ABSTRACT: In temperate climates, the cost of providing feed is greater in winter than in other seasons, causing ewes to be fed restricted rations during some periods of pregnancy. Epidemiological information indicates that undernutrition of the fetus may affect its health and performance in later life (i.e., fetal programming), and these effects may be passed between generations. The primary focus of the results presented in this paper is to examine the effects of feeding levels during pregnancy on a variety of traits from offspring at the fetal stage to 3.5 yr of age and also traits in the grand-offspring. Two studies are reported in which ewes were fed restricted diets during pregnancy, with a variety of fetal traits, offspring traits up to 3.5 yr of age, or grand-offspring traits up to 8 mo of age being measured. Study 2 also considered differences in dam size (heavy vs. light). In study 1, several fetal mammary gland measures indicated that milking ability may be enhanced in offspring from dams fed ad libitum during pregnancy. However, study 2 showed that mammary mass was greater in fetuses from dams fed at maintenance levels during pregnancy and that contemporaries of these fetuses produced greater protein and lactose yields in their first lactation. In the second lactation, the advantages in protein and lactose yields did not reoccur and ewes from ad libitum-fed dams produced greater fat yield. In study 2, grand-offspring whose granddams were fed at maintenance levels during pregnancy were lighter at birth in both the first and second parturitions than those whose granddams were fed ad libitum during pregnancy. First-parity grand-offspring whose granddams were fed maintenance levels during pregnancy achieved heavier BW by 40 to 50 d of age in the first lactation, which reflected the greater protein and lactose yields; however, no BW differences were present in second-parity lambs at the same age. A smaller proportion of first-parity ewe grand-offspring from heavy granddams that were fed ad libitum during pregnancy reached puberty at approximately 8 mo of age relative to the other granddam size and feeding groups. These results indicate that dam nutrition can affect the yield and composition of milk in their offspring and the BW and reproductive capability of their grand-offspring. Molecular and physiological mechanisms for these changes are being sought.

Key words: fetal programming, milk yield, reproduction

INTRODUCTION

There is now sufficient experimental evidence to accept that events around the time of conception, during pregnancy, and in early life can affect the future health and productivity of the resultant individual (e.g., Vickers et al., 2005; Burdge et al., 2007). This is not a surprising outcome because developmental biologists and animal breeders have long recognized consequences exist, such as permanent environmental effects and canalization (Lush, 1940; Waddington, 1961). What is relatively new is the recognition of potential mechanisms that allow these events to occur; these mechanisms are...
often grouped under the term epigenetics (Probst et al., 2009).

A challenge for sheep farmers using a grazing pastoral system is the year-round supply of quality feed for the ewe, particularly around the time of conception to optimize the number of eggs shed, during pregnancy for fetal growth and maternal body reserves, and during lactation for milk supply. In countries with a temperate environment, such as New Zealand, pasture growth typically declines during winter months. This has led to the practice of restricted feeding of ewes during early pregnancy to enable the conservation of feed supply for use later in pregnancy and possibly early lactation, on the expectation that the early-gestation fetus(es) require minimal nutrition because of their small mass (Robinson, 1977). However, the new knowledge mentioned previously indicates that fetuses may be exposed to programming effects (Symonds et al., 2007) in early pregnancy, indicating that this practice should be reinvestigated, with an emphasis on the long-term effects of restricted feeding during early pregnancy. Whereas several sheep studies have investigated the effects of different feeding regimens around the time of conception and during pregnancy on offspring phenotypes (e.g., Redmer et al., 2004; Wu et al., 2006; Poore et al., 2007; Rhodes et al., 2009; Todd et al., 2009), none has considered the impact of dam feeding on offspring lactation.

In sheep meat-producing systems, approximately 60 to 80% of feed is consumed by the reproducing ewe (Byerly, 1967). This has led to a debate regarding the most efficient ewe BW. Relatively few recent data exist on the effects of ewe size on the BW of lambs weaned and whether any interaction exists between ewe size and feeding amounts during pregnancy.

This paper reports on 2 studies. Study 1 compared the effects of various dam feeding regimens during pregnancy on fetal growth and organ size. Study 2 was designed to examine whether the fetal mammary gland differences found in study 1 were repeatable and whether the differences in fetal mammary tissues translated into differences in milk production. In addition, study 2 incorporated differing ewe production. In addition, study 2 incorporated differing ewe production.

**MATERIALS AND METHODS**

The studies were conducted at Massey University, 5 km south of Palmerston North, New Zealand. All animal handling procedures were approved by the Massey University Animal Ethics Committee.

**Study 1**

**Dams.** Dams bred over a 4-d progesterone-synchronized period that had not returned to estrus by d 19 of pregnancy (P19, n = 136) were randomly allocated to 1 of 2 nutritional treatments, either 1.0× maintenance (M; initial BW 54.5 ± 1.5 kg) or 1.5× maintenance (H; initial BW 55.3 ± 1.5 kg) from P19 to d 47 of pregnancy (Figure 1). Pregnant H dams (n = 27) remained on the H regimen until P102. At d 103 of pregnancy (P103; range: d 102 to 104 of pregnancy), ewes were subdivided further into either H- or M-feeding levels until d 136 of pregnancy.

**Pasture (i.e., ryegrass and white clover sward mixture) was the only nutritional source. Pre- and post-grazing pasture covers were 1,300 and 1,000 kg of DM/ha for the M-feeding regimen and 3,300 and 2,600 kg of DM/ha for the H-feeding regimen, respectively.**

**Dam Euthanasia and Measurement of Fetal Material.** During P103 (range: d 102 to 104 of pregnancy), 4 twin- and 6 singleton-bearing dams from each treatment group (MM dams, n = 10; MH dams, n = 10; and HH-dams, n = 10) were weighed and allocated randomly to daily euthanasia groups, thereby ensuring that the treatment groups were balanced across the
euthanasia dates. At the second euthanasia (d 137 of pregnancy (P137); range: d 136 to 140), a total of 53 ewes were killed across all 6 feeding treatments: MMM, n = 8; MMH, n = 10; MHM, n = 10; MHH, n = 10; HMM, n = 8, and HHH n = 7. Data from 6 ewes were excluded because of missing data or information.

The dams were killed by captive bolt pistol, immediately followed by exsanguination. After euthanasia, the abdominal cavity was opened and the gravid uterus was removed. The cervix and ovaries were then removed and the uterus was weighed to obtain total gravid uterus weight. The fetus(es) were then removed and the umbilical cord was ligated at the abdomen before being severed. Fetuses were killed by intracardiac injection of sodium pentobarbital (Pentobarb 500, Chemstock Animal Health, Christchurch, New Zealand). Each fetus was gently squeezed to remove amniotic fluid from the wool, and fetal BW, crown-rump length, and thoracic girth measurements were recorded, as well as birth rank and sex.

**Tissue Collection and Histology.** Fetal heart, liver, lungs, kidneys, adrenal glands, and thyroid glands of both sexes were collected and weighed. The mammary glands of female fetuses were collected and separated into left and right glands, weighed, and placed in Bouin’s fixative (within 15 min of euthanasia) for 20 h. After this time, excess fixative was washed out in 2 changes of 70% ethanol and the glands were stored in 70% ethanol before processing into paraffin wax (Leica Histoc embedding, Meyer Instruments Inc., Houston, TX). Sections 6 to 7 μm thick were cut from the left gland of each animal, running through and parallel to the long axis of the teat in the anterior-posterior plane. Once the ducts became visible, every 10th section was taken using a digital camera (Spot RT, Diagnostic Instruments Inc., Sterling Heights, MI) and a differential interference contrast microscope (Axioskop, Zeiss, Göttingen, Germany). Images were processed with the digital camera and Image-Pro Plus software, version 4.1 for Windows (Media Cybernetics, Bethesda, MD). The intensity of IGF-1 receptor immunostaining for each image was quantified at high throughput using an automated image analysis journal (i.e., for high content analysis) written by M. Dragunow (http://www.fmhs.auckland.ac.nz/sms/pharmacology/discovery1/default.aspx) using Metamorph software, version 6.2.6 (Molecular Devices, Downingtown, PA). Briefly, color images were converted to gray scale and then thresholded for dark objects. The Show Region Statistics tool in Metamorph was used to measure the average intensity of the thresholded region, and the results were saved to Microsoft Excel (Microsoft Corporation, Redmond, WA) spreadsheets. Because these are bright-field images, smaller scores correlate with a stronger intensity of immunostaining. Further details on the use of high content analysis for tissue processing can be found in the report by Dragunow (2008).

**Study 2**

**Background Information.** Reproductive performance and growth-to-weaning information from the original ewes and lambs in this study were reported by Kenyon et al. (2009), and the first-lactation yields from female offspring were reported by van der Linden et al. (2009). This paper reports on second-lactation yields and puberty of grand-offspring of the original ewes. Approximately 450 heavy (HV; 60.8 ± 0.18 kg) and 450 light (Lt; 42.5 ± 0.17 kg) Romney dams were selected from the extremes in a commercial flock of 2,900 ewes, on the basis of size, as determined by BW (Kenyon et al., 2009). From d 21 to 140 (P140) of pregnancy, the dams were randomly allocated, within size, to maintenance (i.e., M) or ad libitum (A) nutritional regimens under New Zealand pastoral grazing conditions. The aim of the M-feeding regimen was to ensure that, throughout pregnancy, total dam BW gain was similar to that of the expected increase in conceptus mass. The aim of the A-feeding regimen was to provide ad libitum grazing conditions throughout pregnancy. From P140 through to weaning, all dams and their lambs were provided with ad libitum feeding. After weaning, female progeny were managed and fed to nutritional requirements as 1 group according to commercial New Zealand farming practices (van der Linden et al., 2007). The study therefore used a 2 × 2 factorial design with 2 nutritional treatments (M vs. A) and 2 dam size treatments (HV vs. Lt).

The BW of male and female progeny to weaning were described previously by Kenyon et al. (2009). Similarly, the BW of female offspring to 12 mo were also reported previously (van der Linden et al., 2007). At 24 mo of...
age, a cohort of ewe offspring were milked (van der Linden et al., 2009).

Present Study. In study 2, a total of 52 twin-bearing, twin-rearing, 36-mo-old ewes, born to the dams described in the study by Kenyon et al. (2009), were managed under commercial farming conditions at Massey University. Postlambing, the ewes were milked once per week, for 6 subsequent weeks, using the oxytocin method (Morgan et al., 2006). The first milking commenced at an average of d 7 (range: d 6 to 11) after parturition. On each milking occasion, ewes were initially milked by machine, followed by hand-stripping, after an intravenous injection of 1 IU of synthetic oxytocin [Oxytocin V, 10 IU/mL; Vetpharm (NZ) Ltd., Auckland, New Zealand]. The time when the udder was empty was recorded. Animals were milked again using both machine and hand-stripping approximately 5 h later, with the time and weight of the milk recorded. Lambs were separated from the ewes and bottle fed as required during the intervening period. Daily milk yield was calculated using the formula (24 h/time between milkings) × milk yield at second milking.

The same group of operators milked all ewes on each occasion in this study by using a milking machine (Otenz, Otorohanga, New Zealand) with vacuum set at −40 kPa and the pulsator frequency set at 60 pulses/min. Milk obtained by machine and hand-stripping was mixed and subsampled for analysis of milk composition (i.e., percentage of fat, percentage of CP, percentage of lactose, and percentage of casein). Milk samples were preserved with bronopol and refrigerated at 4°C until analyses of composition with the Fourier-transform infrared spectroscopy technique, using a MilkoScan FT120-FTIR machine (Foss Electric, Hillerød, Denmark) calibrated for sheep milk (DairyNZ, Hamilton, New Zealand). Two reference checks (i.e., fat and CP) were taken during lactation to account for changes in the matrix of the milk attributable to stage of lactation.

Lambs. A total of 104 lambs (grand-offspring of the original ewes; Kenyon et al., 2009) were ear tagged, identified to their mother, and weighed within 24 h after birth. Lambs were weighed weekly for the first 6 wk of life. Lamb growth rates were calculated for the period from birth to d 42 of age. A crayon-harnessed vasectomy ram was placed with ewe lambs in mid-May for 17 d. The presence of crayon marks on the rump of ewe lambs was recorded on removal of the ram. Ewe lambs were classified as having attained puberty by the end of this period if a clear crayon mark was present.

Statistical Analyses

In study 1, ewe BW, fetal BW, and weights of fetal organs were analyzed using the GLM procedure with a linear model that included the fixed effects of pregnancy rank (singleton vs. twin) and ewe nutritional treatment (M vs. H; SAS Inst. Inc., Cary, NC). The ANOVA of SAS was used to determine the effects of maternal nutrition at P103 and P137 of fetal mammary gland development. All measures of mammary gland development were adjusted to a common fetal BW and birth rank, where applicable. Data are expressed as least squares means ± SE for the treatment groups and are considered different when \( P < 0.05 \), unless otherwise stated.

In study 2, daily yields of milk and composition data were fitted using a third-degree orthogonal polynomial for each animal:

\[
y_i = \alpha_0 \phi_{0i} + \alpha_1 \phi_{1i} + \alpha_2 \phi_{2i} + \alpha_3 \phi_{3i} + \epsilon_i,
\]

where \( y_i \) is the record of milk or composition taken at day \( i \), \( \alpha_n \) is the \( n \) regression, \( \phi_{ni} \) is the rescaled value of days in milk \( i \), calculated as \( \phi_{0i} = 1 \), \( \phi_{1i} = x \), \( \phi_{2i} = [(3x^2 - 1)/2] \), \( \phi_{3i} = [(5x^3 - 3x)/2] \) {where \( x = 2[i - (50 + 1)]/(50 - 1) \)}, and \( \epsilon_i \) is the error term associated with each milk record.

Accumulated yields of milk, lactose, CP, fat, and milk NE (Holmes et al., 2002) were calculated over a 50-d lactation period for each animal using the estimates of the regression coefficients of the third-degree orthogonal polynomial. Accumulated milk, lactose, CP, fat yield, milk NE, lamb growth rates, and repeated measures analyses of milk yield, milk composition, and lamb BW were undertaken using the MIXED procedure of SAS with a linear model that included the fixed effects of dam nutrition, dam size, and the interaction of dam nutrition × dam size. Sex of lamb was fitted as a fixed effect in the linear model for lamb BW and growth analyses.

Ewe lambs that attained puberty by 9 mo of age were assigned a value of 1, and those that did not attain puberty were assigned a value of 0. The attainment of puberty was analyzed using the GENMOD procedure of SAS. Body weight at 8 mo of age was fitted as a covariate and rearing rank was fitted as a fixed effect. Values are reported as logit least squares means (±SE) accompanied by back-transformed values to provide an indication of the proportion attaining puberty.

RESULTS

Study 1

Dam BW. No differences (\( P > 0.05 \)) in BW between singleton- and twin-bearing dams were found at P19 or P102 (data not shown). At P19, no differences (\( P > 0.05 \)) in BW between nutritional groups were found (Table 1). The HH dams killed at P103 were heavier (\( P < 0.05 \)) than the MM dams, but did not differ from the MH dams (Table 1). The HHH dams killed at P137 were heavier (\( P < 0.05 \)) than the MMM, MMH, MHM, and HHH dams (Table 2). The MHH dams killed at P137 were heavier (\( P < 0.05 \)) than the MMM dams (Table 2).

Fetal Measures at P103 and P137. Dam nutrition had no effect (\( P > 0.05 \)) on fetal crown-rump
length and thoracic girth circumference at P103 or P137 (Tables 1 and 2). Dam nutrition did not affect fetal thyroid gland weights at P103; however, at P137, fetuses carried by HHH dams had heavier (\(P < 0.05\)) thyroid glands than fetuses carried by MMM, MMH, MHH, and HHM dams. Dam nutrition had no effect (\(P > 0.05\)) on fetal heart, liver, lung, kidney, and adrenal gland weights at P103 and P137 (data not shown).

Dam nutrition had no effect (\(P > 0.05\)) on fetal mammary gland weights at P103 and P137 (Tables 1 and 3). At P103, fetuses carried by MM dams had smaller (\(P < 0.05\)) total duct areas than fetuses carried by MH and HH dams (Table 1 and Figure 2). In addition, fetuses carried by HH dams had less (\(P < 0.05\)) IGF-1 receptor staining intensity in the mammary gland compared with fetuses carried by MM dams, indicating greater numbers of IGF-1 receptors in HH fetuses (Table 1 and Figure 2). At P137, fetuses carried by MMM dams had smaller (\(P < 0.05\)) total duct areas and fewer ducts compared with fetuses carried by MMH, MHH, HHM, and HHH dams. In addition, MMM ewes had smaller (\(P < 0.05\)) secretory cell areas compared with MMH, MHM, HHM, and HHH dams (Table 3). However, maternal nutrition had no effect (\(P > 0.05\)) on the area of the lumen at P137. Fetuses carried by HHH dams had more (as indicated by less staining intensity; \(P < 0.05\)) IGF-1 receptors stained in the mammary gland than fetuses carried by dams from the other nutritional groups at P137.

**Study 2**

**Ewe BW and Body Condition.** No dam nutritional or dam size effects (\(P > 0.05\)) were found on ewe BW at d 0 of pregnancy (67.3 ± 1.65 kg and 69.1 ± 1.81 kg for M and A ewes, respectively; 67.9 ± 1.88 kg and 68.4 ± 1.57 kg for Lt and Hv ewes, respectively). Furthermore, no differences were found at P140 (75.5 ± 1.85 kg).
Table 3. Effect of maternal nutrition treatment during pregnancy on fetal mammary gland development at d 137 of pregnancy1

<table>
<thead>
<tr>
<th>Trait</th>
<th>MMM</th>
<th>MMH</th>
<th>MHM</th>
<th>MHH</th>
<th>HHM</th>
<th>HHH</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of fetuses2</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Fetal mammary gland wt, g</td>
<td>11.5 ± 0.8</td>
<td>13.0 ± 0.8</td>
<td>11.2 ± 1.0</td>
<td>11.5 ± 0.8</td>
<td>11.2 ± 0.8</td>
<td>11.0 ± 1.0</td>
</tr>
<tr>
<td>Total duct area, mm2</td>
<td>1.761 ± 0.575a</td>
<td>4.370 ± 0.599b</td>
<td>3.025 ± 0.724ab</td>
<td>3.720 ± 0.558b</td>
<td>3.771 ± 0.572b</td>
<td>4.641 ± 0.726b</td>
</tr>
<tr>
<td>Total lumen area, mm2</td>
<td>0.892 ± 0.320</td>
<td>1.767 ± 0.333</td>
<td>1.487 ± 0.402</td>
<td>1.884 ± 0.310</td>
<td>1.508 ± 0.318</td>
<td>2.150 ± 0.403</td>
</tr>
<tr>
<td>Total secretory cell area, mm2</td>
<td>0.868 ± 0.315</td>
<td>2.693 ± 0.328</td>
<td>1.538 ± 0.396</td>
<td>1.836 ± 0.306</td>
<td>2.264 ± 0.313</td>
<td>2.491 ± 0.397</td>
</tr>
<tr>
<td>No. of ducts</td>
<td>137.4 ± 28.3a</td>
<td>327.1 ± 31.7c</td>
<td>170.5 ± 32.2a</td>
<td>129.9 ± 33.0b</td>
<td>235.6 ± 30.9c</td>
<td>220.0 ± 32.2d</td>
</tr>
<tr>
<td>IGF-1 staining intensity4</td>
<td>190.4 ± 2.6</td>
<td>182.4 ± 2.1</td>
<td>185.6 ± 2.6</td>
<td>189.7 ± 2.5</td>
<td>189.9 ± 2.6</td>
<td>171.1 ± 2.1</td>
</tr>
</tbody>
</table>

*Means within rows with different superscripts are significantly different (P < 0.05).
1Table shows least squares means ± SE.
2M = 1.0× maintenance; H = 1.5× maintenance planes of nutrition from d 19 to 47, d 48 to 102, and d 102 to 136 of pregnancy.
3Includes only female fetuses.
4The smaller the value, the greater the staining intensity.

1.27 kg and 78.2 ± 1.39 kg for M and A ewes, respectively; 76.2 ± 1.45 kg and 77.6 ± 1.21 kg for Lt and Hv ewes, respectively).

**Lactation Curve and Milk Composition.** No dam size effects (P > 0.05) were found on accumulated milk yield, lactose, CP, fat yield, and milk NE over a 50-d lactational period (Table 4). No dam nutrition effect (P > 0.05) was found on accumulated milk yield, lactose, and CP yields over a 50-d lactation period; however, A ewes produced greater accumulated fat yield (P < 0.05) and milk NE (P < 0.05) than M ewes.

The A ewes tended to show greater milk yield (P = 0.08) and fat percentage (P = 0.06) at d 14 of lactation compared with M ewes (Figures 3 and 4). No dam nutritional effect (P > 0.05) was found on lactose and CP percentages at any time point. The Hv ewes tended to show a greater CP percentage (P = 0.08) at d 7 of lactation compared with Lt ewes. At d 21 of lactation, Lt ewes tended to show greater fat percentage (P = 0.09) compared with Hv ewes. No dam size effect (P > 0.05) was found for lactose percentage at any time point.

**Lamb Birth Weight and BW.** No granddam size effects (P > 0.05) were found for lamb BW at d 38 or on lamb growth rates from birth to d 38 (Table 5). However, lambs with Hv granddams fed ad libitum throughout pregnancy were heavier than the other 3 groups at weaning. The birth weights of lambs whose granddams were M-fed during pregnancy was greater (P < 0.05) than those of lambs whose granddams were fed ad libitum, but only the birth weights for those with Lt granddams reached significance. Body weights at d 38 and growth between birth and d 38 were not different (P > 0.05) between feeding groups.

**Ewe Lambda Puberty.** A smaller proportion of ewe lambs born to first-parity 2-yr-old ewes with Lt granddams and A-fed throughout pregnancy reached puberty (51%) compared with the other 3 groups, of which between 69 and 81% achieved puberty (Table 5). Only 7 out of 105 ewe lambs born to 3-yr-old ewes did not reach puberty. Those not achieving puberty were distributed across all granddam feeding and size groups, resulting in no significant treatment effects.

**DISCUSSION**

The primary focus of the results presented in this paper is to examine the effects of feeding levels during pregnancy on a variety of traits from offspring at the fetal stage to 3.5 yr of age and also traits in the grand-offspring. In addition, study 2 superimposed the effect of Hv or Lt ewe size on feeding level during pregnancy. Ewe size effects were largely nonsignificant, except for grand-offspring weaning weight and proportion of grand-offspring reaching puberty; thus, little discussion is devoted to this aspect of the study. Kenyon et al. (2009) and van der Linden et al. (2009) reported the effects of ewe feeding during pregnancy and ewe size on lamb and ewe offspring performance to 2.5 yr of age.

In study 1, the different feeding regimens during pregnancy changed ewe BW but did not alter fetal BW. The lack of change in fetal BW was unsurprising, given that M-fed ewes would not have needed to draw significantly on their own reserves. In addition, ewes were in good body condition, which would have allowed those on M feeding to mobilize reserves for fetal growth if required. Despite the lack of differences between fetal BW in study 1, changes were observed in fetal mammary characteristics, which led to the hypothesis that offspring born to ewes fed at restricted levels in early to mid-pregnancy might have compromised lactational capability. However, this hypothesis could not be tested because all offspring were killed. Kenyon et al. (2009) and van der Linden et al. (2009), in a follow-up study (study 2), reported that 1) ewe BW changes were decreased by feeding pasture at M levels during pregnancy; 2) fetal mammary gland weights were greater in the M group (relative to the A group); 3) the birth weights of twins from dams fed M levels were reduced; 4) ewes whose dams were fed at M levels during pregnancy produced greater protein and lactose yields in their first
lactation at 2 yr of age than did ewes whose dams were A-fed during pregnancy, and 4) weaning weights of lambs whose granddams were fed M levels during pregnancy were heavier.

There is an inconsistency between the 2 studies for fetal mammary gland results. The results of study 1 indicated that better dam feeding during pregnancy enhanced mammary gland development, whereas study 2 indicated that poorer dam feeding promoted greater fetal mammary size. The authors cannot provide any clear reason for the basis of this difference, and a new study is underway to further examine this issue.

The greater milk protein and lactose yields from 2-yr-old ewes whose dams were M-fed during pregnancy reported by van der Linden et al. (2009) is consistent with the observation that there is a cyclical environmental effect between dam lactation and calf growth rate to weaning such that slower growing calves that have dams with poorer lactations will tend to produce greater lactation yields when they become mothers and will therefore have faster growing calves, who will in turn have poorer lactations, and so on (Koch, 1972; Pala and McCraw, 2005). During their second lactation (study 2), ewes born to M-fed dams produced yields of milk similar to those of their counterpart dams who received A feeding during pregnancy. However, ewes born to dams that received M feeding throughout pregnancy produced decreased yields of fat and had less milk energy output than those of ewes born to A-fed dams throughout pregnancy. The greater milk energy output in the A group did not result in any advantage in lamb growth rate to 38 d of age. With the information that is currently available, it is not possible to speculate on whether the change in lactational performance was due to in utero or early postnatal effects, although fetal mammary changes in study 2 do provide some support to changes in utero. Resolution of this matter will require either cross-fostering of lambs born to ewes fed differently during pregnancy soon after birth or hand rearing.

The loss of advantage from the first to second lactation is consistent with the selfish gene hypothesis (Dawkins, 1976) with a potential epigenetic mecha-

### Table 4

<table>
<thead>
<tr>
<th>Trait</th>
<th>Treatment</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Nutritional effect</th>
<th>Size effect</th>
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<tr>
<td></td>
<td>A</td>
<td>M</td>
<td>Hv</td>
<td>Lt</td>
<td></td>
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<tr>
<td>n</td>
<td>23</td>
<td>29</td>
<td>31</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk yield, kg</td>
<td>119.2 ± 2.9</td>
<td>113.5 ± 2.6</td>
<td>116.9 ± 2.5</td>
<td>115.8 ± 3.0</td>
<td>0.15</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>Lactose yield, kg</td>
<td>6.23 ± 0.15</td>
<td>5.91 ± 0.14</td>
<td>6.10 ± 0.13</td>
<td>6.04 ± 0.16</td>
<td>0.12</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>CP yield, kg</td>
<td>5.84 ± 0.15</td>
<td>5.58 ± 0.14</td>
<td>5.76 ± 0.13</td>
<td>5.66 ± 0.16</td>
<td>0.22</td>
<td>0.64</td>
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<tr>
<td>Fat yield, kg</td>
<td>8.91 ± 0.26</td>
<td>8.17 ± 0.24</td>
<td>8.57 ± 0.23</td>
<td>8.51 ± 0.27</td>
<td>0.04</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>Milk NE, MJ</td>
<td>573.6 ± 14.6</td>
<td>534.5 ± 13.3</td>
<td>556.8 ± 12.6</td>
<td>551.4 ± 15.2</td>
<td>0.05</td>
<td>0.79</td>
<td></td>
</tr>
</tbody>
</table>

1Table shows least squares means ± SE.
2No maternal nutrition × dam size interactions were detected (P > 0.10); therefore, only the main effects are reported.

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Figure 2. A) Photomicrographs of ovine fetal skin showing skin epidermis and sebaceous glands. B) Photomicrographs of ovine fetal mammary gland showing the lumen of a secondary duct, basement membrane, and fibrous connective tissue. Magnification in both photomicrographs is 400×. Ovine fetal skin was used as a control and did not exhibit immunoreactivity to IGF-1 receptor (IGF-1R) antibody when the primary antibody was omitted from the immunohistochemical procedure (no photomicrograph shown). Addition of the primary antibody caused staining of the skin epidermis and the cells of the sebaceous glands (panel A). Immunoreactivity to IGF-1R antibody in mammary tissue from a fetus whose dam was fed on a high plane of feeding from d 19 to 101 of pregnancy (HH) is shown in panel B.
nism, or with the predictive adaptive response hypothesis (Gluckman and Hanson, 2006). It is conceivable that either the dam or the fetus experiencing restricted nutrition “programs” the lactational potential of the fetal mammary gland to ensure survival of young from the first pregnancy. Having invested resources in that initial lactation, there may be no advantage in later lactations when the “prediction” could be that the animal would no longer be alive.

In both the first (van der Linden et al., 2009) and second parities (study 2), the birth weights of lambs whose granddams received M feeding during pregnancy were greater than those of lambs from granddams that received A feeding. Thus, ewes that had decreased birth weights after poorer in utero growth (Kenyon et al., 2009) were themselves able to produce lambs of greater birth weights at 2 and 3 yr of age. This oscillation is consistent with the negative genetic correlation be-

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**Figure 3.** Milk yield (top) and lactose percentage (bottom) during the first 50 d of lactation for ewes born to heavy (n = 31) or light (n = 21) dams fed ad libitum (n = 23) or maintenance (n = 29) during pregnancy. Data are presented as least squares means (±SE). †P < 0.10, maternal nutrition effect. No maternal nutrition × dam size interactions were detected (P > 0.10); therefore, only the main effects are reported.
between direct and maternal effects (Hagger, 1998; Neser et al., 2001). The explanation for such a correlation has been that animals with favorable genes for fetal growth have poorer genes for uterine environment. The current results indicate that this effect might be achieved through inherited environmental effects, with the underlying mechanism being one or more of the epigenetic mechanisms (Probst et al., 2009).

Lambs whose granddams were heavy and received ad libitum feeding throughout pregnancy were heavier at weaning at approximately d 100 than lambs from the other 3 treatment groups. It is not possible to determine whether the greater weaning weight is a carryover genetic effect or is due to some advantage set up after access to more milk energy in the first several weeks of life. It seems unlikely that all the difference, of

Figure 4. Crude protein percentage (top) and fat percentage (bottom) during the first 50 d of lactation for ewes born to heavy (n = 31) or light (n = 21) dams fed ad libitum (n = 23) or maintenance (n = 29) during pregnancy. Data are presented as least squares means (±SE). †P < 0.10, maternal nutrition effect; ‡P < 0.10, maternal size effect. No maternal nutrition × dam size interactions were detected (P > 0.10); therefore, only the main effects are reported.
Table 5. Second-parity grand-offspring BW and puberty traits for lambs born to heavy (Hv) or light (Lt) granddams fed ad libitum (A) or maintenance (M) during pregnancy\(^4\)

<table>
<thead>
<tr>
<th>Grand-offspring trait trait</th>
<th>Granddam size</th>
<th>Granddam feeding</th>
<th>A</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight, kg</td>
<td>Hv</td>
<td>4.99 ± 0.11(^a)</td>
<td>5.16 ± 0.12(^a)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lt</td>
<td>4.76 ± 0.14(^b)</td>
<td>5.16 ± 0.13(^b)</td>
<td></td>
</tr>
<tr>
<td>BW at d 38, kg</td>
<td>Hv</td>
<td>23.89 ± 0.57</td>
<td>22.47 ± 0.59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lt</td>
<td>23.06 ± 0.65</td>
<td>23.84 ± 0.64</td>
<td></td>
</tr>
<tr>
<td>(\text{GR}_{\text{birth-d38}}) g/d</td>
<td>Hv</td>
<td>338 ± 9</td>
<td>322 ± 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lt</td>
<td>343 ± 11</td>
<td>327 ± 11</td>
<td></td>
</tr>
<tr>
<td>(\text{GR}_{\text{d38-wng}}) g/d</td>
<td>Hv</td>
<td>205 ± 5(^a)</td>
<td>188 ± 6(^b)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lt</td>
<td>184 ± 6(^b)</td>
<td>193 ± 6(^b)</td>
<td></td>
</tr>
<tr>
<td>Weaning weight, kg</td>
<td>Hv</td>
<td>29.29 ± 0.61(^a)</td>
<td>27.30 ± 0.64(^b)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lt</td>
<td>27.55 ± 0.71(^b)</td>
<td>28.50 ± 0.70(^ab)</td>
<td></td>
</tr>
<tr>
<td>Puberty(^4)</td>
<td>Hv</td>
<td>-1.44 ± 0.44(^a) (81)(^5)</td>
<td>-0.82 ± 0.43(^b) (69)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lt</td>
<td>0.04 ± 0.47(^b) (51)</td>
<td>-1.09 ± 0.53(^b) (75)</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a,b}\)Least squares means with different superscripts within a trait are significantly different (\(P < 0.05\)).
\(^1\)Data are presented as least squares means ± SE.
\(^2\)Lamb growth rates (g/d) from birth to d 38.
\(^3\)Lamb growth rates (g/d) from d 38 to weaning.
\(^4\)Proportion of ewe lambs reaching puberty; first-parity grand-offspring lambs born in 2007.
\(^5\)Back-transformed logit values (\%).

approximately 2.5 kg, can be ascribed to a genetic advantage from being born to heavier granddams because the original phenotypic difference of 18 kg would likely have regressed to approximately 0.5 kg after allowing for 2 meioses, a heritability of 40%, and a genetic correlation of 0.3 between mature and weaning weights. Therefore, it would seem likely that there must be a delayed contribution to weaning weight from the greater milk energy.

Attainment of puberty before 1 yr of age was assessed in the first 2 cohorts of ewe grand-offspring born to 2- and 3-yr-old ewes in study 2. Ewe lambs should reach puberty at approximately 50 to 70% of mature BW (Dyrmundsson, 1981). In the first cohort produced by primiparous ewes, fewer grand-offspring whose granddams were Lt and A-fed during pregnancy achieved puberty compared with the other 3 groups. However, ≥90% of the second cohort of grand-offspring ewe lambs achieved puberty in all 4 groups because of the attainment of heavier BW by autumn; all lambs were >40 kg. The reason behind the decreased proportion of grand-offspring from primiparous ewes reaching puberty whose granddams were lighter and A-fed throughout pregnancy could be due to either the granddam treatment, requiring an intergenerational effect, or the poorer lactation of the ewes, leading to poorer early postnatal growth of the grand-offspring lambs. However, differences in grand-offspring BW caused by differences in lactation had disappeared by 6 mo of age. Once again, it is not possible to ascribe any cause to the differences in the proportion of ewe grand-offspring reaching puberty, although the lack of a difference in the second-parity grand-offspring is suggestive of a postnatal growth effect rather than a granddam treatment effect.

Summary and Conclusions

Although it appears that the lactation of daughters can be affected by the feeding level their dams receive during pregnancy, it is not clear from the studies presented here whether the effect is due to in utero mechanisms or is caused by consequent changes in the lactation of the dam. Regardless of what the underlying mechanism is, there is an inconsistent long-term carryover effect of dam feeding during pregnancy on ewe offspring lactational ability. Additionally, maintenance feeding of granddams during pregnancy results in increased birth weights of grand-offspring. This result is consistent with the negative genetic correlation between direct (i.e., fetal growth) and maternal (i.e., uterine capability) effects and is deserving of reflection by scientists with an interest in animal breeding and genetics regarding whether the apparent genetic effect might in fact be an epigenetic effect.

Since the Barker (1995) hypothesis was advanced, and molecular mechanisms were proposed to explain the long-term consequences of early life events, there has been a rapid growth in studies manipulating dams of reproductive age in a variety of ways. To date, these studies have not achieved a great deal of clarity regarding recommendations to farmers. Unfortunately, the studies presented here do little to improve the required clarity. There are significant challenges to both researchers and research funders when trying to coordinate research efforts in this rapidly expanding discipline: What are the early-life events that affect later-life phenotypes? At what stage of early life do the events have consequences? What are the important later life phenotypes? What are the mechanisms that underlie the changes? Can these mechanisms be modified by
intervention? Can agricultural and medical interests be coordinated? Medical interests have become largely coordinated through the International Society for Developmental Origins of Health and Disease (University of Southampton, Southampton, UK), but as yet, agricultural efforts appear fragmented, and it would be timely for a new international organization to represent agricultural interests.

LITERATURE CITED


