BRIEF COMMUNICATION: Impact of maternal plane of nutrition, ewe weight and twinning on fetal mammary gland development in sheep

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Abstract

This study evaluated the effects of maternal nutrition, weight and gestation rank on fetal mammary gland development. Light (L) and heavy (H) single- and twin-bearing ewes were fed *ad libitum* (A) or maintenance (M) nutritional regimens from day 21 to day 140 of gestation and euthanised for tissue collection. Plane of maternal nutrition or maternal weight did not affect histomorphometry of fetal mammary glands. Singleton fetuses had 43% greater total duct area (P<0.05), 62% greater secretory cell area and 32% fewer ducts (P<0.05) but similar total lumen area, cell size and total cell number than twins. These results indicate that twinning reduces area of mammary ducts and secretory cell area while maternal weight and plane of nutrition from day 21 have little impact on mammary morphology at day 140 of gestation.

Keywords: mammary gland; sheep; nutrition; twinning; ewe weight

Introduction

There is substantial cellular development of the mammary gland during prenatal life in sheep providing the foundation of the future mammary gland (Jenkinson 2003). From 100 to 140 days of gestation there is significant development of the secondary ducts which are lined with epithelial cells that form the future parenchyma (i.e., milk secretory tissue). Our research has demonstrated that both maternal weight and plane of maternal nutrition during gestation can influence the resulting offsprings' lactation performance (van der Linden et al. 2009; Paten et al. 2017). While the effect of maternal weight and plane of nutrition on histomorphometry of the fetal mammary gland at day 100 of gestation has been described (van der Linden et al. 2009) the impact of maternal nutrition, maternal weight and twinning on morphology of the mammary gland in late gestation has not. Fetal nutrient requirements and uterine space requirements during the last six weeks of gestation increase exponentially, especially in multiple-bearing ewes (McCoard et al. 2017). Therefore, morphological differences observed at day 100 of gestation may not reflect the morphology of the mammary gland in late gestation. Although the magnitude of the aforementioned changes in lactation performance (i.e., greater milk and lactose yields) of the offspring was not sufficient to influence grand-offspring growth rates to weaning in that study (Paten et al. 2017), it provides an interesting model system to understand how the *in utero* environment affects fetal mammary development which can influence future lactation performance.

The objective was to evaluate the effect of maternal weight and plane of nutrition during gestation, and twinning, on mammary mass and morphological development of the fetal mammary gland at 140 days of gestation.

Materials and methods

All procedures in this study were approved by the Massey University Animal Ethics Committee. Mammary samples used in this study were derived from the larger studies previously described (van der Linden et al. 2009; Blair et al. 2010). Briefly, light (L; 42.5 ± 0.2 kg) and heavy (H; 60.8 ± 0.2 kg) single- and twin-bearing ewes were randomly assigned to treatment groups and fed either *ad libitum* (A) or maintenance (M) nutritional regimens from day 21 to day 140 of gestation.

At day 140 of gestation, fetal mammary glands from a random subset of ewes were collected (HA: n=8; HM; n=10; LA: n=11; LM n=8) and histomorphometric analysis undertaken to determine the number of ducts and epithelial cells, area of ducts and secretory cell area (i.e., duct area minus the ductal lumen area), estimated epithelial cell size, using previously described methods (van der Linden et al. 2009). Mammary epithelial cell number was estimated by randomly selecting four ductal regions per gland. Within each region, two separate areas of secretory cells each containing approximately 50 epithelial cells were individually measured and the number of epithelial cells in each area was counted. Average size of epithelial cells and number per unit area were calculated by dividing the mammary duct area by the number of cells in that area.

Histomorphometric data were analysed using the GLM procedure in SAS (v9.2, SAS Inst. Inc., Cary, NC) with a linear model that included the fixed effects of maternal weight (L or H), nutrition (A or M) and twinning (S or T) and the interaction of maternal weight, nutrition and twinning. Covariates (maternal live weight, fetal weight, or fetal mammary gland weight) that were not significant (P>0.05) were removed from the model. Data are expressed as least-square means ± SEM.

Results and discussion

Modifying the plane of maternal nutrition is an established model to study mammary gland development in the ruminant during the productive cycle (Villeneuve et al.

2010, Paten et al. 2017). At day 140 of gestation, there was no effect (P>0.05) of maternal plane of nutrition on fetal mammary histomorphometry (Table 1) which is consistent with previous observations at day 100 of gestation in a separate cohort of fetuses from the same animal trial (van der Linden et al. 2009). Our previous studies using animals from the same trial reported that mammary glands of fetuses carried by M- compared with A-dams were heavier at 100 days gestation (van der Linden et al. 2009) but tended to be lighter at day 140 of gestation (Sciascia et al. 2015). A smaller mammary gland in day 140 gestation fetuses from nutrient restricted ewes coupled with no change in ductal histomorphometry at day 140 gestation indicates a smaller mammary fat pad mass which is consistent with our prior hypothesis of lower fat pad hyperplasia (Sciascia et al. 2015). The reverse is evident at 100 days of gestation (van der Linden et al., 2009). These results highlight the plasticity of the fetal mammary gland. Collectively, results of these studies indicate that changes in lactation performance of ewes whose dams were exposed to different planes of nutrition during gestation are unlikely to be associated with alterations in ductal and epithelial-cell development of the mammary gland (future parenchyma). Rather, changes in protein synthetic capacity for tissue accretion via alterations in MAPK/mTOR signalling pathways in the fat pad during the late fetal period may play a greater role (Sciascia et al. 2015). However, while the fat pad is almost fully developed before birth (Hovey et al. 1999), early postnatal life is also a critical time for mammary development and future lactation performance (Koch 1972, Akers 2017). Further work is required to establish whether lactation performance of offspring from ewes fed differentially during gestation is also influenced by the early postnatal environment.

A positive correlation has been observed between maternal weight and offspring mammary gland weight and lamb live weight (Kenyon et al. 2009; van der Linden et al. 2009). In the present study, maternal weight had no effect on the histomorphometry of the fetal mammary glands at day 140 of gestation, which contrasts with observations at day 100 of gestation where greater duct area was observed in fetuses from heavy compared with light ewes (van der Linden et al. 2009). It was speculated that because the ducts form the base for secretory cell development (Gardner & Hogue 1966), greater milk yield in offspring from heavy compared with light ewes was associated with larger duct area (van der Linden et al. 2009). However, the lack of effect of maternal weight on ductal morphology at day 140 of gestation challenges this notion. Further evaluation of developmental changes such as biochemical signalling in the mammary gland (e.g., MAPK/mTOR) and evaluation of protein synthetic capacity for tissue accretion, previously observed to mediate, at least in part, the impact of maternal plane of nutrition on offspring lactation performance (Sciascia et al. 2015) may provide further insights.

Singleton fetuses had 43% greater total duct area, 62% greater secretory cell area and 31% fewer ducts but similar total lumen area, cell size and total cell number compared with their twin counterparts. These results indicate fetal growth restriction as a result of twinning reduces mammary ductal development and secretory cell area. The greater duct and associated secretory cell area but reduced number of ducts with no change in epithelial cell number or size in singletons compared to twins was unexpected and is difficult to explain. Irrespective of the number of ducts, it is the total amount of potential secretory tissue that is likely to be important for future milk production. There is growing evidence for the importance of mammary development in early life on future milk production potential (Akers 2017). However, the impact of being born a twin compared with a single on secretory cell area of the mammary gland prior to birth and the implications for future milk production has not been evaluated to our knowledge, but is worthy of investigation given the increased milk demand on ewes rearing multiples and high incidence of multiples in New Zealand sheep flocks (McCoard et al. 2017).

Table 1 Effects of maternal size (heavy *vs.* light), maternal nutrition from day 21-140 of gestation (*ad libitum vs.* maintenance) and birth rank (twin *vs.* single) on total duct wall area (TDA), total lumen area (TLA), secretory cell area (SCA), total number of ducts (TDN), estimated epithelial cell size (duct area/number of cells) and total epithelial cell number in the fetal lamb mammary gland at day 140 of gestation[1].

Treatment	TDA (µm^2)	TLA (µm^2)	SCA (µm^2)	TDN	Epithelial cell size (µm^2)	Epithelial cell no.
Maternal nutrition						
Ad libitum	65886±7337	27706±34.35	38180±4475	216±23	70±1.6	949±104
Maintenance	64848±6834	22264±3200	42583±4168	183±22	71±1.5	996±99
Maternal weight						
Heavy	62411±7486	24763±3505	37647±4565	174±24	71±1.6	879±104
Light	68324±6699	25208±3137	43116±4086	225±21	70±1.5	1066±99
Birth Rank						
Single	76888±7234^a	26937±3387	49951±4412^a	162±23^a	70±1.5	1102±100
Twin	53846±6815^b	23034±3191	30812±4157^b	236±21^b	70±1.6	843±103

[1]No interactions between maternal nutrition, maternal size or birth rank were detected (P>0.10), therefore, only the main effects are reported. Different superscripts with main effects indicate significant differences (P<0.05).

This study contributes to our understanding of how the *in utero* environment influences fetal mammary development and future lactation performance, which is important for sheep meat production and for the emerging dairy sheep industry. It is important to note that the biochemical and molecular changes that occur in the mammary gland from late gestation to first lactation and the influence of maternal plane of nutrition, maternal weight and litter size, and the cell-cell interactions involved, remain to be evaluated.

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