MODULATION OF SEXUAL DIMORPHISM IN GROWTH IN TWIN SHEEP

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SUMMARY

Within-sex variation in foetal size, shape and organ growth, lamb birthweight and average daily gain, has been observed in twin lambs ($P<0.05$). These phenomena appear attributable to the sex of an individual's *in utero* cohabitant; analogous to the well documented *in utero* position phenomenon described in fecund mammals. Gestation length differed between mono-sex female, mono-sex male and mixed-sex twin sets ($P<0.05$).
Keywords: intrauterine position, sex, lambs, growth, development

INTRODUCTION

Experiments reporting permanent alterations to growth-related characteristics following the exogenous administration of gonadal steroids during prenatal development highlight the importance of these hormones in predetermining growth (DeHaan *et al.* 1987; Jenkins *et al.* 1988; Gill and Hosking 1995a, 1995b). Events in normal prenatal development also provide evidence for the role of steroid hormones in the programming of growth. The intrauterine position (IUP) phenomenon, first observed in rodents, attributes permanent alterations in physiology, morphology and behaviour to the sex of the animal being considered and the sex of its proximal *in utero* cohabitants (eg Clemens *et al.* 1978; vom Saal and Bronson 1978; vom Saal 1983). vom Saal(1983) also provided evidence of *an in utero* hormonal mechanism, showing differences in prenatal steroid environment between IUP groups. Foetuses situated between 2 male foetuses had higher circulating levels of testosterone and bathed in amniotic fluid with a higher testosterone titre, than foetuses located between 2 female foetuses. Oestradiol-17β concentrations followed an opposing trend, and was lower in foetuses situated *in utero* between 2 male foetuses and higher in foetuses located between 2 female foetuses (vom Saal 1983). The hormone environment of differentiating mammals appears to be a powerful modifier of genetic potential.

The range of mammalian species in which the IUP phenomenon has been described includes rats, mice, gerbils, pigs and hyenas (Clemens *et al.* 1978; vom Saal 1983; Rohde-Parfet *et al.* 1990; Clark *et al.* 1993; Yalcinkaya *et al.* 1993). Donald and Purser (1957) described within-sex variation in twin-bearing sheep. These authors attributed the variation to the sex of the *in utero* cohabitant of the pertinent ovine foetus. McDonald *et al.* (1981) and Dingwall *et al.* (1987) have also described within-sex differences in foetal size in twin sheep. Since the argument about position is tenuous when considering twin foetuses, Gill *et al.* (1995) discussed within-sex variation of postnatal growth in sheep as an intrauterine cohabitant (IUC) phenomenon. The following paper presents both prenatal and postnatal growth data from twin sheep and examines the impact that the sex of a twin's IUC has on the well-established sexual dimorphism in the expression of growth.

MATERIALS AND METHOD

Prenatal data collection

Twenty oestrous-synchronised Border Leicester x Merino ewes were mated on known dates to Romney Marsh rams. The ewes were tested for pregnancy and fecundity by real-time ultrasound at 50 days post coitus. Twin-bearing ewes were identified and at 60 days post coitus the ewes were weighed and then sampled according to the protocol outlined below.

Ewes were fitted with indwelling jugular catheters (0.8 mm I.D., 1.0 mm O.D.; single lumen vinyl tubing) and anaesthetised using Nembutal (pentobarbitone sodium 60 mg/mL; 0.4 mL/kg liveweight; Boehringer Ingelheim Pty. Ltd., Artarmon, NSW, 2064). The gravid uterus was exposed and the vaginal end of the cervix was ligated. A radical hysterectomy, removing the reproductive tract up to and including the vaginal end of the cervix, was performed on the unconscious ewes. The ewe was immediately euthenased (Lethobarb; pentobarbitone 325 mg/mL; 0.5 mL/kg liveweight; Arnolds of Reading Pty. Ltd., Peakhurst, NSW, 2210) and the foetuses were removed and euthenased (Lethobarb; 1 .0 mL) before examination. The foetuses were sexed and their weight, heart girth, crown-rump length and occipital width was recorded. The foetuses were then dissected and the brain, liver and kidneys were removed and weighed. The functional cotyledons were collected and counted as described by McCrabb *et al.* (199 1).

Twin foetuses were classified on the basis of their sex and the sex of their *in utero* cohabitant (IUC), thus generating 4 IUC groups. The nomenclature used to describe these IUC groups is according to that used by vom Saal (1983). Male foetuses that belonged to a mono-sex twin set, i.e. *in utero* with another male foetus, were designated 1M foetuses. Male foetuses from mixed-sex twin sets were designated OM foetuses and had been resident *in utero* with a female foetus. Female foetuses from mixed-sex twin sets were designated 1F foetuses and the remaining group, the female foetuses from mono-sex twin sets, were designated OF foetuses. Litter type was also identified as mono-sex female, mixed-sex and mono-sex male.

Postnatal data collection:

Twenty oestrus-synchronised Border Leicester x Merino ewes were weighed and mated to harnessed Romney Marsh rams. These ewes were from the same flock and managed under the same conditions as those ewes involved in the prenatal data collection. The mating date was recorded and the ewes were grazed as 1 flock, from 2 months prior to mating, until the lambs were weaned. The lambs were born over a 9 day period; their birth date was recorded and the gestation length was calculated. Lambs were weighed and sexed within 14 hours of birth and litter size was recorded. Only data from twin lambs was included in statistical analyses. Twin lambs were classified by sex and the sex of their inferred womb-mate, also generating four IUC groups. The nomenclature applied to these groups is the same as that described above. Litter type was also identified as mono-sex female, mixed-sex and mono-sex male. Once lambing had ceased, lambs were weighed at weekly intervals to weaning. All ram lambs were castrated 31 days after the commencement of lambing.

Statistics

Dimensional measures, weights, average daily gain (ADG) and cotyledon number were analysed using a general linear model (Minitab v8.2; Minitab Inc., 3081 Enterprise-Drive, State College PA 16801 USA) with ewe liveweight at mating used as a covariate.

RESULTS

At day 60 post coitus, OF foetuses were significantly lighter than either 1F or OM foetuses (Table 1; P<0.05). Male foetuses that belonged to a mono-sex twin set had shorter crown-rump lengths and heart girths than OM, 1F or OF foetuses (P<0.05). Female foetuses from mono-sex twin sets had narrower occipital widths than OM or 1M foetuses (P<0.05). Female foetuses from mixed-sex twin sets had intermediate occipital widths. The brains collected from OF foetuses were heavier than 1M brains but lighter than 1F brains (Table 1; P<0.05). The mean weight of OM brains did not significantly differ from either OF or 1F brains (P<0.05). Male foetuses that belonged to a mono-sex twin set had significantly smaller brains and kidneys in comparison to all other groups (P<0.05). The kidney weights of OF, 1F and OM foetuses did not differ significantly (P>0.05).

Table 1. Description of day 60 twin foetuses by in *utero* cohabitant (IUC)

	Foetal sex-Twin's sex				
IUC (n)	0F (4)	1F (4)	0M (4)	1M (4)	SED
Weight (g)	64.2^{aA}	72.3^{b}	73.5^{b}	68.1^{ab}	5.29
Crown-Rump length (mm)	168^{b}	168^{b}	167^{b}	161^{a}	4.19
Heart girth (mm)	94^{b}	95^{b}	95^{b}	82^{a}	4.28
Occipital width (mm)	14^{a}	15^{ab}	16^{b}	15^{b}	0.64
Brain weight (g)	2.7^{b}	2.9^{c}	2.8^{bc}	2.3^{a}	0.15
Liver weight (g)	5.8	5.8	5.9	5.3	0.57
Kidney weight (g)	1.0^{b}	0.9^{b}	0.9^{b}	0.7^{a}	0.08

[A] Means within rows with different superscripts differ significantly (P<0.05).

0F = Female-female twin; 1F = Female-male twin; 0M = Male-female twin; 1M = Male-male twin.

The placentas from litters containing at least 1 female foetus possessed a higher number of cotyledons. The mono-sex female and mixed-sex twin sets were nourished by 106 and 107 cotyledons respectively, while the mono-sex male twin sets were nourished by 99 cotyledons ($P<0.05$).

Gestation length was greater in litters containing at least 1 female foetus. The mono-sex female and mixed-sex twin sets gestated for 145 days, while the mono-sex male twin sets gestated for 142 days ($P<0.05$).

Table 2. The effect of *in utero* cohabitant (IUC) on birth weight (kg) and ADG (g/day) in twin lambs

	Foetal sex-Twin's sex (n)				
	0F (6)	1F (5)	0M (5)	1M (6)	SED
Birth weight (kg)	4.2[aA]	5.0[a]	5.2[b]	4.8[ab]	0.47
ADG (g/day)	280[a]	330[b]	346[b]	323[b]	29.7

[A] Means within rows with different superscripts differ significantly ($P<0.05$).
0F = Female-female twin; 1F = Female-male twin; 0M = Male-female twin; 1M = Male-male twin.

Birth weight and average daily gain (ADG) were significantly increased by the presence of a male foetus (Table 2; $P<0.05$). Male foetuses that shared the uterus with a female foetus (OM) tended to have higher birth weights than male-male (1M) twins ($P<0.15$).

DISCUSSION

The weight of a twin ovine foetus at day 60 post coitus appears to be sensitive not only to the sex of the foetus, but also to the sex of its intrauterine cohabitant (IUC) (Table 1). The differences in weight are evidence of induced accelerations in growth rates. Donald and Purser (1957) have previously described this within sex variation in the sheep. In their extensive study (822 twin pairs), OM foetuses were heavier than 1M foetuses, as in this study. The experiment being reported in this paper suggests that OF foetuses were lighter than 1F foetuses (Table 1). This finding differs from that reported by Donald and Purser (1957). This may be due to the small group-sizes within the data presented in this paper, genotype differences, or seasonal effects. These last 2 possibilities are not excluded by the data presented in Donald and Purser (1957) which indicated differences in the response of the various IUC classes due to genotype and seasonal effects. The data from foetuses collected at day 60 post coitus (Table 1) appear analogous to responses attributed to the intrauterine position phenomenon, as do the postnatal data (Table 2).

The birth weights and postnatal growth rates of lambs have also been altered by the effect of IUC (Table 2; Gill *et al.* 1995). The physical evidence suggests that the 4 IUC groups; female mono-sex twins (OF), female mixed-sex twins (1F), male mixed-sex twins (OM) and male mono-sex twins (1M), differ in their prenatal programming. Furthermore, the evidence from the IUP phenomenon (vom Saal 1983) suggests that these IUC groups may differ in their steroid, especially androgen and oestrogen, environments *in utero.* Varying IUC from OF to 1F, OM and 1M, can also alter placental morphology, prenatal growth and foetal dimensions in the sheep. The alterations in foetal size and shape reported following exposure to increased testosterone concentrations are consistent with this hypothesis (Gill and Hosking 1995b). Gestation length appears sensitive to effects of litter type as well as the altered *in utero* steroid environment generated by acute prenatal androgen treatment (Gill and Hosking 1995a).

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