Maternal constraint in sheep breeds with diverse birth weight

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ABSTRACT

Reciprocal crosses of Shire and Shetland ponies and transferring embryos between small and large breeds of horses and pigs have shown that maternal size, rather than foetal genotype, is the primary determinant of birth size.

In the present study, 40 Cheviot (C) ewes and 40 Suffolk (S) ewes were split into two groups. Each group was mated to either two Suffolk rams or two Cheviot rams to generate CC, CS, SC and SS lambs.

Birth weights differed significantly ($P<0.01$) between the four lamb genotypes (CC 4.12 ± 0.21; CS 4.43 ± 0.23; SC 5.08 ± 0.19; SS 5.17 ± 0.19, kg). Furthermore, lower birth weight was significantly ($P<0.01$) associated with lower maternal circulating plasma placental lactogen (oPL) concentrations in the Cheviot dams at days 90 (C 25.8 ± 10.0 *vs*. S 71.8 ± 11.6, ng/ml) and 110 (C 84.5 ± 16.8 *vs*. S 175.9 ± 19.0, ng/ml) but not at day 130 of gestation.

These results indicate that the size of the ewe affects the intrauterine development of her lamb. Moreover, the lower concentrations of oPL in the Cheviot dams may have contributed to the constraint observed in foetal growth.

Keywords: sheep; birth weight; placental lactogen; maternal constraint.

INTRODUCTION

Size at birth, which is reflected in birth weight, body dimensions, body composition and organ size, is determined by the interaction between foetal genotype and the uterine environment. Classically, this has been demonstrated in crossbreeding studies between Shetland ponies and Shire horses (Walton & Hammond, 1938). They produced crossbred offspring with birth weights similar to those of the purebred progeny of the maternal strain, despite the nearly four-fold size difference in stature between the two breeds. Reciprocal crosses in cattle (Joubert & Hammond, 1958) and in sheep breeds (Hunter, 1956) have also utilised large differences in parental size to demonstrate maternal effects. More recently, 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 shown that birth size is primarily determined by the *in utero* environment rather than by genetic factors, suggesting that there are limitations in the capacity of the uterus to support foetal growth. This constraint is evident in sheep, in which the mean birth weight in multiple pregnancies is less than that for singletons (Stafford *et al*., 2007) and in polytocous species such as rodents (Cowley *et al*., 1989) in which there is an inverse relationship between litter size and mean birth weight. Such limitations in foetal growth are advantageous in that they reduce the risk of foetal overgrowth and dystocia, the latter accounting for up to 50% of all singleton deaths in lambs (Scales *et al*., 1986; Kerslake *et al.,* 2005). Other factors, such as age at first birth (Wallace *et al*., 2001) may also influence foetal size.

Foetal growth is limited by the capacity of the uteroplacental unit to supply nutrients to the foetus; the supply of which is influenced by maternal endocrine factors (Gluckman, 1997). Placental lactogen and growth hormone induce insulin resistance, which appears to be important in ensuring glucose availability to the placenta and placental diffusion capacity (Gluckman & Pinal, 2002; Harding *et al*., 1997). In the foetus, secretion of insulin-like growth factors and insulin, which are regulated by the transplacental nutrient supply (Bauer *et al*., 1998), interact to promote protein deposition and foetal linear growth (Gluckman, 1997).

The present study was designed to determine whether reciprocal crosses in Cheviot and Suffolk sheep could generate a repeatable genetic animal model of maternal constraint and to examine the mechanisms involved in limiting foetal growth.

MATERIALS AND METHODS

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

Experimental design

Forty Cheviot (C) ewes and 40 Suffolk (S)

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ewes were used in this study. The trial was conducted at Massey University's Keeble Farm, 5 kilometres south of Palmerston North (latitude 40.23º S and 175.37º E). In April 2003, ewes from each breed were split into two groups, balanced for age. Each group was mated over a 17-day oestrous cycle to either two Cheviot rams or two Suffolk rams to generate CC, CS, SC and SS lambs. Pregnancy diagnosis was conducted using ultrasound on day 60 from the start of mating.

Blood sampling

Ewes were blood sampled by jugular venipuncture on mean days 90, 110 and 130 of gestation to determine circulating concentrations of oPL, insulin, insulin-like growth factor-1 (IGF-1), free fatty acids (FFA) and glucose.

Ewes were blood sampled immediately off pasture at 0900 hours. Samples (8 mL) were withdrawn into vacutainers (Becton Dickinson Vacutainer Systems, USA) containing heparin as the anticoagulant and immediately placed on ice. Within one hour the samples were centrifuged at 3000 g for 15 minutes. Plasma was pipetted into duplicate Eppendorf tubes and stored at -20ºC until assayed.

Ewe measurements

Ewes were weighed, unfasted, at the start of mating (Day 0), days 90 and 136 (set-stocking) of pregnancy and, at weaning. Condition scores of ewes were determined only at day 136 of pregnancy.

Lamb measurements

Lambs were identified to their dams, tagged and weighed within 24 hours of birth, regardless of whether they were found dead or alive. Lambs were weighed again at docking and weaning.

Assays

Plasma glucose and FFA concentrations (Kunst *et al*., 1984) were measured by standard colorimetric methods modified for assay using a 96-well plate reader (Ashour *et al*., 1987). Plasma hormone concentrations were measured by specific RIA, established and validated for ovine plasma. Plasma insulin was measured according to previously published methods (Oliver *et al*., 1993) except that ovine insulin (Sigma Chemical, St. Louis, MO, batch #I9254) was used as the standard. The standard curve displaced in parallel with ovine plasma samples and cross-reactivity with IGF-I or IGF-II was 0.01%. The minimal detectable concentration was 40 pg/ml plasma and the inter- and intra-assay coefficients of variation were 11.1% and 6.7%, respectively. Plasma IGF-I was measured using an IGFBP-blocked RIA (Blum & Breier, 1994). The detection limit was 0.7 ng/mL and the inter- and intra-assay coefficients of variation were <10% and <5%, respectively. Plasma oPL was measured by RIA as described by Oliver *et al*. (1992). The minimal detectable dose was 0.05 ng/mL and inter- and intra-assay coefficients of variation were 9.7 and 5.1%, respectively.

Statistical analyses

Analysis of variance was used to determine the effects of breed on maternal weight, liveweight gain and condition score (adjusted to a common maternal live weight and pregnancy rank) and on lamb weight (adjusted for maternal live weight, birth rank and sex).

Univariate and multivariate (repeated measures) analyses of variance were used to analyse differences in hormone and metabolic concentrations in the ewes (covariate-adjusted where appropriate).

Data are expressed as least-square means ± standard error. There were no significant interactions. Statistical analyses were conducted using the Proc GLM procedure in SAS version 8.02 (SAS 2005).

RESULTS

Cheviot ewes were significantly lighter ($P<0.001$) than Suffolk ewes at mating (55.0 ± 1.2 versus 68.3 ± 0.8 kg) and at set stocking (61.7 ± 1.5 versus 76.2 ± 0.9 kg). However, maternal liveweight gain from day Day 0 to Day 136 of pregnancy did not differ (C: 6.7 ± 1.1 versus S: 7.9 ± 0.7 kg, $P>0.10$). Condition score at Day 136 of pregnancy was also similar between the two breeds (C: 2.30 ± 0.17 versus S: 2.14 ± 0.11, $P>0.10$).

Of the 80 ewes mated, 61 ewes gave birth, producing 80 lambs (18 CC, 16 CS, 21 SC and 25 SS). Cheviot dams had 28 singletons and 3 pairs of twins. Suffolk dams had 14 singletons and 16 pairs of twins.

Table 1 shows the birth, docking and weaning weights of the four lamb genotypes. Lambs born to Suffolk dams had higher ($P<0.01$) mean body weights at birth, docking and weaning than those lambs born to Cheviot dams. Breed of sire had no effect on any of the weights measured and there were no significant ewe breed by sire breed or ewe breed by rank interactions.

Table 1: Effect of lamb genotype on birth, docking and weaning weights (lsmean ± sem).

Breed (dam first)	n	Lamb live weights (kg)		
		Birth	Docking	Weaning
C x C	18	4.1 ± 0.2[a]	14.6 ± 0.8[a]	28.9 ± 1.4[a]
C x S	16	4.4 ± 0.2[a]	14.5 ± 0.9[a]	30.2 ± 1.6[a]
S x C	21	5.1 ± 0.2[b]	19.0 ± 0.6[b]	34.3 ± 1.2[b]
S x S	25	5.2 ± 0.2[b]	18.0 ± 0.7[b]	32.8 ± 1.3[ab]

Means within columns with different superscripts are significantly different (P<0.05)

Table 2 shows maternal circulating concentrations of oPL, IGF-1, insulin, glucose and FFA at days 90, 110 and 130 of pregnancy. Maternal oPL concentrations were significantly lower (P<0.01) in Cheviot dams at days 90 and 110, but did not differ at day 130 of pregnancy. In the Cheviot, oPL concentrations increased steadily from days 90 to 130 of pregnancy. Concentrations increased in the Suffolk ewes from days 90 to 110, then remained stable through to day 130 of pregnancy.

There were no significant differences in circulating maternal IGF-1 concentrations from days 90 to 130 of pregnancy. However, there was a tendency (P<0.06) for Cheviot ewes to have higher concentrations of IGF-1 than Suffolk ewes at day 110 of pregnancy.

Maternal plasma insulin concentrations in the Cheviot ewes were nearly three-fold higher (P<0.05) than those in the Suffolk ewes, but only at day 130 of gestation.

Breed of ewe had no effect on plasma glucose or FFA concentrations at any of the stages measured.

Table 2: Effect of breed of ewe on maternal circulating concentrations of oPL, IGF-1, insulin, glucose and FFA at days 90, 110 and 130 of pregnancy (lsmean ± sem).

	n	Day of gestation		
		Day 90	Day 110	Day 130
oPL (ng/ml)				
Cheviot	32	25.8 ± 10.0[a]	84.5 ± 16.8[a]	136.5 ± 19.2
Suffolk	31	71.8 ± 11.6[b]	175.9 ± 19.0[b]	154.2 ± 22.2
IGF-1 (ng/ml)				
Cheviot	32	106.0 ± 7.7	114.6 ± 7.5	132.1 ± 7.7
Suffolk	31	104.2 ± 8.7	90.3 ± 8.4	128.0 ± 8.9
Insulin (ng/ml)				
Cheviot	32	0.18 ± 0.04	0.67 ± 0.15	0.17 ± 0.03[b]
Suffolk	31	0.24 ± 0.05	0.53 ± 0.17	0.06 ± 0.03[a]
Glucose (mM)				
Cheviot	32	4.22 ± 0.26	4.29 ± 0.33	3.87 ± 0.24
Suffolk	31	4.12 ± 0.29	4.65 ± 0.35	4.08 ± 0.28
Free Fatty Acids (mM)				
Cheviot	32	0.60 ± 0.09	0.46 ± 0.08	0.69 ± 0.11
Suffolk	31	0.48 ± 0.09	0.61 ± 0.08	0.55 ± 0.10

Means within columns with different superscripts are significantly different (P<0.05)

DISCUSSION

The effect of maternal size on lamb birth weight observed in this study is consistent with the findings of Walton & Hammond (1938), Hunter (1956), Joubert & Hammond (1958), and Allen *et al*. (2002). However, our results, like those reported in earlier crossbreeding and embryo transfer studies, suggest that the effect is not due solely to differences in maternal size. Rather, maternal and uteroplacental factors act to limit the growth of the foetus. For example, oPL, insulin and IGF-1 appear to be important in ensuring glucose availability to the placenta and placental diffusion capacity (Gluckman & Hanson, 2004).

Maternal size, as reflected by the ewe's live weight, is a marker for nutrient availability and physical space, and therefore has a major influence on foetal growth. Although the Cheviot ewes were lighter than the Suffolk ewes at mating and set stocking, both breeds followed a similar pattern of liveweight change as pregnancy progressed and did not differ in condition score at day 136 of gestation. However, mean liveweight gain from mating until parturition was below that of the gain in predicted conceptus weight (Rattray *et al*., 1974) indicating that some ewes were likely to be in negative energy balance. It might therefore be expected that FFA concentrations would increase in late pregnancy, indicating mobilisation of maternal fat stores. Interestingly, FFA concentrations did not differ between the Cheviot and Suffolk ewes and remained relatively stable from days 90 to 130 of gestation.

Maternal endocrine factors influence the supply of nutrients to the foetus (Gluckman & Pinal, 2002; Harding *et al*., 1997). Several studies have suggested that placental lactogen, insulin-like growth factors and insulin are likely to interact in the regulation of foetal growth (*e.g*. Oliver *et al*., 1992). Ovine placental lactogen is present in the foetal and maternal circulation and is postulated to be involved in the repartitioning of maternal nutrients to the foetus; stimulating the foetus to use substrates for growth (Gootwine, 2004). Concentrations of oPL in the Cheviot and Suffolk ewes were consistent with those reported in mid- to late pregnancy in sheep (Gluckman *et al*., 1979). The higher birth weight of the SC lambs compared to the CS lambs was associated with higher maternal oPL concentrations in the Suffolk dams at days 90 and 110, but not at day 130 of gestation, which may account for the enhanced foetal development experienced by the SC offspring. In support of this observation, lambs born to ewes actively immunised against oPL at five months of age were significantly heavier than lambs born to

control ewes. Moreover, immunised ewes showed enhanced oPL production by the placenta and an increase in serum oPL bioactivity in late gestation relative to controls (Leibovich *et al*., 2000).

Placental lactogen also induces insulin resistance, which appears to be important in ensuring glucose availability to the placenta (Gluckman & Pinal, 2002). At days 90 and 110 of gestation, plasma insulin concentrations were similar between the breeds. However, at day 130 of gestation, the Cheviot ewes (who had smaller lambs) exhibited insulin resistance. Although there were no differences in plasma glucose concentrations between the Cheviot and Suffolk ewes, it is possible that glucose uptake by the four foetal genotypes may have differed.

IGF-1 is a major foetal growth-promoting hormone in late gestation, however plasma concentrations of IGF-1 did not differ between the breeds at any of the stages measured. In the rat, maternally administered IGF-1 overcomes maternal constraint without affecting placental size, possibly by augmenting the ability to supply nutrients to the foetus and support growth (Gluckman *et al*., 1992). However, in lines of sheep selected for either high or low levels of IGF-1, lambs born to ewes from the high line have lower birth weight than lambs born to the low line (Blair *et al.,* 2002). It appears that the influence of maternal levels of IGF-1 may differ depending on what caused the IGF-1 levels to be different. This in turn would suggest that the IGF-1 levels are reflective of some other mechanism, rather than being the causative factor.

Models of maternal constraint have shown that duration of pregnancy is often reduced when crossbred foetuses are gestated in a small dam (Hunter, 1956; Joubert & Hunter, 1958). Conversely, a crossbred foetus gestated in a large dam may be carried *in utero* for longer (Hunter, 1956). Gestation length was not measured in the present study but most likely accounts for only a small proportion of the total variation in birth weight.

The present study tentatively suggests that Cheviot dams are able to constrain the growth of a crossbred foetus that has the genetic potential to grow larger. This finding is only tentative because while the breed by rank interaction was not significant, the statistical power of this test was poor due to low sub-group numbers. There are 3 possible scenarios for the dam breed by rank interaction. Firstly, twins suffer the same degree of constraint in both dam breeds (as suggested in these results), secondly twins born to Suffolk dams are proportionately heavier than twins born to Cheviot dams and thirdly, twins born to Cheviot dams are proportionately heavier than twins born to Suffolk dams. If an interaction of the second scenario occurs, twins born to Cheviot dams in this trial would have received a greater weight adjustment in the analysis making it more difficult to detect maternal constraint by the Cheviot dam. In the unlikely event of the third scenario the birth weight of twins from Cheviot dams would have been under-compensated, and the implication of maternal constraint could be wrong. Therefore we have a "tentative" rather than "conclusive" result of maternal constraint by the small Cheviot ewes, requiring that further studies be undertaken.

CONCLUSION

In summary, we have shown, albeit not conclusively, that ovine foetal growth can either be restricted below (maternal constraint) or enhanced above, the normal genetic potential for the breed by varying maternal size. The markedly lower concentration of oPL in the plasma of Cheviot ewes compared with Suffolk ewes at days 90 and 110 of gestation may have impacted on placental growth and vascular development, thereby altering placental transport capacity, and hence, contributing to the constraint in foetal growth.

Recent knowledge about epigenetics and foetal programming suggest that maternal diet and maternal programming should also be considered as further causes of maternal constraint (Harding, 2001; Gluckman & Hanson, 2006).

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REFERENCES

Allen, W.R.; Wilsher, S.; Turnball, C.; Stewart, F.; Ousey, J.; Rossadale, P.D.; Fowden, A.L. 2002: Influence of maternal size on placental, fetal and postnatal growth in the horse. I. Development *in utero*. *Reproduction* **123**: 445-453.

Ashour, M.B.; Gee, S.J.; Hammock, B.D. 1987: Use of a 96-well microplate reader for measuring routine enzyme activities. *Analytical Biochemistry* **166**: 353-360.

Bauer, M.K.; Harding, J.E.; Bassett, N.S.; Breier, B.H.; Oliver, M.H.; Gallaher, B.H. 1998: Fetal growth and placental function. *Molecular Cell Endocrinology* **140**: 115-120.

Blair, H.T.; McCutcheon, S.N.; Breier, B.H.; Gluckman, P.D.; 2002: Correlated response in lamb birthweight following about 5 generations of selection for high

or low plasma IGF-1. In *'7th World Congress on Genetics Applied to Livestock Production'. Montpellier, France pp.* Communication No 19–04.

Blum, W.F.; Breier, B.H. 1994: Radioimmunoassays for IGFs and IGFBPs. *Growth Regulation* **4** *Supplement 1*: 11-19.

Cowley, D.E.; Pomp, D.; Atchley, W.R.; Eisen, E.J.; Hawkins, B. 1989: The impact of maternal uterine genotype on postnatal growth and adult body size in mice. *Genetics* **122**: 193-203.

Ferrell, C.L. 1991: Maternal and fetal influences on uterine and conceptus in the cow. I. Growth of tissues of the gravid uterus. *Journal of Animal Science* **69**: 1945-1953.

Gluckman, P.D.; Uthne, K.; Styne, D.M.; Kaplan, S.L.; Rudolph, A.M.; Grumbach, M.M. 1979: Hormone ontogeny in the ovine fetus IV. Serum somatomedin activity in the fetal and neonatal lamb and pregnant ewe: correlation with maternal and fetal growth hormone, prolactin, and chorionic somatomammotropin. *Pediatric Research* **14**: 194-196.

Gluckman, P.D.; Morel, P.C.H.; Ambler, G.R.; Breier, B.H.; Blair, H.T.; McCutcheon, S.N. 1992: Elevating maternal insulin-like growth factor-I in mice and rats alters the pattern of fetal growth by removing maternal constraint. *Journal of Endocrinology* **134**: R1-R3.

Gluckman, P.D. 1997: Endocrine and nutritional regulation of prenatal growth. *Acta Paediatrica* **423** Suppl.: 153-157.

Gluckman, P.D.; Hanson, M.A. 2004: Maternal constraint of fetal growth and its consequences. *Seminars in Fetal and Neonatal Medicine* **9**: 419-425.

Gluckman, P.D.; Hanson, M.A. 2006: The Fetal Matrix: Evolution, Development and Disease. Cambridge, UK, Cambridge University Press, 2005.

Gluckman P.D.; Pinal, C.S. 2002: Maternal-placental-fetal interactions in the endocrine regulation of fetal growth. *Endocrine* **19**: 81-89.

Gootwine, E. 2004: Placental hormones and fetal-placental development. *Animal Reproduction Science* **82-83**: 551-566.

Harding, J.E. 2001: The nutritional basis of the fetal origins of adult disease. *International Journal of Epidemiology* **30**: 15-23.

Harding, J.E.; Evans, P.C.; Gluckman, P.D. 1997: Maternal growth hormone treatment increases placental diffusion capacity but not fetal or placental growth in sheep. *Endocrinology* **138**: 5352-5358.

Hunter, G.L. 1956: The maternal influence on size in sheep. *Journal of Agricultural Science* **48**: 36-64.

Joubert, D.M.; Hammond, J. 1958: A crossbreeding experiment with cattle, with special reference to the maternal effect in South Devon-Dexter crosses. *Journal of Agricultural Science* **51**: 325-341.

Kerslake, J.I.; Everett-Hincks, J.M.; Campbell, A.W. 2005: Lamb survival: a new examination of an old problem. *New Zealand Society of Animal Production* **65**: 13-18.

Kunst, A.; Draeger, B.; Ziegenhorn, J. 1984: Colorimetric methods with glucose oxidase and peroxidase. In: Bergmeyer, H.U. ed. Methods of Enzymatic Analysis. Verlag Chemie, Weinheim, Germany. Pp. 178-185.

Leibovich, H.; Gertler, A.; Bazer, F.W.; Gootwine, E. 2000: Active immunization of ewes against ovine placental lactogen increases birth weight of lambs and milk production with no adverse effect on conception rate. *Animal Reproduction Science* **64**: 33-47.

Oliver, M.H.; Harding, J.E.; Breier, B.H.; Evans, P.C.; Gluckman, P.D. 1992: The nutritional regulation of circulating placental lactogen in fetal sheep. *Pediatric Research* **31**: 520-523.

Oliver, M.H.; Harding, J.E.; Breier, B.H.; Evans, P.C.; Gluckman, P.D. 1993: Glucose but not a mixed amino acid infusion regulates plasma insulin-like growth factor-I concentrations in fetal sheep. *Pediatric Research* **34**: 62-65.

Rattray, P.V.; Garrett, W.N.; East, N.E.; Hinman, N. 1974: Growth, development and composition of the ovine conceptus and mammary gland during pregnancy. *Journal of Animal Science* **38**: 613-626.

SAS 2005: SAS 8.02, SAS Inst. Inc.

Scales, G.H.; Burton, R.N.; Moss, R.A. 1986: Lamb mortality, birthweight and nutrition in late pregnancy. *New Zealand Journal of Agricultural Research* **29**: 75-82.

Stafford, K.J.; Kenyon, P.R.; Morris, S.T.; West, D.M. 2007: The physical state and metabolic status of lambs of different birth rank soon after birth. *Livestock Science.* In Press, Corrected Proof, *Available online 24 January 2007.*

Wallace, J.; Bourke, D.; Da Silva, P.; Aitken, R. 2001: Nutrient partitioning during adolescent pregnancy. *Reproduction* **122**: 347–357.

Walton, A.; Hammond, J. 1938: The maternal effects on growth and conformation in Shire horse-Shetland pony crosses. *Proceedings of the Royal Society of London, Series B, Biological Science,* **125**: 311-335.

Wilson, M.E.; Biensen, N.J.; Youngs, C.R.; Ford, S.P. 1998: Development of Meishan and Yorkshire littermate conceptuses in either a Meishan or Yorkshire uterine environment at day 90 of gestation to term. *Biology of Reproduction* **58**: 905-910.