BRIEF COMMUNICATION: Are fetal programming effects, due to maternal early pregnancy nutrition, evident in adult male offspring in sheep?

SJ Pain*, K Asmad, LM Fermin, 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: S.J.Pain@massey.ac.nz*

Keywords: sheep; maternal nutrition; fetal programming; male offspring

Introduction

Manipulation of fetal development processes via maternal nutritional intervention can affect the physiology and cellular function of adult offspring through a process known as fetal programming (Harding & Johnson 1995). Nutritional status of the dam in the first trimester of pregnancy is crucial to fetal development because, although fetal energy requirements in early pregnancy are relatively small, and thus, total nutrient demand is typically low, fetal metabolic activity is very high and organogenesis is occurring (Harding & Johnson 1995).

Evidence from a variety of animal species indicates that maternal nutritional status during pregnancy can influence fetal weight, fetal metabolism and endocrine axis function, birth weight and subsequent postnatal metabolic and endocrine function, growth and reproductive performance, and carcass quality (Whorwood et al. 2001; Da Silva et al. 2001; Bielli et al. 2002; Bloomfield et al. 2003; Kind et al. 2003; Daniel et al. 2007; Kenyon 2008; Kotsampasi et al. 2009; Fermin 2013; Asmad 2014)

The study reported here examines the effects of early pregnancy maternal nutrition on the gross physiology of twin-born male offspring in adulthood.

Materials and methods

All animals were maintained under commercial farming conditions at Massey University's Keeble Sheep and Beef farm, five kilometres south of Palmerston North. This study was conducted with approval from the Massey University Animal Ethics

Table 1 Live weight, body condition score and organ weight/measurements of rams (3 years of age) born to dams fed sub-maintenance, maintenance or *ad libitum* nutrition in early pregnancy (day 21 to 50 of gestation).

	Maternal nutrition treatment in early pregnancy		
	Ad libitum	Maintenance	Sub-maintenance
Ram offspring:			
Live weight (kg)	90.0 ± 2.64	94.0 ± 1.97	93.6 ± 2.10
Body condition score (1-5)	3.8 ± 0.13	3.8 ± 0.25	3.7 ± 0.18
Heart (g)	375.5 ± 18.82 *	414.0 ± 14.78 †	391.3 ± 12.11 *†
Spleen (g)	114.4 ± 7.95	129.9 ± 9.62	133.1 ± 8.71
Liver (g)	1279.8 ± 45.62	1386.8 ± 59.00	1358.4 ± 43.96
Thyroid (g)	7.5 ± 0.65	7.7 ± 0.42	8.3 ± 0.71
Adrenal gland (g)			
Left	2.3 ± 0.17	2.4 ± 0.08	2.6 ± 0.17
Right	1.9 ± 0.18	2.4 ± 0.13	2.6 ± 0.17
Kidney (g)			
Left	104.5 ± 5.41	112.7 ± 3.90	106.5 ± 3.71
Right	102.8 ± 5.04 *	114.2 ± 4.49 †	108.0 ± 4.09 *†
Pancreas (g)	108.4 ± 8.37	113.7 ± 4.26	111.6 ± 5.82
Omental fat (g)	2094.2 ± 237.94	2080.7 ± 299.08	1978.5 ± 198.08
Semitendinosus muscle [1]			
Weight (g)	239.9 ± 5.36	255.1 ± 9.01	252.4 ± 7.17
Length (mm)	153.1 ± 3.89	152.5 ± 4.10	150.0 ± 3.87
Testis			
Left weight (g)	244.2 ± 14.07	241.3 ± 8.04	226.4 ± 11.27
Right weight (g)	249.8 ± 15.50	241.7 ± 7.68	226.7 ± 11.33
Left circumference (mm)	192.9 ± 5.21	185.4 ± 1.86	182.8 ± 3.63
Right circumference (mm)	192.6 ± 4.66	186.9 ± 2.41	182.0 ± 2.82

*† indicate values in rows that tended to differ, P<0.10

[1] semitendinosus muscle was taken from the left hind leg

Committee.

The ram offspring used in the present study were taken from a larger study described by Kenyon et al (2011). Romney ewes (n = 879, three- to five-year-old multiparous ewes) from a commercial flock, conceived to artificial insemination using fresh semen from one of five Romney rams, were randomly allocated to one of three nutritional treatments from day 21 of pregnancy (P21) until day 50 (P50) of pregnancy: sub-maintenance (S: total ewe liveweight change achieved P21-P50, –0.15 ± 0.02 kg/day), maintenance (M: total ewe liveweight change achieved P21-P50, 0.02 ± 0.02 kg/day) or *ad libitum* (A: total ewe liveweight change achieved P21-P50, 0.15 ± 0.02 kg/day) (Kenyon et al. 2011).

The study reported here examines the effects of early pregnancy (P21-P50) nutrition (S vs M vs A) of the dam on the gross physiology of twin-born-and-reared male offspring at three years of age (S-rams (n=11), M-rams (n= 10), A-rams (n=8)). Rams were body condition scored (Jefferies 1961, scale 0-5 including half units) weighed and then euthanased by a commercial slaughterman, and organs (heart, spleen, liver, thyroid, adrenal glands, kidneys, pancreas, visceral (omental) fat, testes and the semitendinosus muscle of the left hind quarter) were immediately collected onto ice and weighed/measured.

All data was analysed using the PROC MIXED procedure in SAS (Statistical Analysis Software, SAS® 9.4, SAS Institute, North Carolina, USA) with dam nutrition fitted as a fixed effect. Organ weights/measurements were analysed with and without ram live weight as a covariate.

Results and discussion

Live weight and body condition score of the rams did not differ (P>0.10) at slaughter (Table 1). The heart and right kidney of M-rams tended (P<0.10) to be heavier compared to those from A-rams (Table 1), however when live weight was included in the statisical analysis as a covariate these effects were no longer apparent. There were no differences (P>0.10) in any of the other organs measured.

These results indicate that maternal nutrition, at the levels applied in this study, had little impact on the post-natal growth and development of male offspring at a gross physiological level. It's likely that more extreme under or over nutrition of the dam during pregnancy would elicit more extreme responses in the offspring.

The larger co-hort of rams (from which the smaller sample of rams used in the present study were taken) were monitored closely from birth to 27 months of age and showed no evidence of growth or reproductive performance differences (Fermin 2013; Asmad 2014). This supports the lack of gross physiological differences observed in the present study. This is likely because the maternal nutritional treatments were not sufficiently extreme to significantly affect fetal growth (Martín et al. 2012) and were unlikely to affect dam lactational performance, and thus, had little impact on postnatal lamb growth.

The study reported here demonstrates that maternal nutrition during early pregnancy does not appear to result in large alterations to the physical phenotype of adult male offspring; however, the tissue collected from this study will be investigated for histological and gene expression differences that may influence physiological function.

Acknowledgements

The authors would like to acknowledge Massey University and Gravida: National Centre for Growth and Development, for funding this research.

References

Asmad K 2014. The effect of ewe nutrition during pregnancy on the reproductive system of the offspring. Thesis for PhD in Animal Science, Massey University, Palmerston North, New Zealand.

Bielli A, Perez R, Pedrana G, Milton JTB, Lopez A, Blackberry MA, Duncombe G, Rodriguez-Martinez H, Martin GB 2002. Low maternal nutrition during pregnancy reduces the number of Sertoli cells in the newborn lamb. Reproduction Fertility and Development 14(6): 333-337.

Bloomfield FH, Oliver MH, Giannoulias CD, Gluckman PD, Harding JE, Challis JRG 2003. Brief undernutrition in late-gestation sheep programs the hypothalamic-pituitary-adrenal axis in adult offspring. Endocrinology 144(7): 2933-2940.

Da Silva P, Aitken RP, Rhind SM, Racey PA, Wallace JM 2001. Influence of placentally mediated fetal growth restriction on the onset of puberty in male and female lambs. Reproduction 122(3): 375-383.

Daniel Z, Brameld JM, Craigon J, Scollan ND, Buttery PJ 2007. Effect of maternal dietary restriction during pregnancy on lamb carcass characteristics and muscle fiber composition. Journal of Animal Science 85(6): 1565-1576.

Fermin L 2013. The effect of nutritional fetal programming on post-pubertal male reproduction in sheep. Thesis for Master of Veterinary Studies, Massey University, Palmerston North, New Zealand.

Harding JE, Johnston BM 1995. Nutrition and fetal growth. Reproduction Fertility and Development 7(3): 539-547.

Jefferies BC 1961. Body condition scoring and its use in management. Tasmanian Journal of Agriculture 32: 19-21.

Kenyon PR, Pain SJ, Hutton PJ, Jenkinson CMC, Morris ST, Peterson SW, Firth EC, Blair HT 2011. The effects of twin bearing ewe nutritional level in pregnancy on ewe and lamb performance

to weaning. Journal of Animal Production Science 51: 406-415.

Kenyon PR 2008. A review of *in-utero* environmental effects on sheep production. Proceedings of the New Zealand Society of Animal Production 68: 142–155.

Kind KL, Clifton PM, Grant PA, Owens PC, Sohlstrom A, Roberts CT, Robinson JS, Owens JA 2003. Effect of maternal feed restriction during pregnancy on glucose tolerance in the adult guinea pig. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 284(1): R140-R152.

Kotsampasi B, Balaskas C, Papadomichelakis G, Chadio SE 2009. Reduced Sertoli cell number and altered pituitary responsiveness in male lambs undernourished in utero. Animal Reproduction Science 114(1-3): 135-147.

Martín NP 2012. Ewe nutrition during pregnancy: effects on the development of twin foetuses. Thesis for Master of AgriScience, Massey University, Palmerston North, New Zealand.

Whorwood CB, Firth KM, Budge H, Symonds ME 2001. Maternal undernutrition during early to midgestation programs tissue-specific alterations in the expression of the glucocorticoid receptor, 11β-hydroxysteroid dehydrogenase isoforms, and type 1 angiotensin ii receptor in neonatal sheep. Endocrinology 142(7): 2854-2864.