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Increased ovarian response following pre-synchrony of superovulated red deer (*Cervus elaphus*) hinds

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ABSTRACT

The effects of two modified superovulation protocols on ovarian response in red deer were compared to a standard protocol. Twenty mixed-age red deer hinds were allocated to one of three treatment groups during the breeding season: Group S (n=7) received our standard superovulation protocol of 12-days treatment with an intravaginal progesterone-releasing device (CIDR', InterAg, NZ), eight injections of 0.45 mg ovine FSH (Ovagen, Immuno-Chemical Products Ltd., NZ) 12 h apart, beginning 72 h before CIDR removal with 200 iu eCG (Folligon, Intervet, Australia) added with the last injection. Group M1 (n=7) hinds received the same CIDR and eCG treatment, but Ovagen was delivered as four daily injections of 0.9 mg Ovagen dissolved in 30% polyvinylpyrrolidone (PVP-40; Sigma Chemical Co, USA). Group M2 (n=6) hinds were pre-synchronised using a 12-day CIDR' treatment and then received seven daily injections of 0.9 mg Ovagen dissolved in 30% PVP beginning four days after CIDR removal. A prostaglandin analogue (2 ml Estrumate, Pitman-Moore NZ Ltd, NZ) was administered concurrent with the sixth Ovagen injection. Ovarian response was highest in M2 hinds. Mean ovulation rates (±S.E.M.) were 4.9 ± 0.83, 3.4 ± 0.70 and 8.7 ± 1.20 for S, M1 and M2 respectively ($P>0.05$). Two S and three M1 hinds were anovulatory following superovulation treatment, but all M2 hinds ovulated ($P>0.05$). Pre-synchrony of red deer hinds before daily injection of Ovagen is an effective superovulation protocol. However, further work is required to ascertain if viable embryos are produced following the extended period of FSH exposure.

Keywords: red deer; FSH; superovulation; ovulation rate.

INTRODUCTION

A feature of red deer superovulation programmes has been the extreme variation in ovulatory response between hinds and between studies (Asher *et al.*, 1995b). Generally, the hormone- treatment protocols used have been based upon those applied successfully to other livestock species. Thompson and Asher (1988) found that a "cocktail" of FSH (multiple injection delivery) and eCG (single injection) in conjunction with use of a double CIDR treatment provided a more consistent ovulatory response in fallow deer than either FSH or eCG alone. This has become our standard red deer superovulation protocol. However, success rates using this protocol are still less than favourable, with up to 30% of hinds remaining anovulatory after treatment (I.C. Scott, unpublished data).

The presence of a dominant follicle at the time of exogenous gonadotrophic stimulation markedly reduces the superovulatory response in cattle (Huhtinen *et al.*, 1992). Reliable detection of oestrus in this species has allowed the initiation of gonadotrophin stimulation to coincide with the emergence of a new follicle wave, leading to subsequent improvement in superovulation response. However, it is difficult to observe natural oestrus in red deer and they, therefore, require synchronisation with a progesterone CIDR treatment to allow the timing of exogenous gonadotrophin treatment to coincide with emergence of a new follicle wave after ovulation (Asher *et al.*, 1997).

The standard red deer superovulation protocol requires hinds to be yarded twice daily for FSH injections over a period of four days. Not only is this inconvenient, but it also induces stress in the animals. Red deer adrenal glands secrete physiological levels of progesterone in response to acute stress (Jopson *et al.*, 1990) and recent studies indicate that release of adrenal progesterone in response to exogenous ACTH may perturb the pre-ovulatory LH surge in red deer (Asher, unpublished data 1998). Reducing the frequency of injections will undoubtedly reduce the frequency or cumulative stress imposed on treated hinds and may result in an improved superovulatory response. Cows given a single injection of FSH dissolved in polyvinylpyrrolidone (PVP) produced a similar number of transferable embryos as cows that were given FSH as eight doses over four days (Yamamoto *et al.*, 1994). However, red deer hinds treated with one dose of FSH only had a single ovulation (I.C. Scott, unpublished data), indicating an increased frequency of injections was required (i.e., daily) to induce superovulation.

This study was conducted to investigate the efficacy of either daily injections of FSH dissolved in PVP, or gonadotrophin treatment initiated to coincide with follicle wave emergence, as superovulation treatments in red deer hinds.

MATERIALS AND METHODS

Animals and treatments

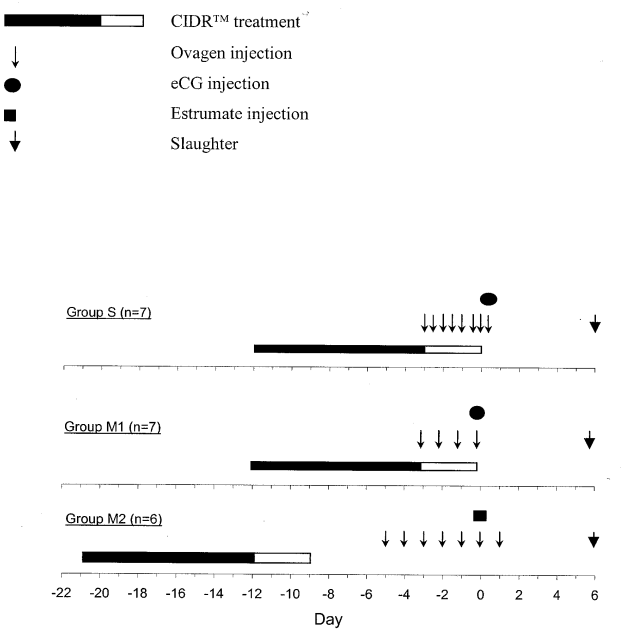
Twenty mixed-age red deer hinds were allocated to one of three treatment groups during the breeding season (Figure 1). Group S hinds (n=7) received our standard superovulation protocol. A CIDR device (Eazi-breed CIDR-G, 0.3 g progesterone per device, InterAg NZ Ltd., Hamilton, NZ) was inserted in the anterior vagina for 8 days after which time this device was removed and replaced with a new device for a further 4 days. Ovine FSH (Ovagen, Immuno-Chemical Products Ltd., Auckland, NZ) was delivered as eight equal-dose (0.45 mg) i.m. injections at intervals of about 12 h starting 72 h before removal of the CIDR' devices and eCG (200 iu Folligon, Intervet, N.S.W., Australia) was added with the last Ovagen injection. Hinds

in Group M1 (n=7) each received the same treatment protocol as for S except that FSH was administered only once daily as 0.9 mg Ovagen dissolved in 30% polyvinylpyrrolidone (PVP-40; Sigma Chemical Co, St Louis, MO, USA). Hinds in Group M2 (n=6) were pre-synchronised using a 12-day CIDR regimen and then received seven daily i.m. injections of 0.9 mg Ovagen dissolved in 30% PVP beginning 4 days after CIDR removal. Luteolysis was induced by i.m. injection of 500 mg cloprostenol (2 ml Estrumate, Pitman-Moore N Z Ltd., Upper Hutt, NZ) administered concurrent with the sixth Ovagen injection.

All hinds were slaughtered 6 days after CIDR' removal/ Estrumate injection, their reproductive tracts were recovered and structures present on the ovaries were recorded. Non-ovulatory follicles were classified as being large if between 6 and 11 mm in diameter, cystic if ≥12 mm in diameter and luteinised if luteal tissue was present but no stigma of ovulation was observed.

This experiment was approved by the AgResearch Invermay Animal Ethics Committee (Approval Number P398), as required under New Zealand law by the Animal Welfare (Codes of Ethical Conduct) Act 1987.

FIGURE 1: Schematic representation of experimental design where red deer hinds received either a standard (S) or Modified (M1, M2) superovulation protocol. Day 0 = day of CIDR removal/Estrumate injection.



Statistical analyses

The number of corpora lutea (CL) and non-ovulating follicles counted were analysed by a Poisson generalised linear model (McCullagh and Nelder, 1989) fitting treatment and adjusting for overdispersion of ovulation rate (number of CLs). Similarly, the proportion of non-ovulating hinds was analysed by a binomial generalised linear model, fitting treatment.

RESULTS

Ovagen induced superovulatory responses in hinds in all treatment groups (Table 1). Hinds that were pre-synchronised with a CIDR before Ovagen delivery (M2) had a mean ovulation rate of 8.7, which was approximately twice that of the other two treatments, although the difference was not significant (P>0.05) because all treatments had a large range of ovulation rate. The corpora lutea of ovulations in all hinds looked at a normal stage of development.

All M2 hinds ovulated four or more follicles, but two S and three M1 hinds were anovulatory (P>0.05). The mean number of follicles that had not ovulated by slaughter differed significantly (P<0.01) between treatment groups (Table 1). Hinds treated with the standard superovulation protocol (S) had more large (6-11 mm) and cystic (≥12 mm) follicles detected on their ovaries after slaughter (P<0.01).

TABLE 1: Mean ± S.E.M. (and range) of ovarian responses and number of non-ovulatory hinds following treatment with differing superovulatory regimens of ovine FSH.

			No. of non-ovulating follicles			
Group	No. of hinds	No. of CLs (range)	Luteinised (range)	6-11 mm (range)	Cystic (range)	No. of anovu-ltory hinds
S	7	4.9 ± 0.83 (0-14)	1.0 ± 0.38 (0-2)	1.6 ± 0.47 (0-5)	0.9 ± 0.35 (0-3)	2
M1	7	3.4 ± 0.70 (0-10)	0.3 ± 0.20 (0-1)	1.3 ± 0.43 (0-5)	0 ± 0 (0-3)	3
M2	6	8.7 ± 1.20 (4-15)	0.8 ± 0.37 (0-2)	0 ± 0 (0-1)	0 ± 0 (0)	0
		n.s.	n.s.	***	**	n.s.

DISCUSSION

Treatment with ovine FSH in conjunction with progesterone CIDR devices induced a superovulatory response in red deer in all treatment groups. Of note, however, was the observation that hinds pre-synchronised so that FSH delivery coincided with the emergence of a new follicle wave (M2) had a mean ovulation rate almost twice that of hinds for which FSH delivery commenced at an unknown stage of the oestrous cycle (S and M1). Furthermore, no M2 hinds remained anovulatory and all had an ovulation rate of four or more with corpora lutea at the expected stage of development.

Saumande *et al.* (1978) hypothesised that a large proportion of the variation of superovulatory responses in cows may be accounted for by variability of ovarian status at the initiation of eCG treatment. This was later confirmed by Monniaux *et al.* (1983), who demonstrated a positive relationship between ovulation rate and the number of growing follicles at the time of eCG treatment, and Lindsell *et al.* (1986), who further demonstrated that time of initiating FSH-P treatment in relation to stage of the oestrous cycle influenced the ovulatory response in cattle. Guilbault *et al.* (1991) hypothesised that response to exogenous gonadotrophin would be reduced in the presence of a dominant follicle, and this was later confirmed by Huhtinen *et al.* (1992). It is well documented that ruminant ovarian follicles grow and regress in non-random patterns or

"waves" (for example, cattle: Sirois and Fortune, 1988; camelids: Bravo *et al.*, 1991; sheep: Noel *et al.*, 1993; goats: Ginther and Kot, 1994; red deer: Asher *et al.*, 1997; fallow deer: Asher *et al.*, 1998), with the main difference between species being the extent to which the dominant follicle(s) exerts a suppressive effect on the development of subordinate follicles.

On the basis of putative stress effects on the competence of the pre-ovulatory LH surge we considered that a reduction in the frequency of injections from twice to once daily might reduce the number of anovulatory hinds. However, this does not seem to be the case, as although all M2 hinds ovulated, three of seven M1 hinds remained anovulatory. It is more likely that follicles present in both M1 and S anovulatory hinds did not have time to shift from FSH to LH dependency before removal of FSH support following CIDR removal (Campbell *et al.*, 1999). Because luteolysis is often incomplete in hinds treated with the prostaglandin analogue cloprostenol before eight days after oestrus (Asher *et al.*, 1995a), M2 hinds received FSH for an extended period. None of these hinds remained anovulatory following superovulatory treatment, suggesting that follicles in M2 hinds had time to switch from FSH to LH dependency before the removal of FSH support.

In conclusion, this study demonstrated that four daily injections of FSH dissolved in PVP were effective at inducing a superovulatory response in red deer hinds. A large, but non-significant increase in both ovulation rate and the proportion of hinds that ovulated was gained when delivery of exogenous gonadotrophin was timed to coincide with the emergence of a new follicle wave. However, it remains to be seen what effect the extended period of FSH exposure has on oocyte quality.

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