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Studies into the mechanisms by which nutrition influences ovulation rate: use of the ovariectomized ewe model.

G.J. CRUICKSHANK, J.F. SMITH, J. KONLECHNER AND J. PARR.

MAF Technology, Ruakura Agricultural Centre, Private Bag, Hamilton

ABSTRACT

Twenty four Coopworth ewes, selected on the basis of an increased ovarian response to lupin grain supplementation, were ovariectomised and fitted with a Foley catheter in the abomasum. They were individually penned and offered a low protein pelleted diet at maintenance levels. Following a five week stabilisation period 12 ewes were infused abomasally with 100g/d whey protein concentrate in 1 litre of water for 8 days. Twelve ewes received 1 litre of water only. Blood samples were collected four times daily for FSH analysis, from 2 days before until 2 days after infusion.

Protein infusion did not influence FSH levels. Mean values were 8.5 ngFSH/ml plasma for both treatments, and means for individual ewes ranged from 4.5 to 11.5 ng/ml. These results indicate that the reported increase in FSH with increased protein intake is not due to a direct effect on the pituitary gland.

Keywords Ewe, ovariectomy, FSH, protein, ovulation rate.

INTRODUCTION

Flushing of ewes to cause an increase in ovulation rate is well established and the effect can occur following as little as 6-7 days prior to ovulation (Stewart and Oldham, 1986; Nottle *et al.*, 1986). The mechanism by which ovulation rate may be modified following increased intake could involve increased protein, or amino acid, absorption (Smith, 1985; 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 oestrous cycle. Others have reported similar findings (Brien *et al.*, 1976; Davis *et al.*, 1981; Knight *et al.*, 1981) although Radford *et al.* (1980), Scaramuzzi and Radford (1983) and Ritar and Adams (1988) failed to record an increase in FSH in conjunction with increased ovulation rate after lupin feeding. Increases in ovulation rate have been observed following FSH administration during the late luteal phase of the cycle (Henderson and McNatty, 1982) and twinning ewes have been shown to have higher FSH levels 15 days before ovulation (McNatty *et al.*, 1985). These findings strongly support the suggestion that increased levels of circulating FSH are involved in the nutritional control of ovulation rate. However, it is not known at

what point nutrients exert their effects on FSH levels.

The interaction between FSH production and ovarian hormones (oestradiol-17 β and inhibin) and the discrete variable of ovulation number limit the application of *in vivo* studies. For these reasons we have studied ovariectomized ewes, using plasma FSH levels as an indicator of potential ovulation rate. Initial screening of ewes for ovulatory response to lupin supplementation was carried out to minimise the possibility that non-responders were included in the experimental programme.

In the experiment reported here the hypothesis was tested that protein exerted its influence on FSH through a direct effect on the hypothalamic-pituitary axis. Previous data (Waghorn, 1986) have shown that increased protein intake was associated with an increase in plasma concentrations of aromatic amino acids, particularly phenylalanine and tyrosine, and that plasma concentrations of these amino acids were strongly correlated with predicted ovulation rate in ewes. Aromatic amino acids are precursors for neurotransmitters (dopamine, norepinephrine, epinephrine, serotonin and 5-hydroxytryptamine) which could play a role in modifying FSH production.

MATERIALS AND METHODS

Animals

148 two year old Coopworth ewes were offered a pelleted low protein diet at maintenance levels for one complete, synchronised, oestrous cycle. Ovulation rate was measured by laparoscopy and 0.5 kg lupin seed substituted for 0.5 kg pellets. Laparoscopy was repeated following the second cycle and 25 ewes which had shown an increased ovarian response were selected for the experimental programme. These were ovariectomised and fitted with a Foley catheter in the abomasum.

Housing and feeding.

Surgically modified animals were housed in individual pens and offered, at 08.00h, 1.0 kg fresh weight of a pelleted ration calculated to provide 9.5 MJME and 100 gCP/kg DM. Average post-surgery liveweight was 43.1±0.53kg.

Infusions and sampling.

Whey protein concentrate (Alacen; New Zealand Dairy Board) was infused at a rate of 100g/d in 1 l water per sheep, following the procedure of Cruickshank *et al.* (1988). Controls received 1 l water only. Infusions were started at 11.00h daily for 8 days. Blood samples were obtained by jugular venepuncture four times daily for 12 days, being the 8 days of infusion plus 2 days pre- and post- infusion.

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-o-FSH-I-1 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. Because of the high values anticipated in ovariectomised ewe plasma a 1:2 dilution of plasma was used in the assay.

Statistical analysis

FSH values were subjected to log transformation prior to analysis. Data are presented as retransformed means. Analysis of variance was performed using the pre-infusion values as covariates.

RESULTS

Animal selection. 119 ewes had a single ovulation at the first, low protein, cycle. Of these 18 (15%) had two ovulations following lupin supplementation and 26 had a single ovulation plus a large, unruptured follicle. Seven of the latter group were selected, along with the twin ovulators, for further experimentation.

Experimental.

Average liveweight gain between housing and the end

TABLE 1 Retransformed mean plasma FSH levels (ng/ml) and as a percentage of day 1 values. Animals (n = 12 for each treatment) were infused with protein or water from day 3 to day 10.

day	1	2	3	4	5	6	7	8	9	10	11	12
Protein infused												
FSH	8.5	8.2	8.3	8.0	8.0	8.0	7.8	7.8	8.0	8.1	8.4	8.6
%	100	96.2	97.3	94.2	94.3	93.8	92.2	92.0	93.6	94.9	99.4	101
Water infused												
FSH	7.6	7.4	7.1	7.1	7.4	7.2	7.5	7.7	7.6	7.6	7.7	7.8
%	100	96.9	92.9	93.3	96.6	94.5	98.5	100.6	99.6	99.9	100.6	100.4

of the experiment was $5.3 \pm 5.62 \text{ g/d}$. The FSH levels of ewes infused with protein did not differ significantly from those infused with water only (Table 1). The retransformed mean FSH values for the 12 days of sampling were 8.1 and 7.5 ng/ml for protein and water infusions respectively.

DISCUSSION

Selection of ewes on the basis of an ovarian response to lupin supplementation should ensure that all experimental animals had the capacity to respond physiologically to nutritional manipulation. Although it was not possible to distinguish between nutritionally induced ovulation and natural seasonal variation the procedure should maximise the responsiveness of the experimental animals. The proportion of animals showing a response to lupin response was lower than observed elsewhere (Knight *et al.*, 1975; Nottle *et al.*, 1988), possibly due to the lower level of supplementation used in the present experiment. There were a large number of ewes exhibiting large, unruptured follicles which suggest that the level of supplementation was slightly less than required to fully realise the potential for twin ovulations. Had these animals shown two ovulations the response to lupin supplementation would have been similar to other studies.

The ovariectomised ewe model is a valuable technique for the study of the physiological regulation of reproduction. By removing the influence of ovarian hormones (oestrogen and inhibin) the full potential for increased FSH can be realised. Subsequent treatment, at a controlled release rate, with oestrogen and/or inhibin (Smith *et al.*, 1990) allows greater experimental control over FSH production. Any effects of nutritional manipulation will then be magnified in comparison with those in entire ewes.

The absence of an FSH response to increased protein absorption in the present experiment agrees with the observation of Wright *et al.* (1988) but not with Nottle *et al.* (1988). The latter authors reported an increase in FSH in ovariectomised ewes within 24h of lupin supplementation and suggested that this was due to a direct effect on the brain.

The conclusion from this study is that protein induced increases in ovulation rate are not mediated through a direct effect on the hypothalamic-pituitary axis.

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