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Effect of the maternal environment on fetal growth at mid-gestation in sheep

CMC Jenkinson, RK Sharma, PR Kenyon* and HT Blair

Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11-222, Palmerston North 4442, New Zealand

**Corresponding author. Email: p.r.kenyon@massey.ac.nz*

Abstract

Our group has previously shown that maternal size can affect embryonic development by Day 19 of gestation and lamb birth weight. This study set out to investigate the effect of the maternal uterine environment on fetal growth at mid-gestation. Pure-breed single embryos were transferred within and reciprocally between large (Suffolk: S) and small (Cheviot: C) breeds of ewes to establish four maternal environments; SinS, SinC, CinS and CinC. On Day 90 of gestation, 37 single-bearing recipient ewes (10 CinC, 7 SinC, 10 CinS and 10 SinS) were euthanased. Fetal body weight, fetal body dimensions and fetal organ weights, and placentome number and total weight were measured. Body weight was significantly ($P < 0.001$) smaller in Cheviot compared to Suffolk fetuses. However, Cheviot fetuses had heavier ($P < 0.05$) kidneys and liver than Suffolk fetuses. SinC fetuses had heavier ($P < 0.05$) pancreas than those from all other pregnancy groups. CinS fetuses tended to have longer ($P < 0.10$) fore- and hind-leg lengths than CinC fetuses. There were no differences ($P > 0.05$) in placental parameters between the pregnancy groups. The maternal uterine environment had no major effect on fetal weight, fetal dimensions and fetal organ mass at mid-gestation.

Keywords: sheep; maternal environment; fetal growth

Introduction

There is considerable evidence to suggest that the genetic potential for fetal growth is rarely completely expressed because it is constrained by the maternal uterine environment (Gluckman & Liggins 1984). Early crossbreeding studies by Walton & Hammond (1938) demonstrated that maternal size has a marked influence on pre- and post-natal growth in foals. Subsequently, similar findings were reported in cattle (Joubert & Hammond 1958) and in sheep breeds (Hunter 1956; Jenkinson et al. 2007). Experimental studies of transferring embryos between small and large breeds of horses (Allen et al. 2002), cows (Ferrell 1991) and pigs (Wilson et al. 1998) have also shown that birth size is primarily determined by the *in utero* environment rather than by genetic factors.

Recently, embryo-transfer experiments in sheep by our group demonstrated that Suffolk embryos, as an example of a large breed, gestated in Cheviot ewes, as an example of a small breed, were shorter at Day 19 of gestation, had smaller heads at Day 55, and were lighter and had smaller body dimensions at birth than Suffolk embryos gestated in Suffolk ewes (Sharma et al. 2009; Sharma 2010a; Sharma et al. 2010b). In this paradigm, the 'restricted' uterine environment of the smaller Cheviot ewe constrained the growth of the Suffolk embryo. Although Cheviot embryos gestated in Suffolk uteri were longer in length than their 'control' embryos (Cheviot in Cheviot) at Day 19, they did not differ in size at Day 55 or at birth (Sharma et al. 2009; Sharma 2010a). This is in contrast to the findings of Scheaffer et al. (2004) who reported that Romanov embryos (small breed) gestated in Columbia ewes (large breed) were

22% heavier at 130 days of gestation than control Romanov fetuses gestated in Romanov ewes.

The aim of the present study was to determine whether the maternal uterine environment has an effect on fetal growth at mid-gestation; and whether that effect is consistent with that found at Day 19 of gestation and at birth.

Materials and methods

All animal manipulations were approved by the Massey University Animal Ethics Committee.

Establishment of pregnancies

In March 2008, pure-breed embryos were collected from donors of Cheviot and Suffolk ewes and transferred into the uteri of the same and opposite breeds to establish Cheviot in Cheviot (CinC), Suffolk in Suffolk (SinS), Cheviot in Suffolk (CinS) and Suffolk in Cheviot (SinC) pregnancy groups. CinC and SinS acted as Control pregnancies. CinS provided a Luxurious *in utero* environment while SinC provided a Restricted *in utero* environment. All donors were four-year-old ewes, in order to avoid introducing any variation in embryonic quality due to age of donors. Mixed age (three- to six-year-old) recipients of each breed were randomly allocated to each group.

To synchronise oestrus, all ewes had progesterone-impregnated, controlled internal drug release devices (Eazi-breed CIDR, Pharmacia, Auckland, NZ) inserted for 13 days. Superovulation of donor ewes was achieved by injection of porcine FSH (Folltropin-V; Bioniche Animal Health; Belleville, Canada) in seven tapering doses of 48, 48, 28, 28, 24, 20, 20 mg/dose at 12 hourly intervals

Table 1 The effect of pregnancy group (CinC = Cheviot in Cheviot (small Control), CinS = Cheviot in Suffolk (Luxurious *in utero* environment), SinC = Suffolk in Cheviot (Restricted *in utero* environment), SinS = Suffolk in Suffolk (large Control)) on mean \pm standard error of maternal live weight and condition score at Day 0 and Day 90 of gestation.

Measurement	Pregnancy group			
	CinC	CinS	SinC	SinS
Day 0				
Live weight (kg)	54 \pm 3 ^a	72 \pm 3 ^b	58 \pm 4 ^a	68 \pm 3 ^b
Condition score	2.9 \pm 0.3	2.9 \pm 0.2	3.2 \pm 0.3	2.6 \pm 0.3
Day 90				
Live weight (kg)	61 \pm 3 ^a	79 \pm 3 ^b	65 \pm 4 ^a	75 \pm 3 ^b
Condition score	2.8 \pm 0.2	3.1 \pm 0.2	3.0 \pm 0.2	2.9 \pm 0.2

beginning at 60 hours prior to CIDR removal. Semen was collected by electroejaculation from two rams of each breed. Donor ewes were inseminated laparoscopically with 0.5 mL semen from the ram of the same breed, 32 hours after CIDR removal. Six days after insemination, embryos were recovered from donors via midline laparotomy performed under general anaesthesia using diazepam and ketamine induction, halothane in oxygen maintenance. The embryos were immediately evaluated for morphological characteristics using light microscopy. Those embryos in the late morula to early blastocyst stage were immediately transferred to recipients by laparoscopy, at the rate of a single embryo per recipient. Embryos were inserted near the tip of the uterine horn ipsilateral to a corpus luteum. The proportion of transferable embryos was similar from the two breeds of donor.

The trial was conducted at the Massey University Keeble Farm, five kilometres south of Palmerston North (latitude 40.23° S and 175.37° E). All ewes were managed as one flock under New Zealand lowland pastoral conditions grazing ryegrass (*Lolium sp.*) and white clover (*Trifolium repens*). Crayon-harnessed vasectomised rams were used to detect pregnancy status of ewes. At Day 19 of gestation, ten pregnant recipient ewes per group (n = 40 in total) were removed for embryo evaluation (Sharma 2010a).

Maternal and fetal sampling

Ultrasound scanning at Day 70 of gestation confirmed pregnancy status and rank of the remaining ewes. On Day 90 of gestation, 37 pregnant, single-bearing recipient dams (10 CinC, 7 SinC, 10 CinS and 10 SinS) were euthanased via captive bolt and exsanguination. The abdominal cavity was opened and the gravid uterus removed. The cervix, vagina and associated tissue were removed. Total conceptus weight was recorded. The fetus was carefully removed from the uterus and the umbilical cord ligated at the abdomen before being cut. Fetal mass, sex and linear measurements of crown-rump length, thoracic girth and fore- and hind-

leg lengths, were recorded. Both thyroids, heart, liver, pancreas, spleen, both kidneys and both adrenal glands were removed from the fetus, blotted dry and the weight recorded. The combined weight of bilateral organs was recorded (Mettler Toledo GmbH, Greifensee, Switzerland).

Placentomes were dissected from the uterus, counted, and their total weight recorded. Maternal live weight and condition score were recorded on a scale of zero to five including half units (Jefferies 1961).

Statistical methods

Analysis of variance was used to determine the effects of pregnancy group on the weight of placental parameters adjusted to a common maternal weight and age, on fetal body weight with fetal sex as a fixed effect, and on dimensions and organ weights adjusted to a common fetal body weight and sex (SAS 2006).

All the main effects and interactions were included in the initial model, with the interaction terms progressively removed if not found to be significant ($P > 0.05$). The interaction between breed of ewe and breed of fetus was always kept in the model irrespective of whether it was significant ($P < 0.05$) or not ($P > 0.05$), since this interaction defines the different maternal environments. The results for the interaction tested between breed of ewe and breed of fetus are shown in all tables. Only significant ($P < 0.05$) main effects of breed of ewe or breed of fetus are reported in the results text.

Data are expressed as least square means \pm standard errors. Statistical analyses were conducted using a generalised linear model (SAS 2006).

Results

Dam live weight and condition score

Suffolk ewes were heavier ($P < 0.001$) than Cheviot ewes at Day 0 (S: 70.2 \pm 2.2 vs C: 55.9 \pm 2.9 kg) and Day 90 (S, 77.0 \pm 2.3 vs C, 62.7 \pm 3.0 kg) but did not differ ($P > 0.05$) in condition score. There was no effect of pregnancy group ($P > 0.10$) for ewe live weight or ewe condition score (Table 1).

Table 2 The effect of pregnancy group (CinC = Cheviot in Cheviot (small Control), CinS Cheviot in Suffolk (Luxurious *in utero* environment), SinC = Suffolk in Cheviot (Restricted *in utero* environment), SinS = Suffolk in Suffolk (large Control)) on mean \pm standard error of fetal body weight (g), fetal body dimensions (cm) and fetal organ weights (g) at Day 90 of gestation.

Measurement	Pregnancy group			
	CinC	CinS	SinC	SinS
Fetal body weight (g)	579 \pm 27 ^a	600 \pm 28 ^a	694 \pm 32 ^b	681 \pm 27 ^b
Crown-rump (cm)	27.1 \pm 0.2 ^a	27.1 \pm 0.2 ^a	27.9 \pm 0.3 ^b	27.3 \pm 0.2 ^{ab}
Girth (cm)	17.5 \pm 0.1 ^b	17.1 \pm 0.1 ^a	17.8 \pm 0.1 ^{bc}	17.9 \pm 0.1 ^c
Fore-leg (cm)	15.3 \pm 0.3 ^a	16.2 \pm 0.3 ^{ab}	17.0 \pm 0.4 ^b	16.3 \pm 0.3 ^b
Hind-leg (cm)	14.5 \pm 0.2 ^a	15.2 \pm 0.3 ^{ab}	15.9 \pm 0.3 ^b	15.3 \pm 0.3 ^b
Thyroid (g)	1.13 \pm 0.16 ^b	1.18 \pm 0.16 ^b	0.56 \pm 0.18 ^a	0.87 \pm 0.15 ^{ab}
Heart (g)	5.4 \pm 0.2 ^{ab}	5.3 \pm 0.2 ^a	6.1 \pm 0.3 ^b	5.4 \pm 0.2 ^a
Liver (g)	39.3 \pm 0.9 ^b	39.1 \pm 0.9 ^b	33.8 \pm 1.0 ^a	35.9 \pm 0.9 ^a
Pancreas (g)	0.83 \pm 0.07 ^a	0.83 \pm 0.06 ^a	1.14 \pm 0.08 ^b	0.81 \pm 0.06 ^a
Spleen (g)	0.86 \pm 0.06	0.88 \pm 0.06	0.78 \pm 0.07	0.79 \pm 0.06
Kidney (g)	6.7 \pm 0.2 ^b	7.1 \pm 0.2 ^b	5.4 \pm 0.2 ^a	5.4 \pm 0.2 ^a
Adrenal (g)	0.21 \pm 0.04	0.17 \pm 0.04	0.16 \pm 0.05	0.17 \pm 0.04

Table 3 The effect of pregnancy group (CinC = Cheviot in Cheviot (small Control), CinS Cheviot in Suffolk (Luxurious *in utero* environment), SinC = Suffolk in Cheviot (Restricted *in utero* environment), SinS = Suffolk in Suffolk (large Control)) on mean \pm standard error of placental parameters (g) at Day 90 of gestation.

Measurement	Pregnancy group			
	CinC	CinS	SinC	SinS
Total conceptus weight (g)	2483 \pm 184	2760 \pm 175	2933 \pm 196	2846 \pm 173
Total placentome weight (g)	622 \pm 95	692 \pm 90	746 \pm 101	717 \pm 90
Number of placentomes	68 \pm 9	71 \pm 9	76 \pm 10	78 \pm 9
Number of empty caruncles	55 \pm 8	45 \pm 8	51 \pm 9	38 \pm 8
Caruncle occupancy (%)	57 \pm 6	60 \pm 5	62 \pm 6	67 \pm 5

Fetal body weight and organs

Suffolk fetuses were heavier ($P < 0.01$) than Cheviot fetuses (S, 688 \pm 21 vs C, 590 \pm 19 g) but there was no effect ($P > 0.05$) of pregnancy group (Table 2). Thoracic girth, fore-leg and hind-leg lengths were smaller ($P < 0.05$) in Cheviot than in Suffolk fetuses (girth: C, 17.3 \pm 0.1 vs S, 17.9 \pm 0.1 cm) (fore-leg: C, 15.7 \pm 0.2 vs S, 16.7 \pm 0.3 cm) (hind-leg: C, 14.9 \pm 0.2 vs S, 15.6 \pm 0.2 cm). There was a tendency ($P = 0.08$) for crown-rump length to be shorter in Cheviot than in Suffolk fetuses (crown-rump: C, 27.1 \pm 0.2 vs S, 27.5 \pm 0.3 cm). CinC fetuses had shorter fore-leg ($P = 0.02$) and hind-leg ($P < 0.05$) lengths than SinC and SinS fetuses.

Cheviot fetuses had heavier ($P < 0.01$) liver (C, 39.2 \pm 0.6 vs S, 34.8 \pm 0.7 g) kidney (C, 6.9 \pm 0.2 vs S, 5.4 \pm 0.2 g) and thyroid gland (C, 1.15 \pm 0.11 vs S, 0.72 \pm 0.12 g) than Suffolk fetuses. There was a tendency ($P = 0.07$) for Suffolk fetuses to have heavier pancreas than Cheviot fetuses (S, 0.97 \pm 0.05 vs C, 0.83 \pm 0.05 g). Pancreas weight was heavier ($P = 0.02$) in SinC fetuses than in CinC, CinS and SinS

fetuses (Table 2). There was an effect ($P < 0.05$) of ewe breed on fetal pancreas weight (C, 0.98 \pm 0.05 vs S, 0.82 \pm 0.04 g). Heart weight in SinC fetuses was greater ($P < 0.05$) than SinS fetuses, however no difference was observed between CinS and CinC fetuses.

Male fetuses were heavier ($P < 0.01$) than female fetuses (M, 685 \pm 19 vs F, 592 \pm 21 g).

Placental parameters

Total conceptus weight tended ($P < 0.10$) to be heavier in Suffolk ewes than in Cheviot ewes (S, 2,887 \pm 139 vs C, 2,619 \pm 114 g). There were no ($P > 0.05$) differences in total conceptus weight, total placentome weight, placentome number or caruncle occupancy between the pregnancy groups (Table 3).

Discussion

The aim of the present study was to determine the effect of the maternal uterine environment on fetal growth at mid-gestation. The key findings were that

fetal body mass, body dimensions and organ weights were not reduced when fetuses were gestated in a Restricted uterine environment (SinC) nor were they enhanced when gestated in a Luxurious uterine environment (CinS). This is in contrast to our findings at Day 19, when conceptuses developing in a Restricted uterine environment (SinC) were smaller than in the Control (SinS) (Sharma *et al.* 2010b); at Day 55, when the head length of SinC fetuses was smaller than in SinS fetuses (Sharma 2010a); and at birth, when SinC lambs were lighter and smaller than SinS lambs (Sharma *et al.* 2009; 2010b). However, embryos gestated in a Luxurious environment (CinS) were bigger than the Controls (CinC) at Day 19. There were no differences in fetal dimensions between the Luxurious CinS and the Control CinC groups at Day 90, which is in agreement with our findings at Day 55 (Sharma 2010a). Thus, the fetuses in the Luxurious environment (CinS) were unable to maintain the apparent benefit they derived from their privileged uterine environment, observed at Day 19. This suggests that once the metabolic demands of the smaller Cheviot genotype were met, they were not able to utilise any advantage from the luxurious uterine environment of the larger Suffolk dam.

The placenta is not only the site for exchange of nutrients between ewe and fetus but is also a metabolically active organ (Gootwine 2004). We found no differences in placental parameters between the Restricted SinC and the Control SinS groups. Similarly, Sharma (2010a) observed no differences in the cross-sectional area of individual placentomes between SinC and SinS placenta on Day 110 of gestation. Placental parameters also did not differ between the Luxurious CinS and Control CinC groups, which is in agreement with the findings of Sharma (2010a) on Day 55 of gestation. However, in contrast to these findings, Sharma *et al.* (2010b) reported, at Day 19 of gestation, that mean epithelial cell height of the trophoblast, a placental precursor, was greater in CinS compared to CinC. Our data suggests that the genetically smaller Cheviot fetuses did not exert any increased incremental demand from the bigger Suffolk dam (CinS). Interestingly, there were no differences in the birth weight and body dimensions of the cohort of lambs born from the CinS and CinC groups (Sharma *et al.* 2009).

Numerous embryo transfer and crossbreeding experiments in large and small breeds of farmed animals (sheep: Dickinson *et al.* 1962, Gootwine *et al.* 1993; horses: Allen *et al.* 2002, Walton & Hammond 1938; cattle: Joubert & Hammond 1958; pigs: Wilson *et al.* 1998) have shown that fetal growth can be altered from the normal genetic potential by varying dam size. However, neither the size nor the morphology of the fetal organs has been investigated in these studies. In the present study, fetal development in the Restricted uterine environment (SinC) was somewhat enhanced compared to Control lambs (SinS) as shown by heavier pancreas and heart weights in SinC fetuses.

However, there were no differences in fetal body weights or body dimensions between these groups. The latter finding is contrary to the results of Sharma *et al.* (2009) who found all the parameters of body morphology to be significantly less in SinC lambs than in SinS lambs at birth. The reasons for such a discrepancy in findings require further investigation. Development was not enhanced when fetuses were developed in a Luxurious environment (CinS) suggesting Cheviot fetuses achieve only their expected genetic growth potential.

Our observations show that development is characterised by a starting point at about Day 19 where the embryos start to exhibit differences in morphology, followed by a stage of reduced variability at about Day 90 and ending at birth, in progressive divergence in development in which phenotypic differences emerge. This may be consistent with the so-called hourglass theory proposed by Duboule (1994) and Raff (1996) which suggests evolution should have the greatest impact in early and late development and less impact in mid-gestation, a period during which general features of the body plans develop. Further studies, with more frequent time points, are required to establish whether the developmental processes observed in the pregnancy groups are consistent with those of the hourglass model.

Conclusion

These findings indicate that, at Day 90 of gestation, fetal development is not impaired in a 'restricted' uterine environment nor is it enhanced in a 'luxurious' uterine environment. This constraint in development could be viewed as a transition between two phases of developmental variability; a pattern of morphological events known as the hourglass model. However, to test the validity of this model further datasets of developmental-timing variation need to be analysed.

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