing placenta in progesterone synthesis and pregnancy maintanence. The fact that only one hind aborted following administration of cloprostenol of Day 60 may be due to the supportive effects of the well developed placenta (large placentomes were visible during ultrasonography). However, conclusive demonstration of the role of the fetal-placental unit in progesterone production and pregnancy maintenance necessitates surgical removal of luteal tissue, either through ovariectomy or lutectomy. Recent studies involving these manipulations in pregnant red deer hinds (M.W. Fisher and G.W. Asher, unpublished data) and reindeer (*Rangifer tarandus*) cows (26) provide contrasting evidence for the role of the placenta in cervids. Ovariectomy and lutectomy of red deer hinds on Day 33 and Day 75 of pregnancy resulted in a dramatic reduction in progesterone secretion and a high incidence of abortion ($^{7}/_{7}$ for ovariectomy and $^{6}/_{7}$ for lutectomy), raising doubts about the ability of the fetal-placental unit to support pregnancy before Day 75. In contrast, ovariectomy of reindeer cows between Days 30 and 70 was associated with abortion in only $^{2}/_{4}$ animals, indicating extra-ovarian sources of progesterone in this species. Clearly, the situation in red deer remains to be resolved.

Second, while a single intramuscular injection of 500 μg cloprostenol may be highly effectively in causing luteolysis in non-pregnant, cyclic red deer hinds (7; G.W. Asher and M.W. Fisher, unpublished data), it is possible that the same regimen has reduced potency in pregnant animals due to decreased luteal sensitivity to prostaglandins. In support of this hypothesis, sheep and rabbit corpora lutea are less sensitive to prostaglandins during early pregnancy than during the oestrous/luteal cycle (27, 28, 29, 30). Thus, the single injection regimen may be associated with incomplete luteolysis in red deer hinds, resulting in sufficient residual progesterone secretion being maintained to safeguard fetal viability until luteal regeneration occurs. This is supported by data on changes in plasma progesterone concentrations following injection of cloprostenol, whereby non-aborting hinds exhibited marginally higher progesterone values than aborting hinds, and also exhibited a tendancy for recovery in progesterone secretion within 72 hours of cloprostenol delivery. As PGF is secreted in a pulsatile manner during the final stages of the oestrous cycle (ie. 5-8 discrete pulses at 6- to 8-hour intervals; 31, 32, 33), it is possible that repeat injections of exogenous prostaglandins may be needed to ensure

complete luteolysis, particularly if the corpus luteum of pregnancy exhibits decreased sensitivity to prostaglandins. Thus, a greater efficacy of induction of abortion may have been achieved in red deer hinds if two or more cloprostenol injections had been delivered at spaced intervals (eg. 12-24 hours apart).

In summary, the single injection regimen of 500 μg cloprostenol has a low level of efficacy in inducing abortion in red deer hinds between 30 and 60 days of pregnancy. The role of the fetal-placental unit in progesterone synthesis and pregnancy maintenance has yet to be determined but may have considerable bearing on the overall effects of exogenous prostaglandins on fetal viability.

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