

## Nutrition of the Ewe and Embryo Growth during Early Pregnancy

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### Abstract

Mature Merino ewes ( $n = 228$ ) were fed rations at levels of either 5, 10, 15, 20, 30 or 40 g/kg daily from day 1 until day 35 after mating. Embryos were then removed from single ovulating ewes and, after being weighed and measured, were dissected and embryo liver weights were obtained. Plasma samples were taken from all ewes on days 5, 10, 15, 20, 27 and 35 after mating and later analysed for glucose concentrations.

During the 35-day treatment period, mean liveweight changes of ewes ranged from  $-6.9$  to  $5.5$  kg in groups receiving the lowest and highest levels of nutrition respectively. Although there were no significant differences in the percentages of ewes pregnant at day 35 the weight and size of embryos, including embryo liver weight, varied with the level of nutrition. Mean wet weights ( $\pm$  s.e.m.) of embryos from ewes receiving rations at the rate of 10 g/kg daily and those receiving 30 g/kg daily were  $1.67 \pm 0.04$  g and  $1.91 \pm 0.04$  g respectively (difference significant at  $P < 0.01$ ). Plasma glucose concentrations of the ewes increased with increasing level of ration and decreased with time.

### Introduction

Investigations involving the feeding of high or low levels of rations to ewes during the first weeks of pregnancy have usually focused upon embryonic mortality (Edey 1976). El-Sheikh *et al.* (1955), Foote *et al.* (1959) and Hulet *et al.* (1969) failed to detect significant effects on embryonic growth of different levels of nutrition offered during early gestation. In contrast Parr *et al.* (1978) found that single embryos removed from ewes undernourished from mating until day 21 of gestation were retarded in growth and development compared with those fed at a higher plane of nutrition for the same period. Williams *et al.* (1978) also demonstrated retardation of size and development in 25-day-old single embryos removed from ewes fed one-third maintenance rations for the first 16 days of gestation. In addition, they found that ewes fed twice maintenance rations tended to have smaller embryos which were less well developed than those fed maintenance rations; however, these differences failed to reach significance. The first 35 days of gestation in the sheep represent a period of rapid specific growth (Robinson and McDonald 1979) and important organogenesis (Green and Winters 1945). It is not known how nutritional extremes during this period influence embryonic and organ growth.

Ellington (1980) reported the beneficial influence of glucose added to *in vitro* culture sera on the growth of rat embryos during organogenesis. Because plasma

glucose concentrations decrease when ewes are undernourished (Reid and Hinks 1962*b*) the availability of glucose might influence embryonic growth in sheep. Little importance has been placed on glucose in sheep nutrition because of its reported inaccuracy as an indicator of nutritional status (Reid and Hinks 1962*a*). In contrast, recent studies have shown that glucose concentrations in the blood are a sensitive indicator of nutritional status (Parr and Williams, unpublished data).

In this paper maternal blood glucose concentrations were monitored and embryo size measured in groups of ewes offered a wide range of nutritional levels during the first 35 days of gestation to ascertain whether a relationship existed between nutritional level and embryo size, and whether blood glucose levels reflected this relationship.

## Materials and Methods

### *Experimental Animals*

Mature Merino ewes ( $n = 228$ ) together with vasectomized rams were grazed on dry annual pastures for 8 weeks prior to the start of the joining period when the mean liveweight ( $\pm$  s.d.) of the flock was  $41.3 \pm 4.0$  kg. The ewes were maintained with body condition scores of 2-3 (Jefferies 1961) and were supplemented with the pelleted diet which was to be used during the experimental period.

### *Nutrition*

Ewes were ranked in descending liveweight and allotted at random to six nutritional groups (A-F). During the experimental period, they were offered daily either 5, 10, 15, 20, 30 or 40 g/kg of the pelleted ration respectively. No other feed was offered. The ration consisted of a pelleted 1 : 1 (w/w) mixture of hammer-milled lucerne hay and crushed barley grain. The pellets contained 10.9% crude protein, 87% dry matter and 12.6 MJ/kg dry matter metabolizable energy.

### *Experimental Program*

In early February 1979, vasectomized rams were replaced with fertile Merino rams fitted with mating harnesses (Radford *et al.* 1960). Vaginal smears (Killeen 1974) were taken from any ewes marked during the previous 24 h, and examined for the presence of spermatozoa. Inseminated ewes were transported 2 km the following day to an experimental sheep shed where they were weighed and condition scored (Jefferies 1961) on days 1, 5, 15 and 35 after mating. If spermatozoa were not evident in the first vaginal smear taken from the marked ewes a second vaginal smear was taken the next day. If this smear was positive, the ewe was taken to the animal house on that day. Inseminated ewes were penned individually in the animal house and fed their allotted daily rations until 35 days after mating. Feed residues were weighed each day. On day 35 each ewe was anaesthetized by intravenous injection of 800 mg of Intraval (sodium thiopentone, May & Baker, Australia). A mid-ventral incision was made and the reproductive tract was exteriorized. Corpora lutea were counted and any embryos were removed by hysterotomy. After recovery from surgery, ewes were returned to pasture. Pregnancy rates were defined as the percentage of ewes pregnant at day 35 of the total number of ewes inseminated by the ram in each nutrition group. Embryos from the few twin-ovulating ewes ( $n = 22$ ) were subsequently discarded. Single embryos were examined for viability, measured under  $\times 12$  magnification and weighed; finally the liver was dissected out and weighed.

### *Blood Sampling and Glucose Determinations*

Blood samples were taken by jugular venipuncture from each ewe at 9.00 a.m. on days 5, 10, 15, 20, 27 and 35 after mating. Samples were collected in heparinized tubes and were immediately placed in ice which inhibited glycolysis (Parr, unpublished data). They were centrifuged under refrigeration and the plasma stored at  $-20^{\circ}\text{C}$  for subsequent glucose determinations. Plasma

glucose concentrations were measured by the glucose oxidase-phenylaminophenazone (Boehringer) method (Trinder 1969) with a Technicon Autoanalyser. The coefficient of variation of 44 sets of standards measured during the assay was  $\pm 1.89\%$ .

#### Analysis of Data

Embryo and liver size data were analysed using single classification analysis of variance with unequal sample sizes (Sokal and Rohlf 1969) and a modified multiple range test (Kramer 1956). These data were also subjected to regression analysis. Glucose data were analysed by means of least-squares analysis (Harvey 1960) using a model which included nutrition, day after mating, pregnancy rate and two-way interactions. Pregnancy rates at day 35 after mating were analysed using a  $\chi^2$  test.

#### Results

Groups A–D consumed their full rations during the 35-day period; groups E and F consumed a daily mean of 28 and 37 g/kg respectively. Results for 16 ewes from group F and four from group E were discarded as these ewes ate less than 90% of the ration offered. During the treatment period liveweight changes ranged from a mean of  $-17.7\%$  (group A) to  $+13.1\%$  (group F) of the initial mean liveweight at mating (Table 1). Body condition closely reflected this trend and the feed level required to maintain liveweight (maintenance level) was approximately 18 g/kg liveweight daily. Pregnancy rates ranged from 63.9% (group C) to 80.6% (group D). These differences were not significant ( $\chi^2 = 3.15$ ;  $P > 0.05$ ).

**Table 1.** Mean liveweight change of ewes from day 1 to day 35 after mating and mean fresh weight of embryos at day 35  
Mean values with different superscripts differ significantly ( $P < 0.01$ )

Group	Daily feed intake (g/kg)	Mean initial liveweight $\pm$ s.e.m. (kg)	Mean change in liveweight $\pm$ s.e.m. (kg)	Mean fresh weight of embryos $\pm$ s.e.m. (g)
A	5	41.9 $\pm$ 0.6	-6.9 $\pm$ 0.3	1.71 $\pm$ 0.04 <sup>bc</sup>
B	10	41.7 $\pm$ 0.5	-4.4 $\pm$ 0.3	1.67 $\pm$ 0.04 <sup>c</sup>
C	15	42.0 $\pm$ 0.7	-1.6 $\pm$ 0.4	1.75 $\pm$ 0.04 <sup>abc</sup>
D	19.5	41.8 $\pm$ 0.5	+0.5 $\pm$ 0.3	1.87 $\pm$ 0.04 <sup>ab</sup>
E	27.9	40.8 $\pm$ 0.7	+2.4 $\pm$ 0.3	1.91 $\pm$ 0.04 <sup>a</sup>
F	37.1	40.7 $\pm$ 0.7	+5.5 $\pm$ 0.8	1.83 $\pm$ 0.08 <sup>abc</sup>

Mean fresh weights of 35-day-old embryos in the three nutrition groups which gained liveweight were greater than embryos in the three groups which lost liveweight (Table 1). A maximum difference in fresh weight of 13% was evident between groups B and E. Embryo crown-rump length and embryo liver weight measurements were closely correlated with embryo weight ( $r = 0.83$  and  $0.71$  respectively). The ratio of liver weight to embryo weight was similar for all groups.

Mean glucose concentrations increased as feed level was increased (Fig. 1) and decreased as period of feeding (day of pregnancy) increased ( $P < 0.01$ ). There were significant interactions ( $P < 0.05$ ) in plasma glucose concentrations between nutrition treatments and days, and between nutrition treatments and pregnancy.

Differences between nutrition levels were less evident in the low-nutrition groups with time and pregnant ewes in low-nutrition groups had lower plasma glucose concentrations than non-pregnant ewes. In the high-nutrition groups, this trend was reversed with pregnant ewes having higher plasma glucose concentrations. Overall, mean plasma glucose concentrations were similar between pregnant and non-pregnant ewes.

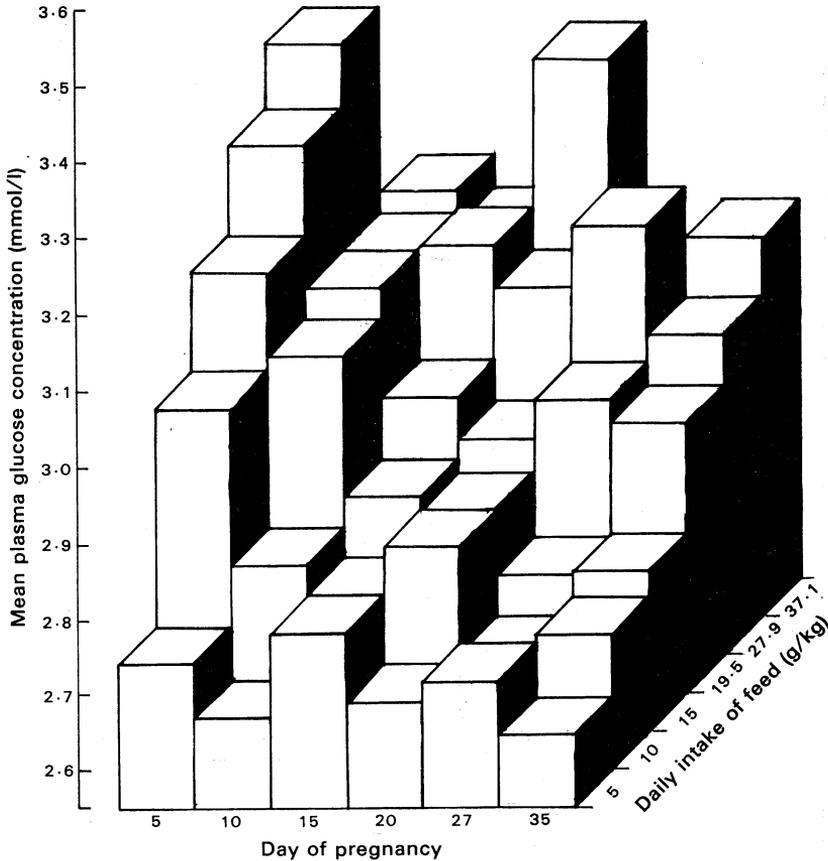


Fig. 1. Mean plasma glucose concentration plotted against mean feed intake and day of pregnancy.

## Discussion

Results showed that maternal undernutrition to day 35 of pregnancy causes live-weight loss and decreases in embryo weight and size. This supports earlier findings of an effect of maternal nutrition upon embryo growth to days 21 (Parr *et al.* 1978) and 25 (Williams *et al.* 1978) of pregnancy. Hulet *et al.* (1969) fed ewes 75, 100 and 150% of maintenance energy requirements from conception to days 21 or 30 of pregnancy. Though differences in the size of embryos from these ewes failed to reach significance, there was a trend of increasing embryo size with increasing level of nutrition. In other work El-Sheikh *et al.* (1955) and Foote *et al.* (1959) failed to show differences in embryo weight or size through differential feeding in early pregnancy. In the former study, ewes were fed on roughage or roughage plus grain

rations which were described as being either 'medium' or 'high' planes of nutrition. Since liveweight changes were not reported it is not possible to determine if liveweight was lost during the treatment period. In the latter study it was shown that growth was limited in young, pregnant ewes fed a restricted ration (hay only). The ewes gained liveweight during the treatment period and produced embryos which were unaffected in size by the nutritional restriction. In our study, post-mating loss in liveweight did occur in three groups which produced embryos with decreased weights. The liveweight loss in the ewes was similar to that which often occurs during drought or when sheep breeders allow liveweight loss in ewes after mating in the belief that it will not be detrimental. It is not known whether embryos with retarded growth to the degree shown in this study are capable of full recovery once the nutritional status of the ewe is improved. The reduction in liver size in embryos could have important consequences in later foetal life. Other organs which develop early, such as the brain, could also be adversely affected. Once organs have passed through sequential phases of development they may be precluded from full compensatory development in later foetal or post-natal life, even if skeletal or muscle tissue is capable of later compensatory gains.

The response of embryos to maternal nutrition evident in this study indicates that a mechanism operates to limit embryonic growth and development when feed is limiting. Ellington (1980) found that rats which had been undernourished for varying periods after mating lost liveweight, their serum glucose concentrations declined, and growth and differentiation of their embryos were retarded. When embryos from untreated rats were cultured in serum from fasted rats, development was also retarded and size decreased unless glucose concentrations in the sera were raised to those of unfasted rat sera. The sheep must synthesize 95% of its glucose via gluconeogenesis (Bergman 1973) and the concentration of blood glucose of the adult ruminant is low compared with the level in adult non-ruminants (Reid 1950). While recognizing the limitations of applying data between species it is postulated that during early pregnancy in the sheep, embryo growth may be controlled by a limitation of the supply of maternal glucose in response to nutritional stress.

In recent years much attention has been devoted to studying the influence on the foetus of nutrition during mid and late gestation. The results of this study suggest that more investigation is required into the influence of nutrition during early gestation on the subsequent growth and development of the foetus and the realization of the animal's full productive potential in later life.

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