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Effect of nutrition on the ovulatory response of Coopworth ewes to varying doses of two FSH preparations

J. G. E. THOMPSON AND J. F. SMITH

Ruakura Agricultural Centre
Ministry of Agriculture and Fisheries, Hamilton

ABSTRACT

A 2 x 2 x 4 factorial trial was performed (n=20, N=320) to investigate the effect of 2 levels of nutrition (high protein diet -23% crude protein v low protein diet - 10% crude protein) on the ovarian response to exogenous follicle stimulating hormone (FSH) administration in mixed-age Coopworth ewes. Two preparations of FSH were examined (FSH-P, Burns-Biotec and Folltropin®, Vetripharm) at 4 different levels (0, 8, 16 and 24 mg equivalents FSH-P).

The ovulation rate of both high and low protein intake ewes increased linearly in response to increasing FSH levels (0 mg = 1.2; 8 mg = 3.2; 16 mg = 7.9; 24 mg = 9.6). There was no difference in response between the 2 sources of FSH. Ewes on a high protein intake had a higher ovulation rate when FSH was not administered (mean ovulation rate \pm SE; 1.40 ± 0.1 v 1.0 ± 0.1 , $P < 0.05$), but no significant differences were observed when exogenous FSH was administered. These results suggest that the mechanism(s) which cause the protein-induced increase in ovulation rate is masked by the administration of exogenous FSH.

Keywords Coopworth, sheep, nutrition, protein, ovulation rate, superovulation, FSH, CIDR.

INTRODUCTION

It is well established that diets of high energy and/or high protein will increase the ovulation rate in cycling ewes. Although ovulation rate is also determined by genetic factors (Bindon, 1984) the nutritional effect is not breed dependent and can be observed within the period of 1 oestrous cycle (Smith *et al.*, 1982; Smith, 1985). However, the mechanism for this effect has yet to be elucidated. It has recently been shown that ewes fed high dietary protein intakes also have increased levels of circulating follicle stimulating hormone (FSH) compared to ewes fed a basal low protein diet (Smith *et al.*, 1986; Smith, 1988). Increased levels of circulating FSH in ewes causes an increase in the ovulation rate. Such an effect can be achieved by a variety of treatments: Administration of exogenous FSH (Wright *et al.*, 1981; McNatty *et al.*, 1985); after a period of bovine follicular fluid administration (Wallace and McNeilly, 1985) or active immunisation against inhibin-enriched fractions of bovine follicular fluid (O'Shea, *et al.*, 1984). Little is known on what effect higher endogenous levels of FSH would have on the response to the various doses of exogenous FSH. Moreover the postulated mechanism of increased nutrition via a stimulated of hepatic steroid metabolism and the ensuing reduced negative feedback (Smith, 1988) may potentiate the response to exogenous FSH.

Data on the ovulatory response to exogenous hormone indicated an increasing variability with

higher doses. It has been suggested that a contributor to this variability could be differences in nutrition level and/or live weight of ewes being treated. Some workers (Allison, 1975; Keane, 1973; Tait, 1971) have shown that heavier and better fed ewes give a greater response than lighter, less well fed ewes. The magnitude of difference appears to be related to the duration of treatment and the extent of difference in ewe live weight, as other workers with smaller differences in ewe live weight have failed to show any response (Eastwood and McDonald, 1975).

This paper describes an experiment which examines the effect of exogenous FSH administration on ovulation rate in ewes maintained on either high or low protein intakes.

MATERIALS AND METHODS

A 2 x 2 x 4 factorial trial (n=20, N=320) was performed in June, 1987 with mature Coopworth ewes randomised for live weight across treatments. The imposed treatments were:

- Two levels of protein in diets (high protein — 23% crude protein (CP) v low protein — 10% CP) with metabolisable energy (ME) equivalent to 11 MJ/kg of feed, fed at a level of approximately 1.5 kg/ewe/d. Feed was prepared as pellets (NRM, Hamilton, New Zealand) formulated from constituents described by Smith (1985).
- Two commercial FSH preparations, FSH—P (Burns-Biotec, Omaha, USA, Batch No. 526K85) and Folltropin® (Vetripharm, Ontario,

Canada, Batch No. V-018). FSH-P has a variable luteinising hormone (LH):FSH ratio (Lindsell *et al.*, 1986), whereas the manufacturer of Folltropin® cites it as having an LH content of $5 \pm 2\%$ by weight. Difficulty in comparing the 2 preparations on a dose basis was found because FSH activity in each is expressed in different units. FSH-P is measured in Armour units, whereas Folltropin® is measured in National Institute of Health (NIH) units. To overcome this difficulty, both FSH-P and Folltropin® vials were reconstituted in 10 ml of saline and then considered equal in potency on a per ml basis, based on the distributor's recommendations.

- (c) Four levels of FSH administration as 0, 1.6, 3.2 or 4.8 ml of reconstituted saline (equivalent to 0, 8, 16 or 24 mg FSH-P).

All ewes had been pre-trained to eat pellets and were housed on sawdust. Water was provided *ad libitum* and pellets fed once daily. Animals were fed the pelleted diets for at least 7 d before synchronisation treatments began. Synchronisation was achieved using 2.9% progesterone impregnated controlled internal drug releasers (CIDR) (type G, AHI, Hamilton, New Zealand). The first CIDR was removed 9 d after insertion and replaced immediately with a fresh CIDR. Double CIDR treatments appear necessary to support normal ovulation in super-ovulating ewes (J.G.E. Thompson, R.W. James and H.R. Tervit, unpublished). Ten days after initial CIDR insertion, gonadotrophin treatments began. All ewes received a single, low dose, intramuscular injection of pregnant mare serum gonadotrophin (PMSG). In addition, ewes receiving FSH were treated twice daily for 3 d in a decreasing dose regimen. CIDRs were removed in the morning of the twelfth day after initial insertion. Mature Suffolk rams, harnessed with crayon markers, were introduced (10% joining ratio) immediately after second CIDR removal. Tups were recorded every 12 h for at least 72 h prior to ewes being removed to the abattoir for overnight starvation. At slaughter,

reproductive tracts were recovered and the number of corpora lutea on each ovary recorded. Carcass weights were also recorded.

Ovulation response was analysed by multiple regression analysis using the Genstat statistical package (Lawes Agricultural Trust, Rothamsted Experimental Station, UK) after ln (ovulation + 1) transformation. Comparisons of mean ovulations between treatments were performed by Student's *t*-test. The cumulative percentage of ewes exhibiting oestrus by various times after CIDR removal were examined by the Kolmogorov-Smirnov test. The time intervals between CIDR removal and oestrous onset for different ovulation rates were examined by analysis of variance.

RESULTS

The ovarian responses to protein and FSH treatments are presented in Table 1. Overall, there was no difference in numbers of ovulations between the 2 protein levels or for the 2 FSH preparations. A significant linear increase in ovulation rate ($P < 0.001$) was observed as FSH levels increased (Fig. 1). Ewes maintained on the high protein diet had a significantly higher mean ovulation rate than ewes on the low protein diet when no FSH was administered (1.4 ± 0.1 v 1.0 ± 0.1 , $P < 0.05$). However this effect was lost when exogenous FSH was administered. In addition, the variability of the ovulation response increased with increasing exogenous FSH (Fig. 1). Neither the type of FSH preparation, nor the level of protein in the diet appeared to affect this variability in response. No significant difference was observed for carcass weights between dietary treatments (overall mean 48.1 ± 0.6 kg).

A marked relationship was observed between time of oestrous onset from CIDR removal and the ovulatory status of ewes (Fig. 2). No heats were recorded by 12 h after CIDR removal. Only 28% (43 out of 153) ewes with 0-2 ovulations had come into heat between 12 and 36 h, whereas 69% (59 out

TABLE 1 Mean ovulation response (\pm SE) to exogenous follicle stimulating hormone (FSH) administration in ewes maintained on high or low levels of dietary protein.

Protein level	FSH preparation	FSH dose (ml)			
		0	1.6	3.2	4.8
High	FSH-P	1.3 ± 0.2	3.5 ± 0.7	7.4 ± 1.5	10.1 ± 1.2
	Folltropin	1.4 ± 0.2	3.4 ± 0.9	8.2 ± 1.4	7.1 ± 1.2
	Mean	1.4 ± 0.1	3.5 ± 0.6	7.7 ± 1.0	8.6 ± 0.9
Low	FSH-P	0.8 ± 0.1	2.7 ± 0.8	9.8 ± 1.3	10.3 ± 1.3
	Folltropin	1.1 ± 0.1	3.1 ± 0.7	6.2 ± 1.0	11.5 ± 1.5
	Mean	1.0 ± 0.1	2.9 ± 0.5	8.0 ± 0.8	10.6 ± 1.0
Overall		1.2 ± 0.1	3.2 ± 0.4	7.9 ± 0.6	9.6 ± 0.7

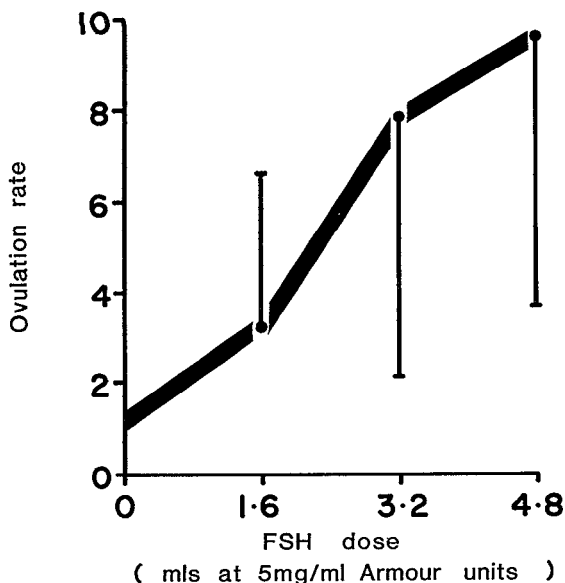


FIG. 1 Mean ovulation rate to FSH administration. Bars indicate standard deviation.

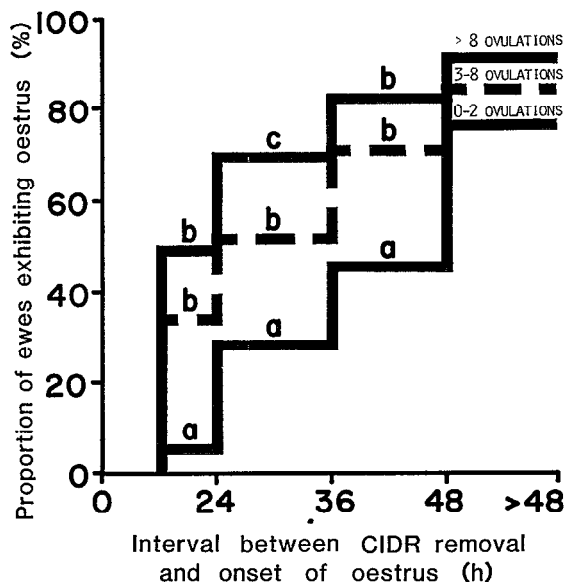


FIG. 2 Effect of ovulation rate on the cumulative percentage of ewes exhibiting oestrus over time from CIDR removal. Different subscripts indicate differences with $P < 0.05$.

of 85) of ewes with greater than 8 ovulations exhibited oestrus onset during the same period of time. Mean time interval to oestrous onset from CIDR withdrawal was significantly shortened as number of ovulations increased (0-2 ovulations -

40.9 ± 1.3 h; 3-8 ovulations - 30.4 ± 1.8 h; > ovulations - 26.2 ± 1.5 h, $P < 0.001$).

DISCUSSION

The higher ovulation rate found in the ewes on the high protein diet when no FSH was administered is in agreement with previous reports (Smith, 1985; 1988). However, while the mechanisms involved are not known, recent results show that ewes maintained on diets containing high levels of crude protein had increased levels of circulating plasma FSH (Smith *et al.*, 1986; Smith, 1988). This phenomenon may be due to either direct stimulation of the hypothalamic-pituitary axis, or to a reduction in the negative feedback mechanisms by ovarian factors, such as oestrogens and inhibin. Results from this present study suggest that exogenous FSH administration completely overrides the effect of dietary protein. Changes in endogenous FSH levels, in response to nutritional changes, appear to have had little influence in the presence of higher doses of exogenous FSH. Furthermore, nutrition did not affect the variability in ovulation response when FSH dose was increased. Little can be determined as to the mechanism of the protein effect in light of the results presented here, except to suggest that it is sensitive to factors which directly effect ovulation rate.

There are now 2 commercial preparations of FSH available in New Zealand — FSH-P and Folltropin®. In our hands, no difference was observed between the 2 preparations in their ability to induce superovulation. This suggests that the use of either preparation will ultimately be based on personal preference, cost and availability. However, overseas data has shown that batches of FSH-P vary in LH content (Lindsell *et al.*, 1986) and that the degree of contaminating LH can have detrimental effects on superovulation rates (Armstrong and Evans, 1984).

It has been reported that a significant proportion of ewes fail to multiple ovulate when FSH is administered alone compared to combinations of PMSG and FSH (Ryan *et al.*, 1984). In this study, a low dose of PMSG was administered to all ewes. In those ewes also receiving 4.8 ml FSH (of either preparation), 15% (12 out of 81) of ewes failed to have 3 or more ovulations. This result is comparable with that of Ryan *et al.* (1984), in which 18% of ewes failed to multiple ovulate when PMSG and FSH were administered together.

While it has been established that progesterone CIDRs will effectively synchronise the oestrous cycle of ewes and hoggets (Harvey *et al.*, 1984; McMillan, 1986; Maxwell and Barnes, 1984) there is little information available on the use of CIDRs in conjunction with superovulatory doses of gonadotrophin in sheep. Boland *et al.* (1983) has reported that the ovulation rate in response to horse

anterior pituitary extract (HAP) was lower in ewes synchronised with 12% progesterone CIDRs than for 30 mg flurogestone acetate (FGA) sponges. We have some evidence that superovulation in ewes synchronised with single 9% CIDRs is associated with very early oestrus onset and abnormal ovarian responses (J.G.E. Thompson, R. James and H.R. Tervit, unpublished). In goats, an early oestrus onset is observed at superovulation with a single CIDR compared to daily progesterone injections (Tervit, 1987), but with normal follicular development. In this study, a double CIDR treatment was applied with the result that a linear response to increasing FSH levels was observed. Furthermore, time of oestrous onset was related to the number of ovulations recorded — the higher the ovulation rate, the shorter the interval between CIDR withdrawal and oestrus onset. This suggests that onset of oestrus is dependant upon the number of oestrogenic follicles present at CIDR removal, and is likely to reflect levels of circulating oestrogens. However, oestrogen levels, as with duration of oestrus and timing of ovulation, were not recorded during this experiment. Little data on the time to onset of oestrus after CIDR removal has been presented to date. Smith *et al.* (1988) has shown that mean time to onset of oestrus in the breeding season is 36.4 ± 0.7 h. A slight advancement of oestrus with increasing ovulation rates has been reported elsewhere (Robinson *et al.*, 1987), but not to the same extent as the 15 h observed here. Results here suggest that the time to onset of oestrus after a double CIDR treatment can be used to estimate the ovulatory response of groups of ewes to superovulation treatments. Further work is being conducted to validate this observation and to assess the effectiveness of CIDR synchronisation in superovulation regimes.

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