Effect on ovulation rate of increasing or decreasing ovarian exposure to follicle stimulating hormone during the preovulatory period in ewes

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ABSTRACT

Ovine FSH was administered hourly to ewes for 48 h from the initiation of luteolysis. The mean ovulation rates of ewes receiving 2.5 μg FSH/h (2.1 ± 0.2 ovulations/ewe, mean ± s.e.m., n = 8) and 5.0 μg FSH/h (6.2 ± 2.0 ovulations/ewe, n = 6) were significantly higher (P 0.01) than that of control ewes (1.3 ± 0.1 ovulations/ewe, n = 16). Mean plasma concentrations of FSH were also raised above normal by these doses of FSH. Plasma concentrations of FSH were reduced below normal by twice daily administration of steroid-free bovine follicular fluid (bFF) for 48 h from the initiation of luteolysis. As a consequence follicles failed to achieve ovulatory maturity. Large follicles (≥5 mm diameter) from ewes treated with bFF for 48 h had reduced concentrations of oestradiol-17β in follicular fluid, fewer granulosa cells, and the cells had a reduced ability to metabolise testosterone to oestradiol-17β and to produce cyclic AMP when challenged with FSH or LH.

Keywords Follicle stimulating hormone; follicular fluid; ovulation rate; granulosa cells; sheep.

INTRODUCTION

The pituitary gonadotrophins follicle stimulating hormone (FSH) and luteinising hormone (LH) are essential for follicles to develop to ovulatory maturity, and at ovulation, release an egg capable of fertilisation. Indeed, ovulation rates in several domestic species can be increased by administration of preparations rich in FSH and LH activity, such as pregnant mares serum gonadotrophin (PMSG). The relative role(s) of FSH and LH in regulating follicular development is, however, still ill-defined. In this study, some aspects of the role of FSH in regulating follicular development in ewes were examined by increasing or decreasing ovarian exposure to FSH during the preovulatory period. Ovarian exposure to FSH was increased by administration of highly purified ovine FSH. Ovarian exposure to FSH was decreased by administration of inhibin (in the form of steroid-free bovine follicular fluid) which specifically suppresses plasma concentrations of FSH (Henderson *et al.*, 1986).

MATERIALS AND METHODS

Studies *in vivo*

Parous New Zealand Romney ewes (aged 2.5 to 3.5 years) were grazed on open pasture and run with a vasectomised ram fitted with a marking harness. The ewes were examined twice daily for signs of oestrus. On the 10th day after oestrus was observed, the ewes received an intramuscular injection of the prostaglandin $F_{2\alpha}$ analogue, cloprostenol (125 μg: ICI Tasman Ltd, Upper Hutt, New Zealand) to initiate luteolysis. Immediately after this injection, treatment with FSH or bovine follicular fluid (bFF) began.

The ovine FSH preparation used was NIADDK-oFSH-16 (biopotency of 20 U/mg; 1 U = 1 mg of NIH-FSH—S1) and its contamination with LH and other pituitary hormones was negligible. Solutions containing 2.5 and 5 μg FSH/ml were prepared in saline (0.9% NaCl w/v) containing 0.2% ovine serum albumin. One ml, once per hour for 48 h was administered to ewes through an intrajugular cannula using an infusion pump adjusted to deliver 1 ml/minute, once/hour. Control ewes received vehicle alone. For comparative purposes, some ewes received a cruder preparation of FSH (F.S.H.-P: Burns-Biotec, Nebraska, USA) at dosages of 0.1 and 0.5 mg/h for 48 h. Jugular venous blood samples were taken from all ewes at 1 to 4 h intervals starting the day before FSH treatment began, and finishing at the end of the treatment period. Ten days after the end of the treatments, the ovaries of each ewe were examined by laparoscopy and the number of ovulations (ovulation rate) was determined by counting the number of corpora lutea present.

Ewes given bFF were injected intramuscularly 0, 12, 24, 36 and 48 h after receiving cloprostenol with 5 ml bFF which had been treated with dextran-coated charcoal to remove steroids, as described previously (Henderson *et al.*, 1986). Control ewes received 5 ml dextran-coated charcoal treated plasma from an ovariectomised cow. Jugular venous blood samples were taken twice daily starting the day before bFF treatment began, through to the end of the treatment period. In some ewes, the ovaries were removed immediately after the final injection of bFF or 'ovariectomised' plasma. Other ewes were left to run with a vasectomised ram to determine the time to oestrus. In these ewes, daily blood sampling for progesterone determination was continued for a further 14 days after the last injection of bFF.

All blood samples collected were centrifuged at

2000 g for 15 min at room temperature. The plasma was removed and stored at -20°C until assayed for FSH and/or progesterone. FSH was measured using a radioimmunoassay kit provided by NIADDK, National Institutes of Health, Bethesda, USA and progesterone was measured by radioimmunoassay as described previously (McNatty *et al.*, 1981).

Studies *in vitro*

Immediately after ovariectomy, all antral follicles ≥1.0mm in diameter were individually dissected from each pair of ovaries. Details of the methods used to collect follicular fluid and measure its oestradiol-17β content, to isolate and quantitate follicular granulosa cells, to measure the ability of granulosa cells to metabolise testerone to oestradiol-17β (aromatose activity), and to produce adenosine 3′, 5′-monophosphate (cAMP) in response to challenge with FSH and LH have all been reported in full elsewhere (Henderson *et al.*, 1986).

groups before injection of cloprostenol or 4, 44 and 48 h after injection of cloprostenol. The mean ovulation rates of groups of ewes receiving each dose of ovine FSH or the 2 doses of F.S.H.-P tested were significantly higher than that of control ewes (Table 1).

Effects of Treatment with Follicular Fluid

By 12 h after the first injection of bFF, the mean plasma concentration of FSH in bFF treated ewes was significantly lower than that of control ewes, and it remained significantly lower for the remainder of the treatment period (Fig. 1b). There was a significant increase ($P<0.001$, Student's t-test) in the mean time to oestrus, after injection of cloprostenol, in the bFF treated ewes (9.3 ± 0.5 d, mean ± s.e.m. for $\bar{N}$ = 6) relative to control ewes (2.2 ± 0.2 d, N = 6). In control ewes mean plasma concentrations of progesterone 8 days after the last injection of 'ovariectomised' plasma was 1.4 ± 0.1 ng/ml (N = 6), indicative of the formation of new corpora lutea. In contrast, in bFF treated ewes,

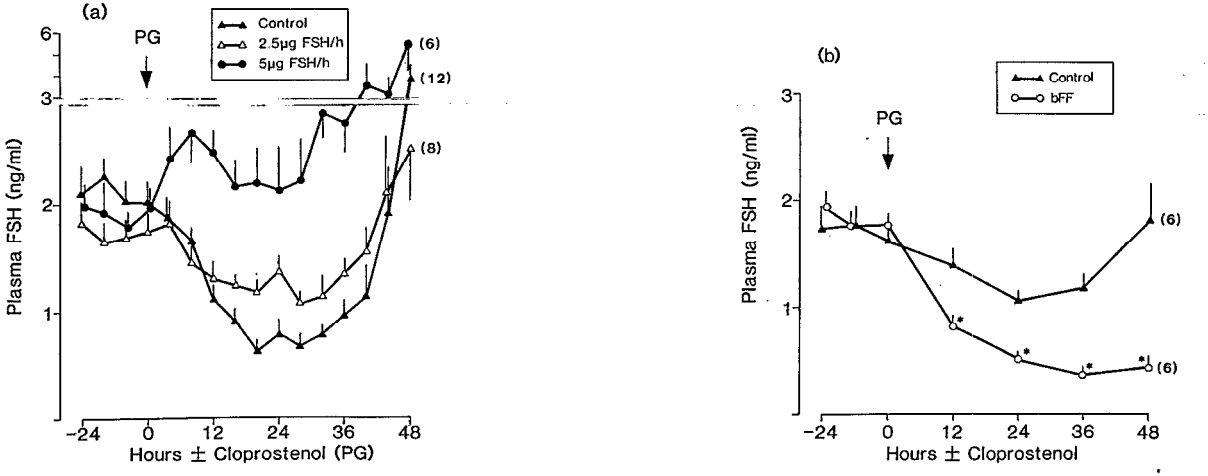

FIG. 1 Effect of administration of (a) ovine FSH or (b) follicular fluid (bFF) on mean plasma concentrations of FSH in ewes (numbers in parentheses). Bars indicate standard errors of the means. Asterisks indicate mean values for bFF treated ewes are significantly different from corresponding control mean values ($P<0.01$).

RESULTS

Effects of FSH

Figure 1(a) shows the effect of administration of ovine FSH on plasma concentrations of FSH, at 4-hourly intervals. In ewes receiving 5 μg FSH/h, mean plasma concentrations of FSH were significantly higher than those of control ewes and ewes receiving 2.5 μg FSH/h from 8 h to 40 h after injection of cloprostenol ($P<0.01$, analysis of variance and Newman-Keuls test). In ewes receiving 2.5 μg FSH/h, mean plasma concentrations of FSH were significantly higher than those of control ewes from 20 h to 28 h after injection of cloprostenol ($P<0.05$). Mean plasma concentrations of FSH did not differ significantly between the 3 plasma progesterone concentrations were still at basal levels (0.2 ± 0.02 ng/ml, N = 6).

Examination of the ovaries of ewes treated for 48 h with bFF showed that relative to control ewes there were no significant differences ($P<0.05$, Student's t-test) in the mean numbers of small (1 to 2.5 mm), medium (3 to 4.5 mm) or large (≥5 mm) diameter follicles. For control (N = 8) *v* bFF treated (N = 12) ewes there were 24 ± 3 *v* 27 ± 4 small follicles, 2.3 ± 0.7 *v* 4.0 ± 0.9 medium diameter follicles and 1.6 ± 0.2 *v* 1.3 ± 0.3 large follicles (values are mean ± s.e.m.). However, relative to control ewes, large follicles from bFF treated ewes had lower concentrations of oestradiol-17β in follicular fluid, contained fewer granulosa cells and the granulosa cells had a reduced ability to metabolise

TABLE 1 Effect of treatment with ovine FSH or F.S.H.-P on ovulation rates in Romney ewes.

Treatment	Ovulation rate 1	2	3	4 to 14	Mean ovulation rate
Controls	10	6	0	0	1.3 (1.1-1.5)
2.5 μg/h FSH	1	5	2	0	2.0 (1.5-2.7)*
5 μg/h FSH	0	3	0	3	3.7 (1.8-12.6)**
0.1 mg/h F.S.H.-P	1	3	0	3	2.6 (1.5-5.3)**
0.5 mg/h F.S.H.-P	1	2	0	4	3.2 (1.6-9.6)**

Data were transformed to reciprocals for analysis and mean values are back transformed means. Ninety five % confidence limits are given in parentheses. Asterisks indicate that mean values are significantly different from control values. Ovulation rates did not differ significantly between the different FSH and F.S.H.-P treatments.

testosterone to oestradiol-17β (aromatase activity) and to produce cAMP in response to challenge with LH or FSH (Table 2). No significant effect of bFF on any of these characteristics was observed in small or medium sized follicles.

2.5 μg/h or 5.0 μg/h for 48 h from the initiation of luteolysis significantly increased mean ovulation rates (Table 1). Interestingly, F.S.H.-P (a crude FSH preparation having considerable contamination with LH-like activity), which is commonly used for super-ovulation, was also effective in increasing mean ovulation rates, but at much higher dosages than ovine FSH. The lowest dose of F.S.H.-P which significantly increased the mean ovulation rate was 0.1 mg/h, whereas just 2.5 μg/h of ovine FSH was effective.

While raising mean plasma concentrations of FSH during the preovulatory period increased mean ovulation rates (Table 1), administration of bFF to reduce mean plasma FSH concentrations below normal was deleterious to the proper development of large follicles. (Previous studies have shown that the effects of bFF treatment can be over-ridden by simultaneous administration of FSH or PMSG (Henderson *et al.*, 1986; McNatty *et al.*, 1985; McNeilly, 1985). Thus the bFF likely acts primarily through reducing plasma levels of FSH). Granulosa cell proliferation, follicular

DISCUSSION

Both FSH and LH are essential for antral follicle development. Although plasma concentrations of LH in sheep increase during the later stages of follicular maturation (Baird and Scaramuzzi, 1976; Baird *et al.*, 1981) those of FSH decrease (Fig. 1; Baird *et al.*, 1981; Miller *et al.*, 1981). Granulosa cells are the target cells for FSH action in the ovary, and recent studies have shown that as follicles enlarge, the granulosa cells become more sensitive to FSH (Henderson *et al.*, 1985). This may be important in ensuring that final maturation of follicles can occur in spite of declining plasma FSH concentrations. Large ovulatory follicles(s) may be protected from the deleterious effects of low plasma FSH concentrations by the increased sensitivity to FSH of the granulosa cells, thereby permitting the follicle(s) to continue to respond to FSH, and so continue development. Follicles not having acquired this increased sensitivity to FSH, when plasma FSH levels decline, likely undergo atresia due to lack of FSH. By this reasoning, one may argue that additional follicles could be brought to ovulatory maturity in synchrony by raising plasma concentrations of FSH during the preovulatory period, thereby increasing follicular exposure to FSH. This was found to be the case as hourly administration of ovine FSH at doses of

TABLE 2 Effect of bFF treatment on properties of large ovine follicles.

	No. of granulosa cells/follicle ($\times 10^{-6}$)	Oestradiol-17β in follicular fluid (ng/ml)	Aromatase activity (ng oestradiol-17β/ 10^6 cells)	Stimulation of granulosa cell cAMP production (pMol/ 10^6 cells) by LH	FSH
Controls	5.3	43	5.1	10.0	6.5
N = 8	4.2-6.4	20-95	2.1-11.0	5.6-17.4	4.6-9.1
bFF treated	3.1**	12*	1.2*	1.3***	0.6***
N = 10	2.0-4.2	6-25	0.2-3.9	0.3-3.1	0.2-1.1

Values are arithmetic means (granulosa cell numbers) or geometric means (others) with 95% confidence limits below. Asterisks indicate that mean values are significantly different from controls.

oestradiol-17β production and granulosa cell responsiveness to FSH and LH, are each FSH-dependent events necessary for proper follicular development, and all were reduced in large follicles of bFF treated ewes (Table 2). The observed ovarian consequences of bFF treatment are thus consistent with inadequate follicular exposure to FSH. The failure of bFF treated ewes to display oestrus at the expected time after cloprostenol treatment (~48 h) is also consistent with impaired development of large follicles. Behavioural oestrus is an event dependent upon the large amounts of oestradiol-17β produced by a follicle as it attains ovulatory maturity.

The results of this study are consistent with the view that plasma concentrations of FSH during the preovulatory period may be critical in determining the number of follicles that can normally achieve ovulatory maturity in sheep. Raising plasma FSH concentrations during this period, thereby increasing ovarian exposure to FSH, allows an increased number of follicles to achieve ovulatory maturity in synchrony. In contrast, reducing plasma FSH concentrations during the preovulatory period prevents follicles from attaining ovulatory maturity.

The normal decline in plasma FSH concentrations during the preovulatory period occurs as a consequence of the negative feedback effects of follicular oestradiol-17β and inhibin. One approach to increasing ovulation rates and hence fecundity in ewes might be to attempt to reduce the negative feedback effects of these 2 compounds, particularly that of inhibin thereby possibly raising plasma FSH concentrations.

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