BRIEF COMMUNICATION: Comparison of early embryo development in Cheviot and Suffolk breeds of sheep

LM Fermin*, SJ Pain, HT Blair and PR Kenyon

International Sheep Research Centre, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.
**Corresponding author. Email: L.M.Fermin@massey.ac.nz*

Keywords: embryonic growth; uterine constraint; sheep

Introduction

Maternal constraint is the major non-genetic factor determining the size of the fetus at term (Gluckman & Hanson 2004). Previous studies in various animal models have reported the effect of maternal size on birth size and postnatal growth, (Allen et al. 2002; Gardner et al. 2007) whereby fetal development and birth weight are reduced in a restricted maternal uterine environment, while enhanced in a luxurious uterine environment.

Interestingly, recent evidence suggests that regulation of fetal growth patterns may be initiated during the early embryonic period, when cell division, implantation and organogenesis occur (Dziuk 1992; van Mourik et al., 2009). To date, reciprocal sheep embryo transfer (ET) studies in breeds of dissimilar mature body size (ie, Suffolk (large) and Cheviot (small)) have demonstrated differences in embryo size at day 19 of gestation and subsequent lamb birth weight (Sharma et al. 2012a, 2013). These studies suggest that differential interactions between the developing zygote and the dam may result in altered development (Gaviria & Hernandez 1994; Fligny et al. 2009) and impact subsequent fetal-growth trajectory. Therefore, there is a need to further understand the effects of maternal genotype on the patterns of embryonic and fetal development. However, it should be noted that the embryo transfer process itself has the potential to confound effects on fetal development and growth (Walker et al. 1996). The present study aims to examine the relationship between the maternal uterine environment and developing purebred Suffolk and Cheviot embryos without the potential confounding influence of embryo transfer. The findings reported in this brief communication focus on Suffolk and Cheviot embryo size measurements at day 19 and 21 of gestation.

Materials and methods

The animals used in this study were managed together under commercial farming conditions at the Massey University's Keeble Farm, Palmerston North, New Zealand. This experiment was approved by the Massey University Animal Ethics Committee. Cheviot (C) and Suffolk (S) sheep breeds were used to provide genotypes with dissimilar mature body size.

Oestrus was synchronised in 160 ewes, C (n=80) and S (n=80), using intravaginal progesterone releasing devices (Eazi-breed CIDR; Pharmacia; Auckland, New Zealand) for 13 days, and a single treatment with PGF2α following removal of the CIDR. Semen was collection via electro-ejaculation from four rams per breed. Ewes of each breed were inseminated (day 0; D0) laparoscopically with 0.5 ml of fresh semen from a ram of the same breed, 32 hours after CIDR removal. From D0 to day 21 (D21) blood samples were collected daily via jugular venipuncture from a subsample of ewes (12 Suffolk and 12 Cheviot). Plasma isolated from these blood samples are currently undergoing analysis for progesterone, insulin like growth factor 1 (IGF-1), insulin and adiponectin.

At D19 and D21 of gestation randomly selected ewes (n=5, 7 respectively) were euthanized via captive bolt and exsanguination. The uterus was removed, placed on ice, weighed and uterine horn length was measured. Each uterine horn was flushed with 20 ml (0.9%) sterile saline for recovery of the embryo. The embryo was identified under a microscope and dissected free of extraembryonic membranes if necessary and preserved in a 5 ml vial containing 10% buffered formal saline. A total of 24 (twin) embryos (10 and 14 for D19 and D21 respectively) were examined.

Multiple maternal uterine tissue samples, both ipsilateral and contralateral to the corpus luteum, were collected at the same time as embryos were recovered (D19 and D21). These tissue samples are currently being used to determine immunohistological localisation and gene expression levels of various hormones/growth factors that may be involved in the mechanism(s) responsible for the observed changes. Analysis of these samples is on-going and as such they are not included in this communication.

Embryo measurements

D19 and D21 embryos were photographed with a stereomicroscope (Leica Mz12 fitted with DFC 320 camera; Leica Microsystems, Heerbrugg, Switzerland). The images were then examined using ImageJ (U. S. National Institutes of Health, Bethesda, Maryland, USA).

Embryo length and width, and heart bulge (HB) width was determine for both D19 and D21 embryos. Embryo length (curved) (CR) was defined as the distance from the medial aspect of the head to the tip of the embryonic tail, following the outer curvature of the embryo. Embryo width was defined as the distance

between the two widest points of embryos with the line passing just below and not including the heart bulge but including somites. HB width was defined as the distance between the two widest points of the HB with the line passing through the midsection of the HB and excluding somites.

Statistical Analysis

Analysis of variance using the Generalised Linear Model procedure in Minitab (v16.2) was used to determine the effect of breed on embryo size measurements at each time point.

Results and discussion

At D19, Cheviot CR was shorter (P<0.05) than Suffolk CR. This result supports previous findings of Sharma et al. (2013) who reported differences in embryo size at D19 in an embryo transfer study. Further, the embryo size measurements reported here were similar in magnitude to those reported by Sharma et al. (2013), suggesting that the embryo transfer process does not greatly impact embryo size at this stage of gestation in this two breed model. The present results combined with those of Sharma et al. (2013) indicate that a Suffolk embryo developing in the Suffolk dam grows at a faster rate to D19 compared to a Cheviot embryo developing in a Cheviot dam. This suggests that between D0 and D19 there is a differential interaction between the dam and the developing, implanting embryo in these two breeds.

Table 1 Suffolk and Cheviot embryo measurements at Day19 and 21 (Crown rump length (CR), embryo and Heart bulge (HB) width) of gestation. Values are least squares means ± standard error of the mean. Different superscripts within main effects indicate significant differences (P<0.05).

		Breed	
		Suffolk	Cheviot
Day19		*n=4*	*n=6*
	CR length (µm)	13.28±1.26^{b}	9.25±0.89^{a}
	Embryo width (µm)	1.46±0.18	1.11±0.15
	HB width (µm)	1.56±0.15	1.23±0.12
Day21		*n=4*	*n=10*
	CR length (µm)	17.20±0.74	17.08±0.53
	Embryo width (µm)	2.70±0.22	2.77±0.14
	HB width (µm)	1.98±0.14	1.73±0.09

It is proposed, that in very early pregnancy, when maternal physical uterine capacity is not a constraint, the apparent differences in embryo size/growth could be due to a number of potential mechanisms involving the interaction of the embryonic trophoblast and the maternal uterus (Sharma et al. 2013). These include circulating maternal progesterone concentrations (P4) (Satterfield et al. 2006), and the expression of progesterone receptor (PR) in the maternal uterus (Sequeira et al., 2012; using the same S/C model) which may play a role in the implantation process. Analysis of maternal blood samples and uterine tissue collected in the current study will help to define the role of P4 and PR on the embryo sizes observed.

Interestingly, there were no embryo size differences (P>0.05) evident at D21. Implantation is completed by D21 in sheep (Gaviria & Hernandez, 1994), which may further emphasise that the mechanisms responsible for the differences seen at D19 are mediated by the implanting trophoblast and/or the receptivity of the maternal uterus to embryo implantation. Further work is required to fully understand the apparent disappearance of size differences. However, in support of these findings, Sharma et al. in a series of studies reported differences at D19 (Sharma et al. 2013); no difference at D55 (Sharma et al. 2012b) but a difference again at birth (Sharma et al. 2012a). This effect has previously been described as the "hourglass model" (Duboule 1994), characterised by observed morphologic differences at Day 19, followed by a period of reduced variability lasting to approximately D90 and ending at birth when a progressive divergence in development occurs and phenotypic differences are observed.

The present results show that differences in embryo size are evident at D19 of gestation and that the mechanisms driving this effect are acting between D0 and D19 and are simply not an artefact of ET. Future studies investigating the mechanisms by which this effect on embryo size occurs are warranted and ongoing.

Acknowledgements

The authors would like to acknowledge Massey University and Gravida for funding this research.

References

Allen WR, Wilsher S, Turnbull C, Stewart F, Ousey J, Rossdale PD, Fowden AL 2002. Influence of maternal size on placental, fetal and postnatal growth in the horse. I. Development in utero. Reproduction 123: 445-453.

Duboule D 1994. Temporal colinearity and the phylotypic progression: a basis for the stability of a vertebrate Bauplan and the evolution of

morphologies through heterochrony. Development. Pg. 135-142.

Dziuk PJ 1992. Embryonic development and fetal growth. Animal Reproduction Science 28: 299-308.

Fligny C, Hatia S, Amireault P, Mallet J, Côté F 2009. Mammalian prenatal development: the influence of maternally derived molecules. BioEssays 31: 935-943.

Gardner DS, Buttery PJ, Daniel Z, Symonds ME 2007. Factors affecting birth weight in sheep: maternal environment. Reproduction 133: 297-307.

Gaviria MT, Hernandez A 1994. Morphometry of implantation in sheep. I. Trophoblast attachment, modification of the uterine lining, conceptus size and embryo location. Theriogenology 41: 1139-1149.

Gluckman PD, Hanson MA 2004. Maternal constraint of fetal growth and its consequences. Seminars in Fetal and Neonatal Medicine 9: 419-425.

Hunter GL 1956. The maternal influence on size in sheep. The Journal of Agricultural Science 48: 36-60. [Accessed 1956].

Satterfield MC, Bazer FW, Spencer TE 2006. Progesterone regulation of preimplantation conceptus growth and Galectin 15 (LGALS15) in the ovine uterus. Biology of Reproduction 75: 289-296.

Sequeira M, Pain SJ, Sartore I, Meikle A, Kenyon PR, Blair HT 2012. Uterine progesterone receptor in Suffolk and Cheviot dams at gestation Day 19, following embryo transfer. Proceedings of the New Zealand Society of Animal Production 72: 35-37.

Sharma RK, Blair HT, Jenkinson CMC, Kenyon PR, Cockrem JF, Parkinson TJ 2012a. Uterine environment as a regulator of birth weight and body dimensions of newborn lambs. Journal of Animal Science 90: 1338-1348.

Sharma RK, Blair HT, Kenyon PR, Jenkinson CMC, Parkinson TJ 2012b. The effects of uterine environment upon fetal development in sheep: an untrasonic study. Proceedings of the 29th Biennial Conference of the Australian Society of Animal Production Pg. 58.

Sharma RK, Parkinson TJ, Kenyon PR, Jenkinson CMC, Blair HT 2013. Uterine environment and early embryonic development in sheep. Small Ruminant Research 115: 67-70.

van Mourik MSM, Macklon NS, Heijnen CJ 2009. Embryonic implantation: cytokines, adhesion molecules, and immune cells in establishing an implantation environment. Journal of Leukocyte Biology 85: 4-19.

Walker SK, Hartwich KM, Seamark RF 1996. The production of unusually large offspring following embryo transfer manipulation: concepts and challenges. Theriogenology 45: 111-120.