New Zealand Society of Animal Production online archive

This paper is from the New Zealand Society for Animal Production online archive. NZSAP holds a regular annual conference in June or July each year for the presentation of technical and applied topics in animal production. NZSAP plays an important role as a forum fostering research in all areas of animal production including production systems, nutrition, meat science, animal welfare, wool science, animal breeding and genetics.

An invitation is extended to all those involved in the field of animal production to apply for membership of the New Zealand Society of Animal Production at our website www.nzsap.org.nz

View All Proceedings   Next Conference   Join NZSAP

Oestrous behaviour and luteal function in anoestrous red deer hinds treated with a GnRH analogue or oestradiol

J.A. DUCKWORTH AND G.K. BARRELL

Animal and Veterinary Sciences Group, Lincoln University, Canterbury, New Zealand.

ABSTRACT

This study investigated apparent reproductive failure occurring in seasonally anoestrous progesterone-primed red deer hinds when they were induced to ovulate with the GnRH analogue, buserelin. In March 1988 (non breeding season), each of 11 progesterone-primed red deer hinds (Groups B and BO) were injected i.m. with 4, 3, 2, 2 and 10 µg buserelin (Receptal, Hoechst) at -48, -24, 0, 24, and 48 h relative to the time of CIDR withdrawal. At CIDR withdrawal 6 of the buserelin-treated hinds (Group BO) and 4 other progesterone-primed hinds (Group O) were injected i.m. with 500 µg oestradiol benzoate. During the breeding season (April), ovulation was synchronised in another 4 hinds (Group C) using CIDRs. Oestrous behaviour was less noticeable and delayed (P>0.05) in Group B hinds and peak LH levels were lower (P>0.01) and occurred later (P>0.01) relative to Group O, BO and C hinds. Two weeks after CIDR withdrawal 3/5 B, 2/4 O, 3/6 BO and 4/4 C hinds had a *corpus luteum* present. During these two weeks plasma progesterone levels were elevated in B, BO and C hinds but were low in the O hinds. None of the buserelin- and/or oestradiol-induced ovulations resulted in pregnancy. These results show that ovulations induced in these anoestrous red deer hinds by the use of a GnRH analogue or oestradiol were not accompanied by normal hormonal and behavioural patterns.

Keywords Ovulation, red deer, oestrus, progesterone, LH, GnRH analogue, oestradiol.

INTRODUCTION

Ovulation can be induced in seasonally anoestrous hinds by using intravaginal progesterone treatment followed by gonadotrophin or gonadotrophin-releasing hormone (GnRH) treatment, but the fertility of the induced ovulations is usually low (Fisher and Fennessy, 1985; Moore and Cowie, 1986; Fennessy *et al.*, 1986; Fisher *et al.*, 1986). We have shown previously that although the GnRH analogue, buserelin, induced ovulation in progesterone-primed red deer hinds prior to the breeding season, no calves were born as a result of the procedure and it was not known if hinds had been mated or even if they had displayed oestrous behaviour (Duckworth and Barrell, 1988). In the anoestrous ewe, failure to establish pregnancy after buserelin treatment has been associated with; absence of oestrous behaviour (Rodway and Swift, 1985), inadequate luteal function (Swift and Crighton, 1980; McNeilly *et al.*, 1981; Rodway and Swift, 1985) or an hormonal environment inappropriate for conception (Rodway and Swift, 1985). In this study oestradiol benzoate was used to induce oestrous behaviour in progesterone-primed red deer

hinds treated with buserelin prior to the breeding season. Mating behaviour, luteal function and plasma LH levels were examined and compared with those recorded in oestrous cycles occurring during the breeding season.

MATERIALS AND METHODS

The trial was conducted at the Research Farm, Lincoln University using adult red deer hinds with an average live weight of 100.5±2.0 kg. On this property the breeding season usually began in early April and calves were born in late November or during December (Barrell, 1985).

On 18 February 1988, two controlled internal drug release devices (CIDR-Type S, AHI Plastic Moulding Co.), tied together, were inserted intravaginally into each of 15 red deer hinds and withdrawn 14 days later. From 2 March 1988, 11 hinds (Groups B and BO) were injected i.m. with 4, 3, 2, 2 and 10 µg buserelin (Receptal, Hoechst) at -48, -24, 0, 24, and 48h respectively from CIDR withdrawal. At CIDR withdrawal 6 of the buserelin-treated hinds (Group BO) and 4 other progesterone-primed hinds (Group O) were

TABLE 1 Incidence and time of oestrus and mating. Means in columns with different superscripts are significantly different (P<0.05).

Treatment group	Oestrous behaviour			Mating		
	No. of hinds detected	Onset (h from CIDR withdrawl) Mean [Range]	S.E.M.	No of hinds mated	Time of mating (h from CIDR withdrawal) Mean [Range]	S.E.M.
B	3/5	41.6[b] [32.5-49.0]	4.8	1/5	52.2 [-]	-
O	4/4	20.4[a] [18.2-23.4]	1.4	2/4	28.5 [27.5-29.5]	1.8
BO	6/6	21.2[a] [18.7-30.2]	2.4	3/6	28.3 [27.0-32.4]	1.8
C	4/4	23.5[a] [18.5-33.1]	3.4	3/4	36.5 [31.5-39.0]	2.2

injected i.m. with 500 µg oestradiol benzoate (Sigma Chemical Company, USA). During the breeding season, ovulation was synchronised in 4 hinds (Group C) by inserting 2 CIDRs into each hind on 1 April and removing the CIDRs 14 days later.

Blood samples were collected via a jugular venous cannula at 3 h intervals for 75 h following CIDR withdrawal for LH analysis and at 2 to 3 day intervals during the mating season for progesterone analysis. Mating behaviour was recorded by running hinds with an entire (Groups B, O and BO) or vasectomised (Group C) red deer stag for periods of 3 h followed by a 3 h separation, repeated over the 75 h following CIDR removal. The onset of oestrus was defined as the time when hinds were first observed to 1) mount the stag, or 2) to stand still when mounted by the stag or 3) to be in standing heat during blood sampling and was equivalent to the third phase of courtship described by Veltman (1985). The entire stag initially run with Groups B, O and BO hinds did not display rutting behaviour and was replaced at 24 h. Ovaries of all hinds were examined by laparoscopy, under general anaesthetic, 14 d after CIDR withdrawal and calving dates were recorded for Groups B, O and BO hinds.

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 0.25 ng/ml. Plasma samples with mean progesterone concentrations ranging from 0.8-5.5 ng/ml had within- and between-assay coefficients of variation (c.v.) between 8.5-14.2% and 11.5-15.9% respectively.

Plasma samples were assayed for LH using the NIADDK ovine LH kit which has been validated for red deer sera by Kelly *et al.* (1982). The sensitivity of the assay in our laboratory was 0.24 ng/ml and plasma samples with mean LH levels of 1.0, 6.4 and 9.6 ng/ml had within-assay c.v. of 5.1, 6.0 and 8.2% respectively. Between-assay c.v. for these samples were 10.1, 6.8 and 7.2% respectively.

Results are expressed as the mean ± the standard error of the mean (S.E.M.). An increase in LH was deemed to be significant when the LH concentration of a sample was greater than 2 ng/ml and exceeded the mean of the previous two baseline values by three times the intra-assay c.v.. Plasma progesterone levels were defined as elevated when plasma progesterone concentrations exceeded 1 ng/ml for 3 consecutive samples. Treatment effects were determined using analysis of variance (SAS Statistical Package) followed

TABLE 2 Magnitude and timing of maximum LH concentration. Means in columns with different superscripts are significantly different (P<0.05).

Treatment group	Maximum LH (ng/ml)			Time of maximum LH (h from CIDR removal)		
	Mean [Range]		S.E.M	Mean [Range]		S.E.M.
B (n=5)	8.3[a] [4.1-11.4]		1.5	54.6[c] [54-57]		0.6
O (n=4)	33.6[b] [20.4-37.8]		3.8	24.5[a] [24-27]		0.8
BO (n=6)	20.4[b] [11.7-37.4]		4.1	28.0[a] [24-33]		1.3
C (n=4)	24.3[b] [10.8-36.8]		6.2	41.3[b] [33-45]		3.3

by Duncan's multiple range test to establish significant differences between means. Progesterone and LH values were log-transformed prior to statistical analysis.

RESULTS

Within 72 h of CIDR withdrawal 3/5 B and all O, BO and C hinds displayed oestrous behaviour and 1/5 B, 2/4 O, 3/6 BO and 3/4 C hinds were mated. The onset of oestrous behaviour was significantly later (P>0.001) in Group B hinds than for other treatment groups (Table 1).

In the three days following withdrawal of progesterone treatment, two peaks of plasma LH were detected in all Group B and 1/6 Group BO hinds while a single LH peak was recorded in all Group O, Group C and 5/6 Group BO hinds (Fig. 1). Group BO, O and C hinds had similar maximum LH values which ranged from 12 to 38 ng/ml. LH maxima in the Group B hinds ranged from 4 to 11 ng/ml, significantly less (P>0.05) than the values recorded in the other groups. The mean interval from the withdrawal of CIDR to maximal LH levels in the buserelin or oestradiol treated animals (Groups B, BO and O) was significantly different (P>0.01) to that recorded in hinds during the breeding season (Group C). Relative to Group C the timing of the LH maxima was significantly earlier (P>0.01) in Group

O and Group BO hinds and significantly later (P>0.01) in the Group B hinds (see Table 2). There was no relationship between the time or magnitude of the LH peak and the incidence of oestrous behaviour or ovulation.
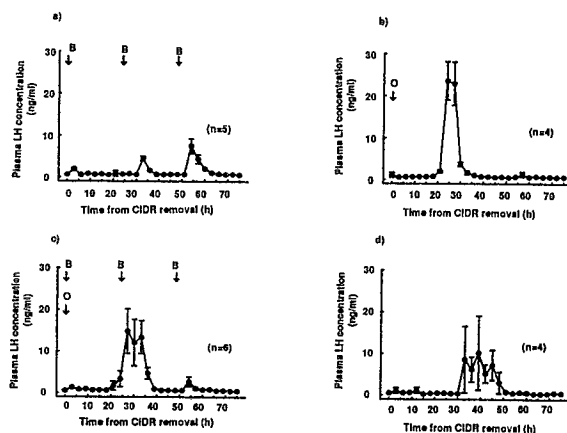

FIG 1 Mean plasma LH concentrations in red deer hinds following withdrawal of progesterone (CIDR) treatment. Hinds received a) buserelin (Group B), b) oestradiol (Group O) or c) buserelin/oestradiol (Group BO) treatment prior to the breeding season or d) progesterone treatment only during the breeding season (Group C). B and O represent the time of buserelin and oestradiol administration, respectively. Vertical bars denote S.E.M.

Two weeks after CIDR withdrawal 3/5 B, 2/4 O, 3/6 BO and 4/4 C group hinds had a single *corpus luteum* CL present at laparoscopy. During these two weeks plasma progesterone levels were elevated in Group B, BO and C hinds with CL and remained high for 11-16 days (Fig. 2) except for one Group B hind whose progesterone levels were high for only 6 days. The time interval from the withdrawal of CIDR to an elevation in plasma progesterone levels was greater in the Group B and Group BO hinds with CL (9.7 ± 0.7 and 11.5 ± 0.5 d after CIDR withdrawal, respectively) than that recorded in the Group C hinds (6.3 ± 1.0 d after CIDR withdrawal). Plasma progesterone concentrations in hinds without CL and in all Group O hinds (including those with CL) generally remained low until at least 20 d after CIDR withdrawal (Fig. 2). There was no relationship between the presence of a CL and the incidence of oestrous behaviour or mating.
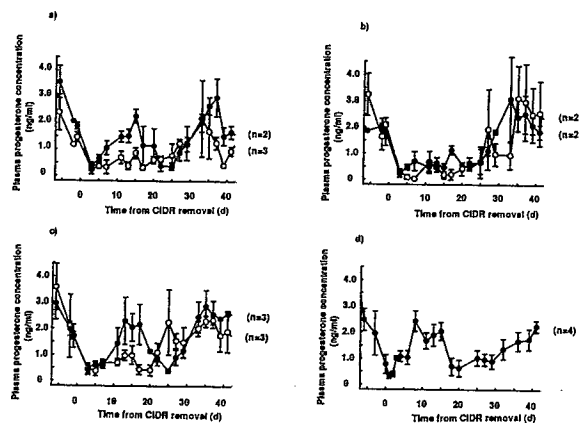

FIG 2 Mean plasma progesterone concentrations in red deer hinds following withdrawal of progesterone (CIDR) treatment. Hinds received a) buserelin (Group B), b) oestradiol (Group O) or c) buserelin/oestradiol (Group BO) treatment prior to the breeding season or d) progesterone treatment only during the breeding season (Group C) and had (•) or did not have (O) a CL present 14 days after CIDR withdrawal. Vertical bars denote S.E.M.

No pregnancies resulted from the induced ovulations but all hinds conceived at a subsequent mating and delivered live calves at term. The mean calving dates of Group B, O and BO hinds were 20

November $\pm$ 3 d, 17 November $\pm$ 2 d and 16 November $\pm$ 2 d respectively with no significant difference between treatment. Calving was advanced approximately 12 days relative to untreated hinds mated in a separate mob on the same property (1 December $\pm$ 4 d, n=6).

DISCUSSION

No conceptions resulted from the ovulations induced in progesterone-primed anoestrous red deer hinds by using the GnRH analogue, buserelin. Unlike anoestrous ewes (Rodway and Swift, 1985), some of the hinds treated with buserelin in this trial displayed oestrus but their oestrous behaviour was less obvious and its onset delayed compared with either progesterone-synchronised oestrous cycles during the breeding season or oestradiol- induced courtship behaviour during anoestrum. In the ewe (see Goodman, 1988) and probably in the red deer hind (Meikle and Fisher, 1990) elevated progesterone concentrations followed by a rise in oestradiol levels are both critical requirements for the expression of oestrous behaviour. This is borne out by the ability of oestradiol benzoate treatment to induce oestrous behaviour in progesterone-primed entire red deer hinds in this study. It is possible that courtship behaviour was delayed or absent in the buserelin-treated hinds because of a late and inadequate increase in oestradiol levels but we are unable to confirm this. Another factor was the inadequate rutting behaviour of one of the stags used in the trial and this highlights the variability in reproductive competence of untreated red deer stags prior to the normal breeding season (Moore and Cowie, 1986; Fennessy *et al.*, 1988) and the need to advance the breeding season of stags for out-of-season mating trials.

In the three days following progesterone treatment, plasma LH concentrations were elevated in the buserelin-treated hinds 3-9 h after each injection of the GnRH analogue but the maximum LH levels achieved were always less than those recorded in the LH surge during the breeding season. This is in contrast to studies in anoestrous ewes where a single buserelin injection (6-40 µg) administered intravenously produced an immediate LH response within the physiological range of the natural preovulatory peak (Siddall and Crighton, 1977; Swift and Crighton, 1980; Rodway and Swift, 1985). The

maximal LH response to buserelin recorded in this trial may have been suppressed or delayed because the analogue was administered intramuscularly instead of intravenously or because the pinnacle of LH peak was missed due to infrequent blood sampling. Regardless of whether hinds were treated with buserelin or not, plasma LH levels increased dramatically 24 to 33 h after administration of oestradiol. Oestradiol elicited large increases in LH secretion in entire (Goodman *et al.*, 1981) or ovariectomised ewes (Karsch, 1987) and in ovariectomised red deer hinds (Meikle and Fisher, 1990) suggesting that this may be a common response in progesterone-primed ungulates. Nevertheless the LH response to oestradiol recorded from hinds in the present study occurred early in comparison with hinds from the other groups. Since the interval from oestradiol injection to LH response is dose-dependent in sheep (Goodman *et al.*, 1981), it is possible that the dose used here was overly high or, alternatively, that it was administered too early.

A single ovulation was induced in the majority of red deer hinds treated with buserelin and/or oestradiol prior to the breeding season or after progesterone treatment during the breeding season. Except for one hind, the elevation in plasma progesterone concentrations in most of the buserelin and buserelin/ oestradiol-treated hinds with CL was similar to that recorded during the luteal phase in the breeding season (in this study; Adam *et al.*, 1985; Jopson *et al.*, 1990) and suggests that most of the CLs observed in these hinds at laparoscopy functioned normally. Luteal function was inadequate in the hinds which ovulated after treatment with oestradiol only. Although a single injection of buserelin induced ovulation in anoestrous ewes, the resulting CL often had subnormal function (Swift and Crighton, 1980; McNeilly *et al.*, 1981) even when ewes were pre-treated with progesterone (Rodway and Swift, 1985). Inadequate luteal function or premature luteolysis arises if the induced CL has developed from an immature follicle (Haresign and Lamming, 1978; Coleman and Dailey, 1983; Murdoch *et al.*, 1983) or from a follicle that was not sufficiently exposed to elevated progesterone levels prior to ovulation (McLeod and Haresign, 1984; Hunter *et al.*, 1988; Southee *et al.*, 1988). Abnormal plasma progesterone profiles in ewes treated with GnRH prior to the breeding season have also been attributed to the presence of

unruptured luteinised cysts in the ovary (Hunter *et al.*, 1989). These cysts, which were macroscopically identical to normal CL, were associated with a late-occurring increase in plasma progesterone levels and a shortened luteal lifespan. The rise in plasma progesterone levels was delayed in hinds treated with buserelin or buserelin/oestradiol in this trial. In the buserelin-treated hinds this may have been because the LH levels peaked later, but the rise in LH was not delayed in the hinds treated with both buserelin and oestradiol.

Lack of an elevation of plasma progesterone levels in the oestradiol-treated hinds with CL present at laparoscopy, indicates that the CL in these cases were nonfunctional. It is unlikely that this luteal incompetence in the oestradiol-treated hinds arose from insufficient exposure of the developing follicle to progesterone because CIDR treatment elevated plasma progesterone to concentrations similar to those occurring during the normal luteal phase (1-4 ng/ml) (Group C hinds in the present study, Adam *et al.*, 1985). Stimulation of the developing follicles by endogenous gonadotrophins may not have been sufficient to ensure that the oestradiol-induced CL in this study were functional. In fact competent CL were induced in oestradiol-treated hinds only when they received the GnRH analogue as well. Also the negative feedback effect of oestrogens on endogenous gonadotrophin secretion prior to the LH surge (Goodman *et al.*, 1981, Meikle and Fisher, 1990) may have retarded follicular development prior to ovulation. Ewe lambs treated with 2.5 mg oestradiol valerate exhibited oestrus but most did not develop a CL (Burfening and Berardinelli, 1986). In the oestradiol-treated ewes which did develop CL the pregnancy rate was significantly reduced suggesting that pharmacological doses of oestradiol may reduce fertility.

Although none of the buserelin and/or oestradiol-induced ovulations resulted in pregnancy the breeding season of the treated hinds was advanced relative to the normal breeding season, which suggests that the treatments used here facilitated subsequent ovarian activity.

It is concluded that, after priming with progesterone, ovulation and formation of a CL was induced in seasonally anoestrous red deer hinds by the use of the GnRH analogue but, because these events may not have been accompanied by appropriate

hormonal and behavioural sequences, pregnancy was not established prior to the normal breeding season. Treatment with oestradiol, separately or in conjunction with the GnRH analogue, did not overcome these problems.

ACKNOWLEDGEMENTS

REFERENCES

Adam C.L.; Moir C.E.; Atkinson T. 1985. Plasma concentrations of progesterone in female red deer (*Cervus elaphus*) during the breeding season, pregnancy and anoestrus. *Journal of Reproduction and Fertility 74*: 631-636.

Barrell G.K. 1985. Techniques for the manipulation of ovulation in red deer. In: *Proceedings of a Deer Course For Veterinarians.* Deer Branch Course No. 2., Deer Branch of New Zealand Veterinary Association. pp. 126-134.

Burfening P.J; Berardinelli J.G. 1986. Effect of feed treatment and exogenous estrogen and progestagen on puberty and lambing rates in ewe lambs. *Journal of Animal Science 63*: 1717-1721.

Coleman D.A.; Dailey R.A. 1983. Effects of repeated removal of large ovarian follicles and treatment with progestin on ovarian function in the ewe. *Biology of Reproduction 29*: 586-593.

Duckworth J.A.; Barrell G.K. 1988. Induction of ovulation in anoestrous red deer hinds with a GnRH analogue. *Proceedings of the New Zealand Society of Animal Production 48*:71-75.

Elder P.A.; Yeo K.H.J.; Lewis J.G.; Clifford J.K. 1987. An enzyme-linked immunosorbent assay (ELISA) for plasma progesterone: immobilised antigen approach. *Clinica Chemica Acta 162*: 199-206.

Fennessy P.F.; Fisher M.W. 1988. Advancing the calving season in red deer hinds. *Proceedings of a Deer Course For Veterinarians.* Deer Branch Course No. 5., Deer Branch of New Zealand Veterinary Association. pp 17-27.

Fennessy P.F.; Fisher M.W.; Webster J.R.; Mackintosh C.G.; Suttie J.M.; Pearse A.J.; Corson I.D. 1986. Advancing the calving season in red deer hinds. *Proceedings of a Deer Course For Veterinarians.* Deer Branch Course No. 3., Deer Branch of New Zealand Veterinary Association. pp 103-120.

Fisher M.W.; Fennessy P.F. 1985. Reproductive physiology of female red deer and wapiti. *Proceedings of a Deer Course For Veterinarians.* Deer Branch Course No. 2., Deer Branch of New Zealand Veterinary Association. pp 88-100.

Fisher M.W.; Fennessy P.F.; Suttie J.M.; Corson I.D.; Pearse A.J.T.;

Davis G.H.; Johnstone P.D. 1986. Early induction of ovulation in red deer hinds. *Proceedings of the New Zealand Society of Animal Production 46*: 171-173.

Goodman R.L. 1988. Neuroendocrine control of the ovine estrous cycle. In: *The Physiology of Reproduction* Ed E. Knobil and J. Neill *et al.*, pp 1929-1969. Raven Press Ltd, New York.

Goodman R.L.; Legan S.J.; Ryan R. D.; Foster D.L.; Karsch K.J. 1981. Importance of variations in behavioural and feedback actions of oestradiol to the control of seasonal breeding in the ewe. *Journal of Endocrinology 89*: 229-240.

Haresign, W.; Lamming G.E. 1978. Comparison of LH release and luteal function in cyclic and LH-RH treated anoestrous ewes pretreated with PMSG or oestrogen. *Journal of Reproduction and Fertility 52*: 349-353.

Hunter M.G.; Ayad V.J.; Gilbert C.L.; Southee J.A.; Wathes D.C. 1989. Role of prostaglandin F-2α and oxytocin in the regression of GnRH-induced abnormal corpora lutea in anoestrous ewes. *Journal of Reproduction and Fertility 85*: 551-561

Hunter M.G.; Southee J.A.; Lamming G.E. 1988. Function of abnormal corpora lutea in vitro after GnRH-induced ovulation in the anoestrous ewe. *Journal of Reproduction and Fertility 84*: 139-148.

Jopson N.B.; Fisher M.W.; Suttie J.M. 1990. Plasma progesterone concentrations in cycling and in ovariectomised red deer hinds: the effect of progesterone supplementation and adrenal stimulation. *Animal Reproduction Science 23*: 61-73.

Karsch F.J. 1987. Central actions of ovarian steriods in the feedback regulation of pulsatile secretion of luteinizing hormone. *Annual Review of Physiology 49*: 365-382.

Kelly R.W.; McNatty K.P.; Moore G.H.; Ross D.; Gibb M. 1982. Plasma concentrations of LH, prolactin, oestradiol and progesterone in female red deer (*Cervus elaphus*) during pregnancy. *Journal of Reproduction and Fertility 64*: 475-483.

McLeod B.J.; Haresign W. 1984. Evidence that progesterone may influence subsequent luteal function in the ewe by modulating preovulatory follicle development. *Journal of Reproduction and Fertility 71*: 381-386.

McNeilly A.S.; Hunter M.; Land R.B.; Fraser H.M. 1981. Inadequate corpus luteum function after induction of ovulation in anoestrous ewes by LH-RH or an LH-RH agonist. *Journal of Reproduction and Fertility 63*: 137-144.

Meikle L.M.; Fisher M.W. 1990. Induction of oestrus in the ovariectomised hinds with exogenous progesterone and oestradiol benzoate. *Proceedings of the New Zealand Society of Animal Production 50: pp 155-160.*

Moore G.H.; Cowie G.M. 1986. Advancement of breeding in non-lactating adult red deer hinds. *Proceedings of the New Zealand Society of Animal Production 46*: 175-178.

Murdoch W.J.; de Silva M.; Dunn T.G. 1983. Luteal phase insufficiency in the ewe as a consequence of premature induction of ovulation by intrafollicular injection of gonadotropins. *Journal of Animal Science 57*: 1507-1511.

Rodway R.G.; Swift A.D. 1985. A comparison of PMSG and LHRH agonist in the induction of ovulation in the anoestrous ewe. *Animal Reproduction Science 9*: 153-162.

Siddall B.; Crighton D.B. 1977. Effects of certain analogues of synthetic LHRH on the release of luteinising hormone and

follicle-stimulating hormone in the anoestrous ewe. *Journal of Endocrinology 75*: 49-57.

Southee J.A.; Hunter M.G.; Haresign W. 1988. Function of abnormal corpora lutea *in vivo* after GnRH-induced ovulation in the anoestrous ewe. *Journal of Reproduction and Fertility 84*: 131-137.

Swift A.D.; Crighton D.B. 1980. The effects of injecting D-Ser (But)[6] Des Gly-NH$_{210}$ in early, mid and late seasonal anoestrus on gonadotrophin secretion and luteal function of the ewe. *Theriogenology 14*: 269-279.

Veltman C.J. 1985. The mating behaviour of red deer. *Proceedings of a Deer Course For Veterinarians*. Deer Branch Course No. 2, Deer Branch of New Zealand Veterinary Association. pp 135-142.