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# Influence of follicle stimulating hormone on ovarian follicular development and ovulation rate in Romney ewes

N. HUDSON, K.P. McNATTY, K. BALL, M. GIBB, D. HEATH, S. LUN,  
L. KIEBOOM AND K.M. HENDERSON

Wallaceville Animal Research Centre  
Ministry of Agriculture and Fisheries, Upper Hutt

## ABSTRACT

The aim of this study was to examine the temporal relationships between FSH concentrations in blood and their effects on ovarian follicular viability, oestradiol synthesis and ovulation rates in Romney ewes.

Collectively, the results showed that: (1) the number of non-atretic antral follicles ( $\geq 1$  mm diameter) in sheep ovaries is influenced by plasma FSH concentrations; (2) the level of follicular oestradiol biosynthesis can be enhanced by FSH supplementation; and (3) sustained elevations of plasma FSH concentrations for 24 but not 6 h within 48 h of the onset of luteolysis significantly enhance the ovulation rate in Romney ewes.

**Keywords** Follicle viability; oestradiol synthesis; granulosa cells; corpora lutea.

## INTRODUCTION

Follicle stimulating hormone (FSH) is thought to act specifically on ovarian follicles to influence their viability and ultimately, their ability to ovulate (Peters and McNatty, 1981). High concentrations of FSH are thought to enhance ovulation rates whereas low concentrations are thought to result in low ovulation rates or infertility. The aim of this study on Romney ewes was to examine the temporal relationships between FSH concentrations in plasma and follicle viability, oestradiol synthesis (by follicle granulosa cells *in vitro*) and ovulation rate.

## MATERIALS AND METHODS

All animals in Experiments 1 to 3 described below were 2.5 to 3.5 year old parous Romney ewes. Experiment 1 was designed to examine the effects of lowering or raising the plasma FSH concentrations on follicle viability and oestrogen-synthetase (aromatase) activity in granulosa cells. Anoestrous ewes ( $n = 28$ ) were injected with saline (0.9% w/v NaCl;  $n = 8$ ), steroid-free bovine follicular fluid ( $n = 6$ ; WA batch V, bFF; 2 injections of 5 ml s.c. 12 h apart), ovine FSH [ $n = 6$ ; 10  $\mu$ g NIH-FSH-S12 (biopotency = 1.25 U/mg; 1 U = 1 mg NIH-FSH-S1) i.v. once per h for 24 h], ovine FSH ( $n = 4$ ; 10  $\mu$ g NIH-FSH-S12 injected as above) plus bFF (injected as above) or ovine FSH ( $n = 4$ , 50  $\mu$ g NIH-FSH-S12 injected at hourly intervals as above) plus bFF (injected as above). The purpose of injecting bFF was to lower the FSH concentrations in blood. The method for removing steroids from bFF was identical to that described by Henderson and Franchimont (1981). The ovaries of all the above animals were excised for further study 24 h after the

treatments began. Preliminary studies showed that the above bFF treatment regimen caused a progressive reduction in the geometric mean plasma FSH concentrations from 88 (76,100) ng/ml at the start of treatment to 26 (21, 32) ng/ml (95% confidence limits in brackets), some 10 h later ( $P < 0.01$ ); the latter concentrations once reached were maintained for at least 14 h ( $n = 8$  treated and 8 control ewes bled hourly for 24 h).

Experiment 2 was designed to compare plasma FSH concentrations in ewes with 2 and 1 ovulations before, and after, an injection of cloprostenol (125  $\mu$ g i.m.) on days 8 to 10 of the oestrous cycle (oestrus = day 0). Forty-four ewes were bled (2.5 ml) via a jugular cannula, once every hour for 120 consecutive hours. Cloprostenol was injected into all ewes after 72 h and 2 vasectomised Romney rams with marking harnesses were introduced to the ewes after 102.5 h. At the end of the intensive blood sampling schedule, all animals which had been housed indoors and exposed to constant artificial lighting, were sent out to pasture and bled daily for 21 days. All animals were laparoscoped after 7 days on pasture to determine the number of new corpora lutea (CL). The blood samples from 12 of the 30 animals with 1 CL and 11 animals with 2 CL were retained for a study of FSH concentrations in blood. The limitation of the study to only 23 animals was due in part to the limited amount of FSH antiserum. All of the 23 animals studied were shown to have experienced a normal pattern of CL regression (i.e., as judged from plasma progesterone levels) and oestrous behaviour for 12 or more hours beginning some 44 h after cloprostenol treatment. Moreover, all animals subsequently formed normal CL which

gave plasma progesterone values in excess of 1 ng/ml for at least 8 days.

In Experiment 3, luteal phase (days 7-10) ewes were injected with FSH (NIAMDD-FSH-S15; biopotency 20 U/mg; 1 U = 1 mg NIH-FSH-S1; 1.6  $\mu$ g i.v. once per h) for 6 or 24 h before, or after, an injection of cloprostenol (125  $\mu$ g i.m.) to induce ovulation. In the 6 h FSH regimen, there were 10 control and 10 treated ewes in each 6 h time frame from 72 h before to 24 h after cloprostenol treatment. In the 24 h FSH injection regimen there were 15 control and 15 treated ewes covering the 24 h time frames -72 to -48, -48 to -24, -24 to 0 and 0 to +24 h from cloprostenol (given at time 0). All ewes were subjected to laparoscopy 6 to 12 days after cloprostenol injection. At no time were any of these ewes exposed to a ram. Preliminary studies with the NIAMDD-FSH-S15 injection regimen showed that the geometric mean FSH concentration during FSH supplementation was  $121 \pm 3\%$  of that before treatment ( $n = 10$  ewes).

All blood samples from the above studies were centrifuged (4000 g at 18 to 20°C for 20 min) within 15 min of collection and the plasmas frozen to -20°C until FSH and/or progesterone analysis.

The excised ovaries were weighed, the gross morphology recorded and all individual follicles ( $\geq 1$  mm diameter) were dissected free of extraneous tissue under a stereomicroscope. Details regarding the classification of follicles as non-atretic or atretic, isolation and quantification of granulosa cells, the granulosa cell aromatase assay, and the radio-immunoassay methodologies for FSH and progesterone are all reported in full elsewhere (McNatty *et al.*, 1984; 1985).

## RESULTS

The effects of bFF and/or FSH treatment on the number of non-atretic follicles and their distribution with respect to follicular diameter are shown in Table 1. None of the treatments altered the total number of

follicles present in each ewe. Treatment with bFF significantly reduced the number of medium to large ( $\geq 3$  mm diameter) non-atretic follicles. Treatment of ewes with the 10  $\mu$ g FSH regimen alone had no effect on the numbers of non-atretic follicles, but the 10  $\mu$ g FSH + bFF regimen neutralised the suppressive effects observed in ewes treated with bFF alone. Treatment of ewes with a 50  $\mu$ g FSH + bFF regimen significantly increased the number of non-atretic follicles and, in particular, the number of medium to large non-atretic follicles ( $\geq 3$  mm diameter).

In control ewes, granulosa-cell aromatase activity was related to follicle size. In 1-2.5 mm, 3-4.5 mm and  $\geq 5$  mm diameter follicles, the respective levels of aromatase activity were  $\leq 0.3$ ,  $2.9 \pm 0.2$  and  $5.3 \pm 0.3$  ng oestradiol-17  $\beta$ /10<sup>6</sup> granulosa cells/3 h (mean  $\pm$  s.e.m., 3 follicles for each size range). The 10  $\mu$ g FSH regimen increased aromatase activity in  $\geq 5$  mm follicles 4.7 fold (i.e., to  $25.1 \pm 0.6$  ng oestradiol/10<sup>6</sup> cells/3 h) without influencing activity in cells from small or medium sized follicles (i.e.,  $\leq 0.3$  ng oestradiol/10<sup>6</sup> cells/3 h in 1-2.5 mm follicles and  $2.6 \pm 0.1$  ng oestradiol/10<sup>6</sup> cells/3 h in 3-4.5 mm follicles). The bFF + 10  $\mu$ g FSH regimen caused no significant change in granulosa cell aromatase activity with respect to the controls; the respective values in small, medium and large follicles were  $5.6 \pm 0.2$ ,  $3.0 \pm 0.3$  and  $\leq 0.3$  ng oestradiol/10<sup>6</sup> cells/3 h ( $n = 3$  follicles for each size range). The bFF + 50  $\mu$ g FSH treated ewes contained significantly higher levels of aromatase activity over all follicle diameters ( $P < 0.01$ ) relative to that in the control animals; the respective values in small, medium and large follicles were  $14.0 \pm 0.6$ ,  $24.2 \pm 3.6$  and  $3.3 \pm 0.3$  ng oestradiol/10<sup>6</sup> cell/3 h.

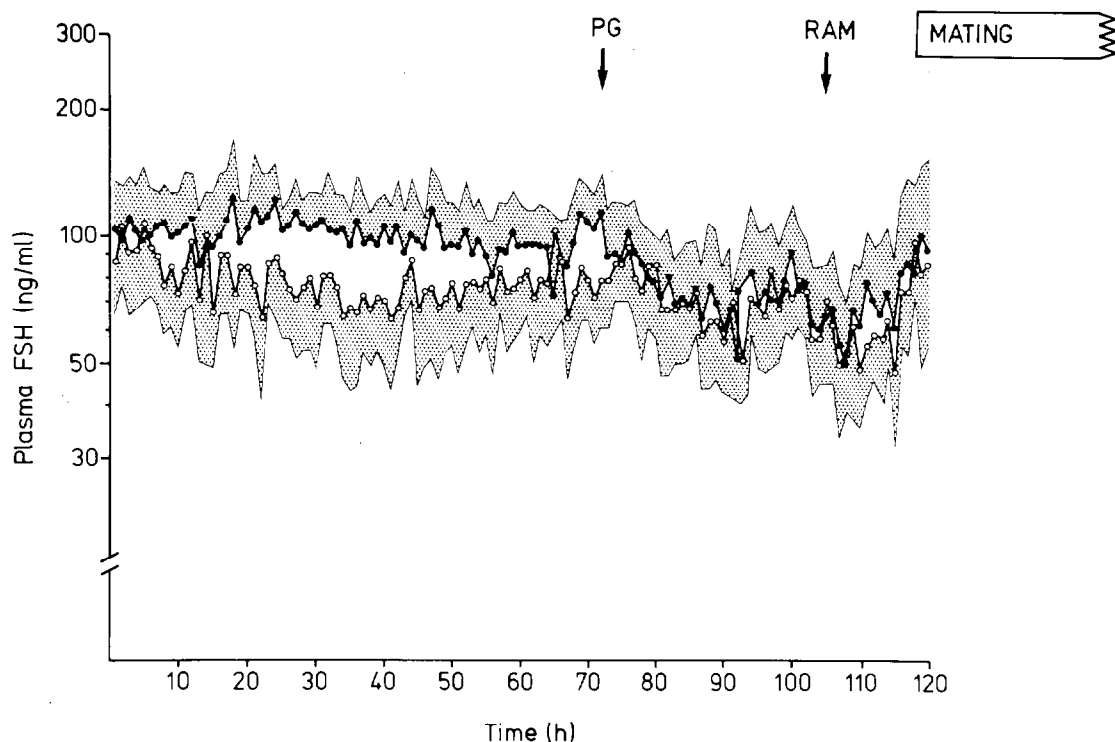
The FSH concentrations in ewes with 1 or 2 ovulations are summarised in Fig. 1. There were no significant differences in the geometric mean FSH concentrations between ewes that subsequently ovulated 1 or 2 follicles over the time frames -72 to -48 h, 0 to +24 h and +24 to +48 h from

**TABLE 1** Effect of steroid-free bovine follicular fluid (bFF) and/or FSH inoculation in sheep on the number of non-atretic follicles and their distribution with respect to follicular diameter (from McNatty *et al.*, 1985).

Treatment	No. of ewes	Total no. of follicles ( $\geq 1$ mm diameter) per ewe	No. of non-atretic follicles ( $\geq 1$ mm diameter) per ewe	No. of non-atretic follicles with respect to follicular diameter (mm)	
				$\geq 3$	1-2.5
Control	8	$33.5 \pm 3.6$	$7.3 \pm 0.7$	$3.3 \pm 0.5$	$4.0 \pm 0.6$
bFF	6	$30.7 \pm 3.9$	$1.5 \pm 0.5^{**}$	$0.2 \pm 0.2^{**}$	$1.3 \pm 0.5$
10 g FSH	6	$29.0 \pm 3.3$	$8.7 \pm 0.7$	$2.2 \pm 0.3$	$6.5 \pm 0.6$
10 g FSH + bFF	4	$36.2 \pm 4.7$	$7.7 \pm 1.5$	$3.2 \pm 0.8$	$4.5 \pm 0.9$
50 g FSH + bFF	4	$39.0 \pm 5.3$	$23.8 \pm 3.7^{**}$	$18.3 \pm 3.5^{**}$	$5.5 \pm 1.1$

Values are mean  $\pm$  s.e.m.

\*\* Value different from that of control ( $P < 0.01$ )



**FIG. 1** Geometric mean concentrations of plasma FSH each hour for Romney ewes from  $-72$  h until  $48$  h after cloprostenol (PG) injection ( $125 \mu\text{g s.c.}$ );  $\bullet$ — $\bullet$  represents the results for animals ( $n = 11$ ) that subsequently formed 2 corpora lutea whereas  $\circ$ — $\circ$  represents the results for animals ( $n = 12$ ) that subsequently formed only 1 corpus luteum. The vasectomised rams were introduced to the ewes at  $30.5$  h and the matings occurred from  $46$  to  $96$  h after cloprostenol injection. Shaded areas = 95% confidence limits. For the sake of clarity only the upper or lower limits are shown although these are equidistant each side of the mean. From McNatty *et al.* (1985).

cloprostenol injection. However, over the time frames  $-48$  to  $-24$  h and  $-24$  to  $0$  h from cloprostenol injection, the respective geometric mean FSH concentrations in the ewes that subsequently ovulated 2 follicles were significantly higher than in those that ovulated 1 follicle (both  $P < 0.01$ , unpaired two-tailed Student's *t* test on geometrically transformed 24 h means).

There were no significant increases in the subsequent ovulation rates in treated ewes compared to control animals when the treated ewes were injected with  $1.6 \mu\text{g}$  ovine FSH (NIAMDD-FSH-S15) once/h for 6 h over any of the consecutive 6 h time frames from  $72$  h before to  $24$  h after cloprostenol injection. However, when the ewes were injected with  $1.6 \mu\text{g}$  ovine FSH (NIAMDD-FSH-S15) once per h for 24 h, the subsequent mean ovulation rates in the FSH/control ewes over the time frames  $-72$  to  $-48$  h,  $-48$  to  $-24$  h,  $-24$  to  $0$  h and  $0$  to  $+24$  h from cloprostenol injection were respectively  $1.00/1.07$ ,  $1.40/1.07$ ,  $1.41/1.07$  and  $1.27/1.07$ . In this study no animals were identified with 3 or more CL. When the numbers of ewes with 1 or 2 CL in the

treated and control groups were compared by  $\chi^2$  analysis, there was a significant effect of FSH treatment over the 24 h time frames of  $-48$  to  $-24$  or  $-24$  to  $0$  h (both  $P < 0.05$ ) from cloprostenol injection but not for the other time frames.

## DISCUSSION

These data show that the viability of ovarian follicles ( $\geq 1$  mm diameter) is influenced markedly by the FSH concentrations in plasma. Compared to control ewes, bFF treatment led to a 70% reduction in the plasma FSH concentrations and a corresponding reduction in the number of non-atretic follicles from 22 to 5% without any alterations in the total number of follicles ( $\geq 1$  mm diameter). Since a  $10 \mu\text{g}$  FSH injection regimen could neutralise the effects of bFF it may be concluded that the effects of bFF were due solely to a reduction in the plasma concentrations of FSH. A  $50 \mu\text{g}$  FSH + bFF regimen caused a significant ( $P < 0.01$ ) increase in the number of non-atretic follicles ( $\geq 1$  mm diameter) compared to the controls. Presumably, in this study, the FSH

treatment dose was in excess of that required to offset the inhibitory effects of bFF so that the ovaries were subjected to a sustained (i.e., 24 h) high level of FSH stimulation.

These data show that FSH stimulates aromatase enzyme activity in ovine granulosa cells from non-atretic follicles as well as influencing follicle viability. Relative to the controls, the 10  $\mu$ g FSH regimen caused a 5 fold increase in aromatase activity in large follicles but had no influence on aromatase activity in medium or small follicles. The 10  $\mu$ g FSH + bFF regimen resulted in granulosa cells having levels of aromatase activity identical to that in cells from control ovaries over all follicle diameters. However, the 50  $\mu$ g FSH + bFF regimen stimulated aromatase activity in cells from all follicle sizes relative to that in the corresponding controls. Presumably, the latter result was due to the FSH supplementation over and above that required to neutralise the bFF influence.

A major finding in this report was that ewes ovulating 2 follicles had significantly higher ( $P < 0.01$ ) plasma FSH concentrations from 48 to 24 h and 24 to 0 h before the onset of luteolysis than did ewes ovulating a single follicle. It seems reasonable to suggest that the 20 to 40% higher FSH concentrations before luteolysis in the sheep with twin ovulations (i.e., compared to those with a single ovulation), were causally related to the increase in ovulation rate. The hourly administration of FSH (1.6  $\mu$ g i.v.; NIAMDD-FSH-S15) increased the mean plasma FSH concentrations by  $\sim 20\%$  ( $P < 0.01$ ); over the 24 h time frames of 48 to 24 h or 24 to 0 h before luteolysis, this FSH regimen also led to a significant increase ( $P < 0.05$ ) in the number of Romney ewes with twin ovulations. The timing and

duration of FSH supplementation relative to the onset of luteolysis was another important finding of this study. FSH supplementation at -72 to -48 h before or 0 to +24 h after luteolysis or for any 6 h time frame from -72 h before to +24 h after luteolysis were ineffective in enhancing ovulation rates.

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