THE INTRAUTERINE ENVIRONMENT OF TWIN LAMBS PREDETERMINES THEIR GROWTH CHARACTERISTICS

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Summary

Within-sex variation in birthweight and weaning weight, attributable to the sex of an individual's in utero cohabitant, has been observed in twin lambs. This ovine intrauterine phenomenon had an highly significant effect on average daily gain. Prenatal nutrition also affected birthweight, weaning weight and average daily gain. There was no significant interaction between the effects of intrauterine cohabitants and prenatal nutrition. The within-sex variation due to the sex of an individual's in utero cohabitants may need to be accounted for in future prenatal nutrition studies on fecund mammals.

I. INTRODUCTION

Many of the postnatal phenomena attributed to inadequate prenatal nutrition appear to result from alterations in the cellular processes involved in differentiation, a process called programming (Barker 1994). Many hormones also participate in the prenatal programming of mammals; this hormonal programming has been called endocrinization (de Pablo and Roth 1990) and imprinting (Dohler 1986). Hormonal programming continues throughout the foetal stage of development (Fisher 1991). Testosterone and its metabolites appear to be important hormones in this process (Dohler 1986). Studies in twin lambs have showed alterations in growth-related and endocrine characteristics following a prenatal hormonal treatment (Gill and Hosking 1995). Elevated testosterone levels in utero appear to alter acute glucose metabolism in singleton lambs (Gill and Hosking 1994). These metabolic alterations suggest that testosterone, and/or its metabolites, participate in the prenatal programming of the sheep.

Events in normal prenatal development provide some evidence for the role of steroid hormones in the programming of growth potential. The intrauterine position (IUP) phenomenon, first observed in rodents, attributes permanent alterations in physiology, morphology and behaviour to the sex of the animal and the sex of its proximal in utero cohabitants (e.g. Clemens et al. 1978; vom Saal and Bronson 1978; vom Saal 1983). vom Saal (1983) also provided evidence of an hormonal mechanism. Foetuses situated in utero between two male foetuses had higher circulating levels of testosterone and bathed in amniotic fluid with an higher testosterone concentration, than foetuses located between two female foetuses. By contrast, oestradiol-17b concentrations were lower in foetuses situated in utero between two male foetuses and higher in foetuses located between two female foetuses (vom Saal 1983). The hormone environment of differentiating mammals appears to be a powerful modifier of genetic potential. Experiments reporting permanent alterations to growth-related characteristics following the exogenous administration of gonadal steroids during prenatal development support this hypothesis (Clarke et al. 1976; DeHaan et al. 1987; Gill and Hosking 1995).

Twin lambs represent a larger metabolic load to a gestating ewe than singleton lambs. They are born smaller than singleton lambs and have a higher rate of preweaning mortality (Owens et al. 1985). Evidence of within-sex variation in growth related characteristics, possibly due to the sex

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of intrauterine cohabitants (IUC), has also been observed in twin lambs (Gill and Hosking unpublished data). In essence, twin lambs appear more sensitive to prenatal stimuli, both hormonal and nutritional, than singleton lambs. This concept is responsible for the selection of the twin lamb as a model for the investigation of the postnatal effects of in utero events in mammals. This paper considers intrauterine environment as a function of both IUC and prenatal nutrition. It examines the variation in growth-related parameters in twin lambs, attempting to relate this variation to the programming experienced by the lamb in utero.

II. METHODS

The data reported in this paper were collected from the three experiments initially described by Holst et al. (1986), who give a detailed account of the design, animal management and measurements taken. During pregnancy (up to six weeks prior to parturition), the Border-Leicester dams grazed on pasture at one of two nutritional levels, either high or low. The pasture availability at the high level of nutrition was visually estimated to be >600 kg green DM/ha and at the low level of nutrition, <100 kg green DM/ha (Holst et al. 1986). Six weeks before their estimated lambing date, each group (both high and low) was re-allocated to two nutritional treatments, either high or low. This generated four nutritional treatment groups designated as HH, HL, LH and LL. Approximately three days prior to parturition, all nutritional treatments were removed from the dams, i.e. the diets were equilibrated. Ewes and lambs from all treatments were grazed together after lambing and retained on high quality pastures until weaning.

In addition to the nutritional treatments, lambs were classified on the basis of their sex and the sex of their inferred womb-mate, thus generating four additional treatment groups. The nomenclature used to describe these IUC groups is in accordance with that used by vom Saal (1983). Male lambs that belonged to a mono-sex twin set were assumed to have been resident in utero with another male foetus and designated 1M lambs. Male lambs from mixed-sex twin sets were designated 0M lambs and were assumed to have been resident in utero with a female foetus. Following a similar rationale, female lambs from mixed-sex twin sets were designated 1F lambs and the remaining group, the female lambs from mono-sex twin sets, were designated 0F lambs.

Lamb birth weight (BWt), weaning weight (WWt) and preweaning growth rate, expressed as average daily gain (ADG), were analysed with a general linear model (GLM) procedure using Minitab v8.8. Ewe mating weight was used as a covariate in all analyses to minimise the effect of any maternal bias within the data. Preliminary analyses had shown BWt to be a significant predictor of the postnatal growth parameters WWt and ADG. To reduce any effect of BWt on these growth-related measures, BWt was included as a covariate in the statistical analyses of WWt and ADG. Individual group means were tested for difference using a Student's T-test. The statistical model used in the analyses of the growth-related characteristics (BWt, ADG and WWt) examined main effects of IUC and prenatal nutrition and their interaction.

III. RESULTS

Female lambs from mixed-sex twin sets (1F) were born significantly lighter than other twin lambs (Table 1; $P<0.05$). Average daily gain was affected by intrauterine position. 1F lambs grew slower than 0F lambs, 0F lambs grew slower than 0M lambs, and 0M lambs grew slower than 1M lambs (Table 1; $P<0.001$). 1F lambs had lighter weaning weights than 1M lambs ($P<0.05$).

Table 1. The effect of intrauterine cohabitants (IUC) on growth-related parameters in twin lambs.

IUP	0F	1F	0M	1M	SED[a]
BWt (kg)	4.1[c] (n=158)	3.9[b] (n=138)	4.2[c] (n=138)	4.2[c] (n=162)	0.07
ADG (g/day)	223[j] (n=132)	218[i] (n=117)	224[k] (n=110)	229[l] (n=130)	0.005
WWt (kg)	29.1[bc] (n=132)	28.5[b] (n=117)	29.1[bc] (n=110)	29.8[c](n=130)	0.51

Pooled data from Holst et al. (1986)
[a]SED = standard error of difference
[b,c], means within rows with different superscripts, differ significantly (P<0.05).
[i,j,k,l], means within rows different superscripts, differ significantly (P<0.001).

Table 2. The effect of prenatal nutrition on growth-related parameters in twin lambs.

Prenatal nutrition	HH	HL	LH	LL	SED[a]
BWt (kg)	4.3[c] (n=136)	3.7[b] (n=134)	4.5[d] (n=154)	3.8[b] (n=172)	0.07
ADG (g/day)	229[k] (n=115)	231[l] (n=120)	220[j] (n=127)	213[i] (n=127)	0.015
WWt (kg)	29.8[c] (n=115)	30.0[c] (n=1120)	28.7[bc] (n=127)	27.9[b] (n=127)	0.50

Pooled data from Holst et al. (1986)
[a]SED = standard error of difference
[b,c,d] means within rows with different superscripts, differ significantly (P<0.001).
[i,j,k,l] means within rows with different superscripts, differ significantly (P<0.001).

Lambs that were exposed to low prenatal nutrition late in gestation, HL and LL, were born lighter than lambs that were exposed to high prenatal nutrition late in gestation, HH and LH (Table 2; P<0.05). HH lambs were born lighter than LH lambs (P<0.05). Prenatal nutrition affected average daily gain (ADG). LL lambs grew slower than LH lambs, LH lambs grew slower than HH lambs, and HH lambs grew slower than HL lambs (Table 2; P<0.001). Lambs that were exposed to high prenatal nutrition early in gestation, HH and HL, had higher weaning weights than LL lambs (P<0.05).

IV. DISCUSSION

The evidence in the analyses presented in this paper suggested that a phenomenon similar to IUP, IUC, occurs in sheep. The similarities between the responses observed in this dataset and the responses reported for other species, eg rat (Clemens et al. 1978), mouse (vom Saal 1983), hyena (Yalcinkaya et al. 1993), gerbil (Clark et al. 1993) and pig (Rohde-Parfet et al. 1990), suggests that the link between IUC and IUP is valid. Further experiments, designed to identify the hormonal vectors of IUC are required to confirm this.

The growth responses of the 1F lambs (BWt, ADG, WWT) were different to those found in a previous study (Gill and Hosking 1995b), where 1F lambs grew faster than 0F lambs, and displayed heavier BWt and WWt. The current studies show a slower rate of growth in the 1F lambs than the 0F lambs (Table 1). Romney Marsh sires were used by Gill and Hosking (unpublished data) and Dorset Horn sires were used in the current studies (Holst et al. 1986).

The highly significant (P>0.001) effects of IOUC and in utero nutrition on ADG illustrate the importance of these factors in programming growth trajectories. No significant interactions between IUC effects and nutritional treatment were observed for the parameters presented in this paper (BWt, P>0.3; ADG, P>0.9; WWt, P>0,9). One implication of the analyses presented in this paper is that in previous studies, differences due to nutritional treatments may have been obscured by pre-programmed within-sex variation. Future studies in fecund mammals may need to account for this variation.

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