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## Effect of oestradiol implants and protein nutrition on plasma FSH levels in ovariectomised ewes

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### ABSTRACT

As part of the study into the mechanism by which nutrition influences ovulation rate two trials have been conducted to examine the effect of protein intake on the negative feedback of oestradiol-17 $\beta$  (E17 $\beta$ ) on plasma FSH levels. In Trial 1 three groups of ovariectomised ewes (n=8) fed on a low protein (LP 11%, 10 MJ ME) diet were treated with silastic implants of E17 $\beta$ . Four days after implant insertion one group was changed to a high protein (HP 22%) diet while another was treated orally for 10 days with phenobarbital (1 g/d). The E17 $\beta$  implants were removed 6 days after treatment started. In Trial 2 groups of ewes (n=6) were given either full (HE) or 1/2 size (LE) implants for 5 days. All ewes were fed on a LP diet but 2 groups were infused abomasally with 100 g/d of protein (HP) commencing 3 days before implant. FSH levels were monitored 4 x d throughout both trials and expressed as a percentage of pre-treatment values. In Trial 1 FSH levels were 33% on day 11 after implant with no treatment difference. However after implant removal the values for the LP ewes returned to 100% by day 17 while for the HP and phenobarbital group values remained at 33%. In Trial 2 the levels 4 days after implant insertion were 37%, and 48% for the HE and LE ewes. Again protein treatment delayed recovery following implant removal. These results do not support the hypothesis that the increased ovulation rate following protein supplementation is due to a reduction in negative steroid feedback on FSH levels.

**Keywords** FSH, protein nutrition, oestradiol, ovariectomised ewes, phenobarbital, steroid feedback

### INTRODUCTION

Feeding ewes increased protein in the diet produces an increase in ovulation rate (Smith, 1985). This has been substantiated by increased ovulation rates following abomasal infusion of protein (Cruickshank *et al.*, 1988). Smith (1988) reported that the increased ovulation rate was preceded by increased levels of FSH in the later luteal phase of the cycle.

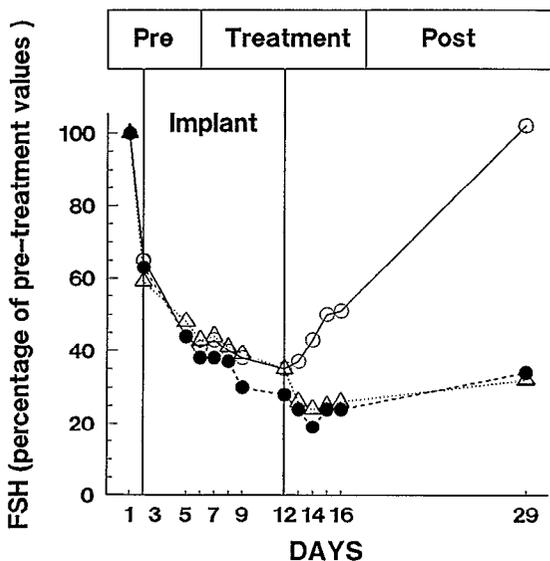
However the mechanism by which this is achieved remains unknown. Two possibilities exist, with increased nutrition producing increases through a direct effect on the pituitary gland or via an indirect effect by alteration of the negative oestrogen feedback system. This paper reports on two experiments undertaken to test the hypothesis that increased protein intake increases FSH by interfering with the oestrogen negative feedback system.

### MATERIALS AND METHODS

#### Trial 1

Twenty-four Coopworth ewes that had been ovariectomised for at least 2 years were housed in individual pens fed on a low protein (11%) pelleted ration at 1.0 kg/d providing 10 MJ ME. Silastic implants (3 mm OD, 1 mm ID and 37 mm in length) containing oestradiol-17 $\beta$  were placed subcutaneously in the wool free area of the pectoral region. Four days after implantation the ewes were divided into 3 groups (n=8). One group remained on the low protein diet, a second group was changed to a high protein ration (22%; 1 kg/d), while the third group remained on the low protein diet but were dosed orally with phenobarbital (1 gm/d) for 10 days. Six days after start of treatment the oestrogen implants were removed. Ewes were bled by

vacutainer 4 x d (0930 h, 1130 h, 1330 h and 1530 h) from 2 days before implantation until 4 days after implant removal and again 14 days later.



**FIG 1** Effect of a low protein diet (o—o), a high protein (•—•) and phenobarbital treatment (Δ....Δ) on mean plasma levels of FSH (expressed as a percentage of pre-treatment values) in ovariectomized ewes during and after treatment with silastic implants containing oestradiol.

## Trial 2

In this experiment 24 Coopworth ewes selected as ovulatory responders to lupin supplement and subsequently ovariectomized and fitted with abomasal catheters (Cruickshank *et al.*, 1990) were used.

Ewes were housed in individual pens and fed on a low protein diet (11%) at 1 kg/d. Ewes were randomly allocated to 4 groups (n=6). Two groups of ewes were infused with 100 g protein (Alacen-whey protein concentrate; N.Z. Dairy Board) per day via the abomasal catheter (1 litre/24 h) while the other two groups were infused with water (1 litre/24 h). Two days after the infusions commenced silastic implants containing oestradiol-17 $\beta$  were inserted. One group on each of the nutrition treatments were given full implants (3 mm, OD; 1 mm, ID; 37 mm long; high oestrogen) while the other groups received half implants (3 mm OD; 1 mm;

ID; 18 mm long; low oestrogen). Five days later the implants were removed and a further 5 days later the abomasal infusions were terminated.

Ewes were bled 4 x d (0930 h, 1130 h, 1330 h and 1530 h) from 2 days before infusions commenced until 3 days after infusions ceased and then once daily each Monday, Wednesday and Friday for a further 50 days.

## FSH assays

FSH was measured using a homologous RIA kit kindly provided by the National Hormone and Pituitary Programme of the University of Maryland School of Medicine. The FSH standard was NIAMDD-RP-1, iodinated NIADDK-oFSH-I-1 was used as tracer and the antibody was rabbit anti-oFSH-RP-1. The mean within and between assay coefficients of variation were 8.4 and 17.3% respectively over the working assay range of 0.5 to 6.0 ng/tube.

For Trial 1 equal volume aliquots of plasma from each of the 4 samples on any one day were combined and then an aliquot of the combined sample used for FSH measurement. In Trial 2 all individual samples were assayed. Because of the high values anticipated in ovariectomized ewe plasma a 1:2 dilution of plasma was used in the assay.

## Statistical analysis

FSH values were all subjected to log transformation prior to analysis. The pre-treatment values were used as a covariate in the analysis. In Trial 1 analyses of variance were performed using the Genstat programme while in Trial 2 in addition to the analysis of variance the slope and shape of the response within each experimental treatment phase was analysed using the REML (Analysis by residual maximum likelihood) programme. The data are expressed as the mean of the percentages of the pre-treatment value with all pre-treatment values expressed as 100 percent.

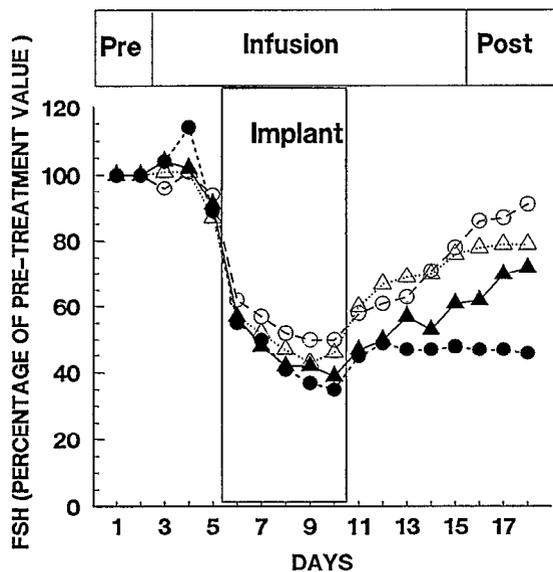
## RESULTS

### Trial 1

Oestrogen implants lowered ( $P < 0.05$ ) the FSH levels to 62% within 1 day and to 55% within 4 days. At day 11

FSH levels were reduced to 37% with no difference between the 3 treatment groups. Following oestrogen implant removal the FSH levels of the ewes on the low protein diet rose and had reached 51% of pre-implant levels within 4 days and by 17 days after implant had fully recovered. This was significantly different ( $P < 0.05$ ) from that seen in the ewes on the high protein and phenobarbital treatments which failed to show a post-implant increase in FSH (Figure 1).

## Trial 2



**FIG 2** Mean FSH levels (expressed as a percentage of pre-treatment values) in groups of ovariectomized ewes fitted with two sizes of silastic implants containing oestradiol (low,  $\Delta$ ; high,  $\circ$ ) infused with either protein (solid) or water (open), before, during and after implant removal.

Though there was no effect of infusion prior to oestrogen implant, the insertion of oestrogen implants significantly lowered the FSH levels compared to pre-treatment values ( $P < 0.01$ ). There was no significant differences between infusion treatments or size of implant in this period, although the ewes receiving the abomasal protein had the greatest depression (Figure 2). After implant removal the FSH values tended to rise and there was a significant ( $P < 0.05$ ) interaction between infusion treatment and implant size (Figure 2). The ewes in the

protein infusion - high oestrogen implant groups failed to show a recovery of FSH levels. The two water infused groups showed a rapid recovery while the protein infused-low oestrogen implant had an intermediate slope. By day 31 however all ewes had shown a recovery in their FSH levels to pre-treatment values.

## DISCUSSION

The results of these trials support the findings of McNatty *et al.*, (1989) and others that subcutaneous implants of oestradiol-17 $\beta$  lowers the FSH levels in the ovariectomized ewe and confirm the negative feedback effect.

The depression in FSH levels caused by oestradiol implants in the ovariectomized ewe to between 30 and 40% of the pre-treatment values was considerably greater than the depression seen in entire ewes (to 75% of pre-treatment value, Payne *et al.*, 1990) treated with the same size implants even though the absolute values were higher in the ovariectomized ewes.

The high values of FSH seen in the ovariectomized ewe may be due to the change in clearance rates of FSH seen after ovariectomy, where the half-life has been shown to increase 10 fold (Fry *et al.*, 1987). It has been assumed that this is due to change in the structure (level of sialic acid content) of the FSH (Peckham and Knobil, 1976). If oestrogen levels do influence the structure of FSH then the negative feedback effect in the ovariectomized ewe may be as much an effect on clearance as it is on secretion and release. There was no difference between level of oestrogen (size of implant) on the extent of depression of FSH and this is in agreement with other reports (McNatty *et al.*, 1989).

The lack of any significant difference between nutrition treatments, in both trials, while the oestradiol implants were inserted is in agreement with findings of Adams and Atkinson (1988) and Ritar and Adams (1988). However it is in contrast to that reported by Wright *et al.*, (1988). The present results show that a doubling of the protein intake of the animal, either by changes in composition of the feed or via abomasal infusion of protein did not reduce the negative feedback effect of oestradiol. In addition the inhibition of the post-implant recovery in FSH levels in ewes exposed to increased protein intakes lends further support to the

conclusion that the effect of protein on increasing ovulation rate is not via a reduction in the negative feedback of oestradiol.

The inhibition of recovery in FSH after implant removal is most unusual. The published data available on post-implant recovery of FSH levels is scant. Webb *et al.*, (1985) reported a similar rate to that seen in the low protein ewes and a logical explanation for the inhibitory effect of protein is difficult to find. Two possible mechanisms are that increased protein intake *either* (1) increases the circulating levels of the aromatic amino acids which are precursors to a number of neurotransmitters and an increase in the level of these may inhibit the release of GnRH once oestrogen suppression has been removed *or* (2) increases the rate of metabolism of FSH by the liver. The difference between Trials 1 and 2 in eventual recovery is probably due to the fact that in contrast to Trial 1 where ewes were put onto the high protein diet and maintained on this diet, ewes in Trial 2 reverted to a low protein diet at the end of the infusion period.

These results coupled with the findings of Cruickshank *et al.*, (1990), who found no effect of protein infusion on FSH in the ovariectomised ewe (without oestrogen implants), indicate that the most likely route of action of nutrition on increased ovulation rate is at the ovarian level. This could be either a direct effect of the branch chained amino acids (Waghorn and Smith, 1990; Downing *et al.*, 1990) or an effect on the ovary through stimulation of a metabolic hormone.

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