Induction of twin ovulations in red deer hinds with steroid-free bovine follicular fluid

M.W. FISHER[1], P.F. FENNESSY[1], K.M. HENDERSON[2], R.E. NEWMAN[1] AND T.R. MANLEY[1]

ABSTRACT

The manipulation of ovulation rate in the red deer hind was investigated by administration of steroid-free bovine follicular fluid (bFF).

Twenty-four adult hinds, treated with intravaginal progesterone (CIDR), received a daily intramuscular injection of either 0, 4, 7.5 or 11 mls of dextran-charcoal treated bFF (n = 6 per group) on days -1, 0 and +1 from the time of CIDR withdrawal.

After CIDR removal, plasma progesterone concentrations declined in all animals. In control hinds, oestrus occurred 2-3 days later and mean plasma progesterone concentrations began to increase thereafter. In bFF-treated hinds, oestrus was delayed by 3-5 days, and plasma progesterone concentrations remained low for at least 7 days after CIDR withdrawal. Plasma LH concentrations were 2-3 fold higher in the bFF-treated groups, relative to the control hinds, for at least 6 days after the end of bFF treatment. Twin ovulations were recorded in 1/6 control and 5/17 treated animals. At pregnancy diagnosis, 10/23 hinds were pregnant having conceived at the treated oestrus (3/6 control and 7/18 treated), but none had more than one foetus. There was no evidence of a dose-response to bFF treatment.

These results indicate that in the hind, bFF can be used to manipulate the timing of oestrus and ovulation rate. A component(s) of bFF may thus have the potential to regulate fecundity in deer.

Keywords Follicular fluid; red deer; oestrus; progesterone; LH; ovulation rate

INTRODUCTION

Although twinning has long been suspected in red deer since there are observations in the wild of hinds accompanied by more than one calf (eg, MacNally, 1977), it is apparently very rare. In Scotland, Lowe (1969) shot over 800 female deer of all ages over an eight-year period and found only one set of twin foetuses although one of the pair had apparently died before the hind was shot. Similarly, Mitchell (1973) examined over 1500 uteri from shot hinds finding no twin embryos. There are few authentic reports of the birth of twin red deer calves (Guinness and Fletcher, 1971; MacNally, 1982) and although twinning in farmed red deer has been reported (eg, Lindeman, 1987), there are no estimates of the incidence.

Studies in other species indicate that the pituitary gonadotrophins, luteinising hormone (LH) and follicle stimulating hormone (FSH), are the main regulators of follicle development and ovulation and that by increasing circulating FSH concentrations in particular, ovulation rates can be increased (Hammond, 1949; McNatty *et al.*, 1985).

Inhibin, a complex glycoprotein produced by the granulosa cells of the ovary (Henderson and Franchimont, 1981) has been implicated in the regulation of FSH secretion (see Findlay and Clarke, 1987) in some species. Active immunisation against a partially purified preparation of inhibin increased ovulation rate in the ewe (Henderson *et al.*, 1984; Cummins *et al.*, 1986). Similarly, treatment of ewes with steroid-free bovine follicular fluid (bFF), an inhibin-rich material, during the luteal phase of the oestrous cycle (Wallace and McNeilly, 1985) or at the time of induced luteolysis (Henderson *et al.*, 1986) delayed oestrus and increased ovulation rate.

The present study was designed to investigate the effect of administration of bFF on ovulation rate in the red deer hind.

[1] Invermay Agricultural Centre, Private Bag, Mosgiel
[2] Wallaceville Animal Research Centre, P.O. Box 40063, Upper Hutt

MATERIALS AND METHODS

Twenty-four adult red deer (*Cervus elaphus*) hinds (live weight 81-113 kg, mean 96.7 kg) were synchronised for oestrus with 15 days of intravaginal progesterone (9% w/w CIDR-S containing 340 mg progesterone, AHI Plastic Moulding Co, Hamilton, New Zealand) commencing on 2 April, 1986. Hinds were treated with a daily i.m. injection of either 0, 4, 7.5 or 11 mls of bFF (obtained and prepared as described by Henderson *et al.*, 1986) on days -1, 0 and +1 after CIDR withdrawal.

Mating was detected by running the hinds in 1 of 2 single-sire mating groups each with a colour-marked adult stag which was removed 12 days later. Ovulation rate was determined by laparoscopy and pregnancy by ultrasound scan under general anaesthesia (Fisher *et al.*, 1989) at 13 and 113 days respectively after stag introduction. In addition, all hinds were blood sampled daily by jugular venepuncture for 10 days beginning the day before the first injection of bFF (day -2). Plasma was removed and stored frozen until analysed.

Plasma progesterone concentrations were determined by solid phase 125I radioimmunoassay (Diagnostic Products Corporation, Los Angeles, USA) and plasma LH by a heterologous double antibody radioimmunoassay which used rabbit antiserum to ovine LH and ovine LH for iodination and as assay standards. Parallelism was demonstrated by serial dilutions of hind plasma in phosphate buffered saline. LH concentrations > 5 ng/ml were assumed to be preovulatory LH surges and were excluded from statistical analyses. The log transformed LH data were grouped as either before, during or after bFF treatment and examined by analysis of variance and Student's t-test.

RESULTS

There were no differences between each of the mating groups so the data have been combined. Oestrus occurred in the control hinds 2 to 3 days after CIDR withdrawal but was delayed in the treated hinds, occurring 5-8 days after withdrawal.

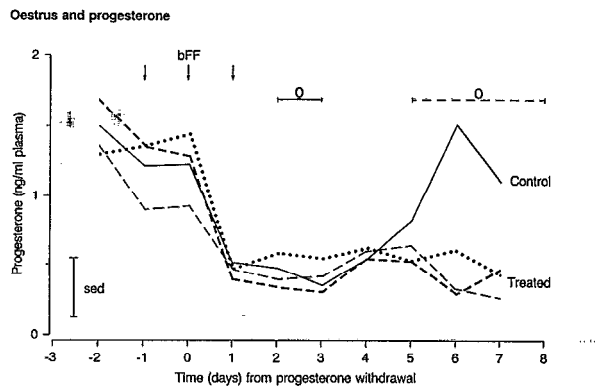

FIG. 1 Mean plasma progesterone concentrations around the time of progesterone withdrawal and oestrus (O) in untreated and bFF-treated (—— 0 mls bFF; ---- 4 mls bFF; – – –7.5 mls bFF; ●●●● 11 mls bFF) hinds.

Plasma progesterone concentrations (Fig.1) averaged 1.30 ng/ml over the last 3 days of CIDR insertion then concentrations declined following device withdrawal. They then began to increase after oestrus (Fig. 1) in control hinds and averaged 1.15 ng/ml over the period 2 - 5 days after oestrus. In contrast, oestrus in treated hinds was delayed by 3-5 days and plasma progesterone concentrations remained low (mean 0.47 ng/ml) until at least day 7.

Mean plasma LH concentrations prior to bFF treatment (Table 1) ranged from 0-0.81 ng/ml

TABLE 1 Mean LH concentrations (ing/ml plasma) in untreated and bFF-treated hinds. Asterisks indicate the level of significant differences between control (0 mls bFF) and treated (4, 7.5 and 11 mls bFF) groups.

Group (mls bFF)	LH concentration Before[1] treatment	During[2] treatment	After[3] treatment
0	0.19	0.35	0.34
4	0.29	1.07***	1.06***
7.5	0.32	0.66**	0.83***
11	0.26	0.73**	1.12***
SED	0.06	0.12	0.15

[1] Days 1 & 2 (days -3 and -2 from progesterone withdrawal)
[2] Days 3 - 5 (days -1, 0 and 1 from progesterone withdrawal)
[3] Days 6 - 10 (days 2-6 from progesterone withdrawal)

(mean 0.27 ng/ml). During treatment with bFF, LH concentrations were elevated significantly, independently of dose-rate in all groups, and this 2-3 fold increase was maintained in the period following treatment at least until sampling ceased.

Twin ovulations were observed in 1/6 untreated and 5/18 treated hinds (Table 2). At pregnancy diagnosis 10/23 (43%) hinds were pregnant having conceived at the induced oestrus (3/6 control and 7/17 treated), none with more than one foetus. Of the 6 hinds that had twin ovulations 5 were pregnant while 5/17 with single ovulations were pregnant.

TABLE 2 Ovarian and pregnancy data obtained from untreated and bFF-treated hinds.

Group (mls bFF)	Number of ovulations	Number of hinds pregnant
0	5 single	3
	1 double	0
4	1 anovulatory	-
	3 single	2
	2 double	2
7.5	5 single	0
	1 double	1
11	4 single	0
	2 double	2

There was no evidence of a dose response to bFF in LH concentrations, ovulation rate or pregnancy rate. There were no significant relationships between progesterone or LH concentrations and ovulatrion or pregnancy rates.

DISCUSSION

The observed effects of bFF treatment on the timing of oestrus, and plasma concentrations of progesterone and LH in deer were similar to the effects found in sheep where there is also a substantial fall in plasma FSH concentrations during bFF treatment. Although, FSH has yet to be measured on the samples collected in the present experiment (a cervine FSH radioimmunoassay is currently being developed), it is likely that plasma FSH concentrations were suppressed during treatment. A decline in plasma FSH concentrations would impair follicular development which would explain the delay in oestrus and subsequent luteal development. It would also reduce follicular oestradiol production so that there would be less negative feedback by oestradiol on the pituitary resulting in enhanced LH secretion.

In sheep (Henderson *et al.*, 1986) cessation of bFF treatment causes plasma FSH concentrations to "rebound" to levels considerably higher than pre-treatment values. Elevated FSH levels persist until follicular development returns to normal, and with it the normal negative feedback effects of oestradiol on pituitary gonadotrophin secretion. The temporary elevation in FSH and LH concentrations are thought to be responsible for the increased ovulation rate observed following treatment with bFF. Similar changes in gonadotrophin secretion may be responsible for the small increase in the number of hinds with twin ovulations following bFF treatment since hinds have been shown to respond to FSH-rich preparations with an increase in ovulation rate (Fisher and Fennessy, 1985).

Although twin births have been noted in red deer they are relatively rare as are natural twin ovulations (1/42 in untreated hinds examined by laparoscopy at Invermay; M.W. Fisher and G.H. Davis, pers. comm.). Thus the twin ovulations induced in the present experiment probably represent a significant effect of treatment. Despite the number of hinds with twin ovulations, none supported twin pregnancies; a finding consistent with the difficulty experienced in inducing viable multiple births in red deer (Kelly *et al.*, 1982; Adam *et al.*, 1985) except prior to the normal breeding season (Moore, 1987).

The reason for the low overall pregnancy rate in the present experiment is unknown particularly since control and treated hinds were similar. It is probably not an effect of synchronisation with exogenous progesterone (CIDR) since these same hinds had a 63% conception rate to a similar synchronisation treatment in the previous breeding season (M.W. Fisher and P.F. Fennessy, pers.comm.). Perhaps the yarding, handling and blood sampling regime imposed around the time of mating and conception had some deleterious

effect on the establishment of pregnancy. Interestingly, 5/7 treated hinds with twin ovulations were pregnant compared with only 2/12 of those with single ovulations. This suggests that multiple ovulation favoured conception and/or embryo survival in these animals perhaps because of enhanced progesterone secretion during pregnancy when multiple corpora lutea are present (Kelly *et al.*, 1982).

It is likely that inhibin is the factor in bFF responsible for the effects observed in this study, although other, as yet unknown, components can not be excluded. Inhibin and/or other factors in bFF may thus have the potential to regulate fecundity in deer. Taken collectively with other studies they also suggest that FSH may be important in preovulatory follicular growth in the red deer hind as in other species.

ACKNOWLEDGEMENTS

Dr A.F. Parlow, National Institute of Diabetes and Digestive and Kidney Diseases, USA, and Dr Y. Combarnous, INRA Station de Physiologie de la Reproduction, France are thanked for generously supplying the reagents for the LH radioimmunoassay. G.H. Davis and I.D. Corson are thanked for performing the laparoscopies and pregnancy diagnoses respectively as are G.M. Caughey for assistance with the assays and P.D. Johnstone for assistance with the statistical analysis. The financial support for M.W.F. provided by Alpine Helicopters Ltd during which this study was undertaken is very gratefully acknowledged.

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.

Cummins L.J.; O'Shea T.; Al-Obaidi S.A.R.; Bindon B.M.; Findlay J.K. 1986. Increase in ovulation rate after immunization of Merino ewes with a fraction of bovine follicular fluid containing inhibin activity. *Journal of reproduction and fertility* 77:365-372.

Findlay J.K.; Clarke I.J. 1987. Regulation of the secretion of FSH in domestic ruminants. *Journal of reproduction and fertility, supplement* 34:27-37.

Fisher M.W.; Fennessy P.F. 1985. Reproductive physiology of female red deer and wapiti. *In* Proceedings of a Deer Course for Veterinarians, Deer Branch Course Number 2, Ashburton. Deer Branch of the New Zealand Veterinary Association. pp 88-100.

Fisher M.W.; Fennessy P.F.; Davis G.H. 1989. A note on the induction of ovulation in lactating red deer hinds prior to the breeding season. *Animal production* 49:(In press).

Guinness F.E.; Fletcher J. 1971. First ever recorded incidence of twins born to a red deer hind in Britain. *Deer* 2:680-682.

Hammond J. (Jr) 1949. Induced twin ovulations and multiple pregnancy in cattle. *Journal of agricultural science, Cambridge* 39:222-225.

Henderson K.M.; Franchimont P. 1981. Regulation of inhibin production by bovine ovarian cells *in vitro*. *Journal of reproduction and fertility* 63:431-442.

Henderson K.M.; Franchimont P.; Lecomte-Yerna M.J.; Hudson N.; Ball K. 1984. Increase in ovulation rate after active immunization of sheep with inhibin partially purified from bovine follicular fluid. *Journal of endocrinology* 102:305-309.

Henderson K.M.; Prisk M.D.; Hudson N.; Ball K.; McNatty K.P.; Lun S.; Heath D.; Kieboom L.E.; McDiarmid J. 1986. Use of bovine follicular fluid to increase ovulation rate or prevent ovulation in sheep. *Journal of reproduction and fertility* 76:623-635.

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.

Lindeman B. 1987. Twins in the herd. *The deer farmer* February: 9.

Lowe V.P.W. 1969. Population dynamics of the red deer *(Cervus elaphus* L.) on Rhum. *Journal of animal ecology* 38:425-457.

MacNally L. 1977. The occurrence of twin calves born to red deer hinds. *Deer* 4:218-219.

MacNally L. 1982. The observed birth of twins to a red deer hind. *Deer* 5:451-453.

McNatty K.P.; Hudson N.; Gibb M.; Ball K.; Henderson K.M.; Heath D.A.; Lun S.; Kieboom L.E. 1985. FSH influences follicle viability, oestradiol biosynthesis and ovulation rate in Romney ewes. *Journal of reproduction and fertility* 75:121-131.

Mitchell B. 1973. The reproductive performance of wild Scottish red deer, *Cervus elaphus*. *Journal of reproduction and fertility, supplement* 19:271-285.

Moore G.H. 1987. Twinning and triplets in red deer. *The deer farmer* October:34-35.

Robinson T.J. 1982. The magic of Hammond. *JOurnal of reproduction and fertility* 66:397-410.

Wallace J.M.; McNeilly A.S. 1985. Increase in ovulation rate after treatment of ewes with bovine follicular fluid in the luteal phase of the oestrous cycle. *Journal of reproduction and fertility* 73:505-515