# Ram-induced inhibition of plasma follicle-stimulating hormone (FSH) concentrations in anoestrous Romney ewes

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## ABSTRACT

The aim of the present study was to examine the influence of the ram on plasma FSH concentrations in progesterone-primed Romney ewes which were: (1) anoestrous; (2) devoid of ovaries; (3) or anoestrous but induced to ovulate by a pulsatile injection regimen of luteinising hormone (LH).

The results showed that there was an abrupt fall in the mean FSH levels in anoestrous or LH-pulsed ewes at 1 hour after ram introduction and that this level of suppression (~40%) was maintained for ~ 22 hours in anoestrous ewes and for 12 hours in LH-pulsed ewes. Also, an FSH reduction (~20%) was observed in the ovariectomised ewes. In the anoestrous ewes, the FSH changes were observed without any associated oestrous or ovulatory behaviour. In the LH-pulsed group, all ewes showed oestrus and 75% produced normal corpora lutea.

Collectively, the evidence suggests that when atempts are made to achieve fertile matings in hormone-treated anoestrous ewes, there may be advantages in delaying ram introduction until ~ 24 hours after progesterone withdrawal.

**Keywords** Plasma FSH; sheep; ewes; rams; oestrus induction.

## INTRODUCTION

Follicle-stimulating hormone (FSH) is known to influence ovarian follicle viability, follicular oestradiol synthesis and ovulation-rate (Hudson *et al.*, 1985). High concentrations of FSH are stimulatory whereas low concentrations are inhibitory. Recently, it was reported that rams may have a rapid and long-lasting inhibitory influence on plasma FSH concentrations in ewes during mid-anoestrus (Atkinson and Williamson, 1985). These authors showed that the FSH concentrations fell within 2 hours of ram introduction and thereafter remained low for some 20 days. These findings raise the possibility that in addition to the stimulatory effects of rams on ovulatory behaviour in ewes (see Knight (1983) for review), there may also be some inhibitory influences of the ram on ovarian function. For example, Boland and Gordon (1970) noted a marked improvement in conception rates when the introduction of rams to anoestrous progesterone/PMSG-primed ewes was delayed until 48 hours after progesterone withdrawal.

The aim of the present study was to examine the influence of the ram on plasma FSH concentrations in progesterone-primed Romney ewes which were: (1) anoestrous; (2) devoid of ovaries; (3) or anoestrous but induced to ovulate by a pulsatile injection regimen of luteinising hormone (LH).

## MATERIALS AND METHODS

Parous Romney ewes (aged 3 years; 54 to 60 kg) were studied during anoestrus (November-January). The animals were housed indoors and fed a mixture of lucerne hay and pellets with water being provided *ad libitum*. All animals were treated for 12 days with an intravaginal sponge containing 60 mg medroxyprogesterone. On the day before sponge withdrawal, the ewes were cannulated via a jugular vein for regular blood sampling purposes. The sponge was withdrawn at 0800 h on the day blood sampling began. During the blood sampling intervals, the animals were exposed to continuous fluorescent lighting in excess of 300 lux.

In Experiment I (November, 1984) the ewes (n = 10) were blood sampled (2 ml/sample) hourly for 60 hours commencing at 2000 h. In Experiment II (November/December, 1984), the ewes (n = 8) were sampled as above and 2 sexually active vasectomised Dorset rams fitted with marking harnesses were introduced after 18 h of sampling at 1315 h for the remainder of the experiment. In Experiment III (January, 1985), ovariectomised ewes (n = 10) were sampled as with Experiment I with the above 2 rams being introduced to the ewes after 18 h of sampling at 1315 h. These ewes had been ovariectomised 12 to 15 months before the present experiment.

In Experiment IV (January/February, 1985), the ewes (n = 8) were subjected to an LH pulse injection regimen to induce ovulation as previously described (McNatty *et al.*, 1981). Briefly, 10 g ovine LH (NIH-LH-S15) in isotonic saline + 0.1% ovine serum albumin were injected intravenously hourly for 24 h starting at 0800 h (i.e., at sponge withdrawal), half-hourly during the next 24 h and then once every 20 minutes for a final 24 h. Hourly blood samples were collected for 52 h from 2000 h on the first day of LH treatment. The vasectomised Dorset rams used in Experiments II and III were introduced at 1315 h after 18 h of blood sampling. At the end of the intensive blood sampling periods, all animals were blood sampled daily for 20 days.

Oestrous activity was monitored hourly while the animals were indoors. Seven to 10 days after the end of intensive blood sampling, all ewes were laparoscoped to record the presence or absence of corpora lutea. All blood samples were collected into heparinised tubes, centrifuged, and the resulting plasmas recovered and frozen at -20° C until analysis for either progesterone (McNatty *et al.*, 1981) or FSH (NIAMDD ovine FSH assay kit) by radioimmunoassay (RIA). The minimum detectable level of progesterone was 0.2 ng/ml. The intra- and interassay coefficients of variations were 10.6 and 14.0% respectively. The FSH detection limit was 0.2 ng/ml. The FSH intra-and interassay coefficients of variation were 6.3 and 9.6%.

For each experiment (I to IV) the mean FSH concentration for each ewe during the first 18 h (i.e., before ram introduction in Experiments II-IV) was determined. This mean value (Xpre) was then used to calculate the proportion of the mean for each subsequent observation (i.e., Xprop = Xactual/Xpre). Resistant lines (Emerson and Hoagland, 1983) were then fitted to both the pre-and post-treatment data for each ewe. Subsequently, for each experiments, the slopes and intercepts of the pre- and post-treatment data were compared by Students *t*-test.

## RESULTS

The mean percentage changes in FSH concentration compared to the first 18 hours of sampling are shown in Fig. 1 (the vertical bars indicate the ranges). In Expts. I, II, III and IV, the respective mean (± s.e.m.) FSH concentrations over the first 18 h were 2.1 (±0.3), 1.8 (±0.3), 18.5 (±3.0) and 2.5 (±0.3) ng/ml. In Expt. I, the fitted resistant lines for the Xpre and Xpost values had slopes and intercepts which were not different from one another indicating that the pattern of FSH secretion after the first 18 h was not different from that during the first 18 h. None of these control animals ovulated and the plasma progesterone concentrations for 20 consecutive days after the end of the intensive blood samplings were not detectable (i.e., $<0.2$ ng/ml).

In Expt. II, the fitted resistant lines for Xpre and Xpost had similar slopes, but the intercept value for Xpost was significantly lower than for Xpre ($P<0.01$), indicating that ram introduction significantly lowered the plasma concentrations of FSH. The data in Fig. 1b indicate that at 1 h after ram introduction the plasma FSH concentrations were ~ 60% of those beforehand and that these lowered values were maintained for 20 h.

In Expt. III, the fitted resistant lines for Xpre and Xpost had similar slopes but the intercept value for Xpost was significantly lower than for Xpre ($P<0.05$) indicating that ram introduction had lowered the plasma concentrations of FSH in the ovariectomised ewes. After ram introduction, the mean FSH concentrations were between 56 and 98% of those beforehand for about 34 h (Fig. 1c).

In Expt. IV, the fitted resistant lines had a significantly higher slope ($P<0.01$) and a significantly lower intercept ($P<0.01$) after ram introduction compared to those beforehand. In these animals there was a 50% reduction in the mean FSH concentration after ram introduction for 12 h followed by marked increases in concentration for the next 22 h (Fig. 1d). In this experiment, all the ewes were in oestrus between 17 and 48 h after ram introduction. All animals produced at least one corpus luteum (CL) (6 ewes, 1 CL; 2 ewes, 2 CL). All but 2 of these ewes appeared to have normal functioning CL since the plasma progesterone levels in those assessed to be normal were in excess of 1 ng/ml for at least 6 of the first 17 days after the expected day of ovulation. In the other 2 animals (1 CL/ewe), the CL were apparently not normal since the plasma progesterone values were in excess of 1 ng/ml for only 3 of the first 17 days after the expected day of ovulation (McNatty *et al.*, 1981).

## DISCUSSION

These data confirm the findings of Atkinson and Williamson (1985) that the introduction of rams to progesterone-primed anoestrous ewes causes a rapid suppressive effect on FSH secretion. The finding that FSH suppression also took place in ovariectomised ewes suggests that the ram effect is mediated via the central nervous system and is, at least in part, independent of alterations in brain sensitivity to steroid-feedback.

The abrupt reduction in FSH concentrations in the ewes being given LH injections (Expt. IV) after ram introduction is also further evidence of a potentially negative influence of rams on hormonally-treated anoestrous ewes. In this study LH therapy began some 29 hours before ram entry and 75% of the animals produced functionally normal CLs. In other studies using gonadotrophin-releasing hormone together with bovine follicular fluid in anoestrous ewes, the consequent reduction in FSH secretion resulted in anovulation (McLeod and McNeilly, 1985). When these data are considered with those of Boland and Gordon (1970; see introduction), it would seem to be advantageous to delay ram entry to hormone-treated anoestrous ewes for at least 24 hours after progesterone withdrawal.

Finally the measurement of plasma FSH concentrations in anoestrous ewes may be a useful method for monitoring male pheromonal substances on the reproductive physiology of female sheep.

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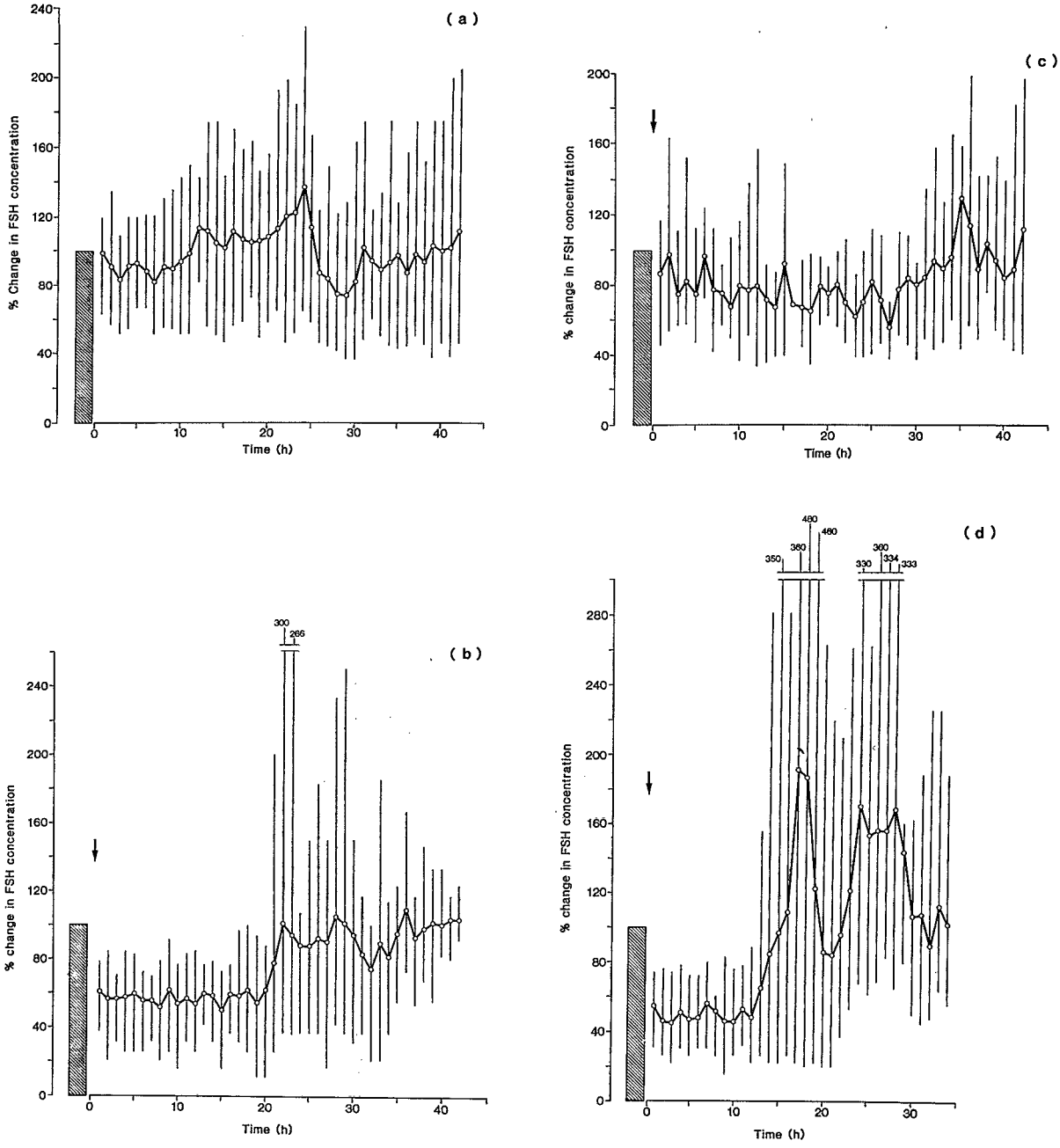


**FIG. 1** Changes in plasma FSH concentrations expressed as mean percentages with respect to the mean levels for the preceding 18 h (indicated by histogram) for anoestrous ewes not exposed to rams (n = 10; Fig. 1a) or anoestrous ewes (n = 8; Fig. 1b), ovariectomised ewes (n = 10; Fig. 1c) or LH-plused ewes (n = 8; Fig. 1d) exposed to rams at time indicated by arrow. Vertical bars indicate the ranges.

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