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Ewe–lamb bonding behaviours at birth are affected by maternal undernutrition in pregnancy

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Maternal undernutrition in pregnancy results in low birth-weights and impaired postnatal survival in sheep. Largely anecdotal evidence suggests that the expression of appropriate maternal and neonate behaviours may also be disrupted by undernutrition. In the present study, we investigated the effect of a moderate (35 %) reduction in ewe nutritional intake in pregnancy on the expression of ewe–lamb bonding behaviours in primiparous Scottish Blackface ewes. Low-intake (L) ewes had significantly higher plasma progesterone than high-intake (H) ewes from mid-gestation onwards (e.g. plasma progesterone at 20 weeks (ng/ml): H 15·72, L 22·38, SED 1·80, $P<0·001$), and a lower oestradiol: progesterone value than H ewes at delivery (H 0·46, L 0·35, SED 0·05, $P<0·05$). Lamb birth-weight was reduced in the L lambs compared with H lambs (mean body weight (kg): H 3·31, L 3·00, SED 0·14, $P<0·05$), but the incidence of malpresentation at delivery was greater in L lambs. L ewes spent significantly less time licking their lambs than H ewes after delivery (time grooming in 2 h after birth (%): H 56·12, L 48·17, SED 2·639, $P<0·01$) and were more aggressive towards the lambs. Lamb behaviours were not directly affected by maternal nutritional treatment, but lamb birth-weight had a significant effect on neonatal developmental progress. Low-birth-weight lambs were slower than heavier lambs to stand and sucked less frequently. In tests of maternal attachment to the lamb, H ewes received higher scores than L ewes at both 24 h after birth (ewes receiving high scores (%): H 41·3, L 21·4, $P<0·05$) and at 3 d postnatal. We conclude that even a moderate level of undernutrition impairs the attachment between ewes and lambs by affecting maternal behaviours expressed at birth. In addition, the results suggest that levels of nutrition resulting in a decrease in birth weight will affect neonatal lamb behavioural progress.

Food restriction: Pregnancy: Maternal behaviour: Neonate behaviour

Reproduction in the sheep is timed so that lamb birth and maternal lactation coincide with the best environmental conditions in the spring. For hill sheep, this means that they are pregnant during winter grazing and periods of undernutrition if supplementary feeding is absent or inadequate. Without supplementary feed, pregnant hill sheep may lose 85 % of their subcutaneous fat during pregnancy and lactation (Russel *et al.* 1968). Maternal nutrition in pregnancy affects both lamb birth-weight (for reviews, see Mellor, 1983; Robinson *et al.* 1999) and the incidence of lamb mortality (Waterhouse *et al.* 1992; Kleeman *et al.* 1993; Hinch *et al.* 1996). Light-weight lambs, particularly of multiple litters, have higher incidences of deaths from exposure and starvation than heavy lambs (Scales *et al.* 1986). In addition, light lambs are at greater risk of hypothermia than heavy lambs (Moore *et al.* 1986; Clarke *et al.* 1997) due to their larger relative surface area, reduced body reserves and reduced thermogenic capability of brown adipose tissue (Robinson & Aitkin, 1985; Budge *et al.* 2000). Maternal undernutrition is also associated with a reduction in udder weight and mammary development (Mellor & Murray, 1985; Mellor *et al.* 1987; Charismiadou *et al.* 2000), resulting in a reduced colostrum yield (Mellor & Murray, 1985; Hall *et al.* 1992; O'Doherty & Crosby, 1996) and total milk production (O'Doherty & Crosby, 1996; Bizelis *et al.* 2000). In addition, maternal undernutrition is associated with a delayed onset of lactation (Mellor *et al.* 1987) and a lower milk secretion rate (Mellor *et al.* 1987; Hall *et al.* 1992).

Maternal undernutrition may also impair lamb survival by affecting the appropriate expression of maternal and neonate behaviours at birth associated with ewe–lamb

Abbreviations: H, high intake; L, low intake.
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bonding. Few studies have, however, considered the effects of nutrition on the behaviour of either ewe or lamb. A preliminary study (Thomson & Thomson, 1949) suggested that undernourished ewes took longer to attend to their lambs, and a larger proportion showed ‘perfunctory or casual’ attentiveness in cleaning the lambs and allowing them to suck than in well-fed ewes. Few other studies have been carried out, although two largely descriptive studies suggest that undernourished mothers have ‘inadequate mothering ability’ (McDonald, 1962), and, in deer, were more likely to ignore, avoid and be aggressive to their young than well-fed mothers (Langenau & Lerg, 1976). Low nutrition in the last 6 weeks of gestation is also reported to increase the number of desertions of twin lambs in comparison with well-fed Merino ewes (Putu *et al.* 1988). In addition, lambs born to undernourished ewes are described as lacking in vitality or being of low vigour (Thompson & Thompson, 1949; Moore *et al.* 1986), although these terms are poorly defined. Some of the apparently poor maternal care, such as desertion, may therefore be attributable to lamb weakness and inability to follow, rather than a failure in maternal ability. Although these results suggest that maternal nutrition in pregnancy may affect the expression of both ewe and lamb behaviour, a quantitative study of the interactions between nutrition in pregnancy and subsequent mother–offspring behaviour is lacking.

The purpose of the present study was to quantify the effects of a reduced feed intake, throughout gestation, on the expression of maternal and neonate behaviours at parturition in Scottish hill sheep, and their consequences for ewe–lamb attachment. The high feed intake (H) ewes were fed a level that were approximately equal to the estimated energy requirements for a gravid 50 kg ewe (Agriculture and Food Research Council, 1993), whereas the low feed intake (L) ewes received approximately 65 % of the intake of H ewes. This level of nutritional restriction was designed to mimic what ewes may experience in a moderately poor winter. In addition to behavioural measures, maternal plasma oestradiol and progesterone concentrations were measured throughout gestation. Plasma oestradiol concentration in late gestation is correlated with the expression of maternal behaviours in sheep (Shipka & Ford, 1991; Dwyer *et al.* 1999) and progesterone has also been implicated in the control of maternal behaviour in the sheep, particularly in reducing aggressive behaviours (Kendrick & Keverne, 1991). Thus, nutrition may affect the expression of maternal behaviour directly through its actions on the hormonal regulators of maternal behaviour or by, for example, the competing motivations of hunger and the desire to express maternal behaviours or remain with the lamb.

Materials and methods

The entire protocol was reviewed and approved by the local experiments and ethics committee (Scottish Agricultural College, Edinburgh, Scotland, UK) and specific procedures were done under an Animal (Scientific Procedures) Act 1986 Project Licence granted by the Home Office.

Animals

Primiparous 2-year-old Scottish Blackface ewes were used in this study. Primiparous ewes were used to eliminate confounding the results by variations in previous maternal experience. The ewes used in the present study were sired by rams resulting from 7 years of divergent selection on an index to alter carcass composition resulting in two lines of animals. Approximately equal numbers of ewes from each selection line were used, and were known to differ in the relative amounts of fat to lean in the carcass. Ewes were synchronised in oestrous using progesterone sponges (Veramix, Upjohn Ltd, Crawley, Sussex, UK), and artificially inseminated with semen from one of five unselected, randomly chosen rams. Ewes were weighed and a measure of backfat (condition score on a scale of 0 (emaciated)–5 (very fat) was determined before conception and then at intervals of 2 weeks throughout gestation. A final weight and condition score were taken 3 d postpartum. Blood samples (7 ml) were collected by jugular venepuncture between 10.00 and 12.00 hours at intervals of 2 weeks from week 4 after conception until week 20, and at 3 d postpartum, in all lambed ewes. Plasma was separated by centrifugation and stored at -70°C until assayed for 17β-oestradiol and progesterone concentration by radioimmunoassay. Additional blood samples were taken at intervals of 1 week over the last 6 weeks of gestation for determination of β-hydroxybutyrate, an indicator of maternal mobilisation of body reserves.

Nutritional treatments

Ewes were brought indoors to large straw-bedded pens in week 4 of gestation and randomly allocated to H or L (approximately 65 % of the intake of H ewes nutritional treatment). Litter size was determined by ultrasonography at week 6 and nutritional intakes were adjusted by litter size as well as treatment group thereafter. Nutritional intakes were manipulated by controlling hay intake initially and then by managing concentrate intake (see Table 1 for daily intakes). Ewes were provided with unlimited fresh drinking water throughout. L ewes also received 10 g mineral supplement (Norvite Sheep, 305W; SCA Nutrition Ltd, Thirsk, Yorks., UK)/d from week 11 until they received concentrate feed to ensure that L ewes were not compromised for trace elements and vitamins compared with H ewes. Ewes were vaccinated with a clostridial vaccine (Heptavac-*P* plus; Intervet Limited, Milton Keynes, Bucks., UK) in week 17. Ewes lambed in large straw-bedded pens (7 × 7 m) in groups of 10–12 animals.

Lambing management

In total, sixty-one ewes (thirty-two H, twenty-nine L) were recorded at lambing in the study, producing 117 lambs. There were eight single and seventeen twin pregnancies recorded in each treatment group, but seven and four triplet litters in the H and L groups respectively. Ewes lambed over a 2-week period and were kept under 24 h surveillance during this time. In addition, a continuous videotape record

Table 1. Daily intakes of hay and concentrates for ewes fed on either a high or low food intake from week 4 of pregnancy
(Mean values)

	High-intake ewes*†									Low-intake ewes*‡								
	Single			Twin			Triplet			Single			Twin			Triplet		
	Food intake (kg/d)		Energy intake (MJ ME/d)	Food intake (kg/d)		Energy intake (MJ ME/d)	Food intake (kg/d)		Energy intake (MJ ME/d)	Food intake (kg/d)		Energy intake (MJ ME/d)	Food intake (kg/d)		Energy intake (MJ ME/d)	Food intake (kg/d)		Energy intake (MJ ME/d)
Time of gestation (weeks)	Hay§	Concentrates‖		Hay§	Concentrates‖		Hay§	Concentrates‖		Hay§	Concentrates‖		Hay§	Concentrates‖		Hay§	Concentrates‖	
4–6	0·80	0·00	5·2	0·80	0·00	5·2	0·80	0·00	5·2	0·70	0·00	4·5	0·70	0·00	4·5	0·70	0·00	4·5
7–9	1·20	0·00	7·8	1·20	0·00	7·8	1·20	0·00	7·8	0·70	0·00	4·5	0·70	0·00	4·5	0·70	0·00	4·5
10	1·29	0·00	8·3	1·23	0·00	8·0	1·24	0·00	8·0	0·70	0·00	4·5	0·80	0·00	5·2	0·80	0·00	5·2
11	1·48	0·00	9·6	1·16	0·20	9·7	1·38	0·20	11·1	0·80	0·00	5·2	0·90	0·00	5·8	0·90	0·00	5·8
12	1·26	0·00	8·1	1·22	0·20	10·0	1·32	0·20	10·7	0·80	0·00	5·2	0·90	0·00	5·8	0·90	0·00	5·8
13	1·32	0·20	10·7	1·17	0·30	10·8	1·26	0·30	11·4	0·80	0·00	5·2	0·90	0·00	5·8	1·38	0·10	8·5
14	1·49	0·20	11·8	1·26	0·30	11·4	1·26	0·30	11·4	0·80	0·00	5·2	0·90	0·00	5·8	1·14	0·10	8·5
15	1·33	0·20	10·8	1·20	0·60	14·2	1·26	0·70	15·7	1·21	0·00	7·8	1·18	0·10	8·7	1·11	0·20	9·3
16–17	0·0	0·40	12·9	0·0	0·75	15·8	0·0	0·80	16·8	0·0	0·0	7·8	0·0	0·20	9·8	0·0	0·30	10·4
18-lambing	0·0	0·40	12·9	0·0	0·75	15·8	0·0	0·80	16·8	0·0	0·20	10·0	0·0	0·50	13·0	0·0	0·60	13·6

ME, metabolisable energy.
* High-intake ewes (*n*): single 8, twin 17, triplet 7; low-intake ewes (*n*): single 8, twin 17, triplet 4.
† High-intake ewes had access to hay *ad libitum* throughout. Values therefore represent their chosen intake.
‡ Low-intake ewes has restricted access to hay until week 12 (triplet) or 15 (single and twin).
§ Hay analysis: 83·2 g crude protein $(N \times 6{\cdot}25)/$kg DM, 7·7 MJ ME/kg DM.
‖ Concentrates (locally milled ewe nuts): 218 g crude protein $(N \times 6{\cdot}25)/$kg DM, 12·5 MJ ME/kg DM.

was made using eight cameras and a Panasonic eight-channel digital field switcher (WJ-FS20/B, Matsushita Communication Industrial, Yokohama, Japan). Ewes were accustomed to the presence of observers in the walkways between pens in the weeks before parturition, and were clearly identified by paint-brands to facilitate recognition on the videotapes.

As far as possible, ewes were allowed to give birth and care for their lambs unaided. However, lambing assistance was given if the ewe had failed to progress through a time schedule of events, i.e. 60 min after the appearance of fluids there was no appearance of parts of the lamb, and/or 120 min after parts of the lamb were seen at the vulva there was no other obvious progress being made. In all cases, intervention was kept to a minimum and mainly involved correcting lamb presentation before the ewe continued the birth process unaided. Assistance at delivery was scored as follows: no assistance, partial assistance (lamb presentation corrected, ewe delivered unaided), manual delivery (ewe assisted to deliver the lamb). Ewes that abandoned or rejected their lambs were placed in an individual pen with their lambs 60 min after birth and behavioural data for these animals were truncated at 60 min.

Ewes and their lambs remained indoors, in the lambing pens, for the first 3 d after delivery. Multiple litters were reduced to two lambs at 24 h by removal of the smallest lambs, unless there were husbandry reasons for removing other lambs. Removed lambs were hand-reared, or fostered to a suitable non-trial ewe, and data for these animals are thus terminated at 24 h old. After 3 d, ewes and lambs were moved outside to a 7·9 ha field.

Data collection

Behaviour at parturition. The overt length of labour (calculated from appearance of fluids until birth of last lamb), the interval between littermates and the degree of assistance required were recorded for all ewes. Lamb presentation at birth was also recorded as normal (both forelegs extended, with the nose lying along the legs) or one of a number of abnormal postures (one or both legs retracted, head back, backwards, breech or two or more lambs presented at the same time).

Data on maternal and neonate behaviour were collected from videotape during four observation periods in the 120 min after the birth of a lamb, using Observer data-recording software (Noldus *et al.* 2000). In addition, vocalisations of the ewe and lambs were collected live, using handheld Psion Workabouts (Psio PLC, London, UK) and Observer software. Data were collected for the first 120 min after the birth of each lamb as follows: the first 30 min, followed by three 10 min periods, every 30 min over the following 90 min. The definitions of ewe and lamb behaviours are as previously described (Dwyer & Lawrence, 1998; Dwyer *et al.* 2001).

Behaviour over the first 3 d. Lamb birth-weight and sex were recorded within 24 h of birth. Once lambs were dry, they were marked with the identification number of their mothers, and for birth order. Additional behavioural data were collected by scan sampling at intervals of 2 h for the first 3 d after parturition when ewes and lambs were kept indoors in the lambing pens. The posture, behaviour and distance between the ewe and her lamb(s) were recorded. Ewe and lamb behaviours were as defined at birth and, in addition, we recorded 'eating' (any behaviours where animals were seen at the feeder and chewing), 'investigatory behaviours' (nosing pen fixtures, animals other than the ewe or own lambs, substrates but not eating), 'ruminating' (characteristic movements of the jaw by adult ewes) and 'other' (all other behaviours which occurred at low frequency).

Ewes were given a maternal behaviour score at 24 h after birth on the basis of their response to the handling of their lambs on a scale from 1 (leaves lamb, does not return)–6 (remains within 1 m of lamb, makes physical contact with lamb) as previously described (after O'Connor *et al.* 1985; modified after Dwyer & Lawrence, 1998). At 3 d after birth, ewes were given a second attachment score based on their response to a handler carrying the lambs slowly out of the home pen from 1 (no concern, does not follow the lambs from the home pen)–4 (follows lambs closely and leaves home pen).

Radioimunoassays

Oestradiol. Plasma oestradiol concentration was determined in duplicate in 500 μl samples (after Mann *et al.* 1995). Samples were first extracted by addition of 2·5 ml diethyl ether and separation of the ether layer. Samples were reconstituted in PBS-gel and assayed using Estradiol Maia radioimmunoassay kits (BioChem ImmunoSystems, Bologna, Italy). Briefly, the samples were incubated with ^{125}I-labelled oestradiol tracer (Amersham International plc, Amersham, Bucks., UK) and rabbit antiserum at 37°C for 2 h. The bound fraction was separated by magnetic sedimentation of the solid phase, and counted in a γ-scintillation counter. The inter- and intra-assay CV were 15·0 and 10·5 % respectively.

Progesterone. Progesterone samples were determined in duplicate in 100 μl samples (after McNeilly & Fraser, 1987). Samples were incubated with rabbit antiserum (a gift from the Scottish Antibody Production Unit, Law Hospital, Carluke, Scotland, UK) and ^{125}I-labelled tracer (Amersham International plc) for 4 h at 37°C. A donkey anti-rabbit second antibody (a gift from the Scottish Antibody Production Unit) was added and tubes were incubated overnight at 4°C. The bound fraction was separated using a PEG and Triton X separation solution and counted in a γ-counter. The inter- and intra-assay CV were 12·7 and 10·4 % respectively.

Statistical analysis

Ewe behaviours were analysed as means for a 10 min period; the initial 30 min sample was incremented as 3 × 10 min repeated samples. Behaviours that occurred infrequently, or were performed by relatively few animals, were analysed by χ^2 tests. There were no interactions between the effects of selection line and nutrition; therefore, the effects of selection have been reported elsewhere (Dwyer *et al.* 2001). In the present analysis, selection line was fitted first and therefore excluded before the other effects were fitted. There were also no interactions of litter size × nutritional treatment for any physical or behavioural traits; therefore, the data from single, twin and triplet ewes could be combined in analysis. The sequential effects of litter size, lamb sex and nutritional treatment, × behaviour and × maternal physiology were analysed using the Residual Maximum Likelihood procedure (Patterson & Thompson, 1971), given the relatively unbalanced nature of the data set. Skewed data were first normalised by square root transformations. For the analysis of ewe behaviours, treatment and litter size were fitted as fixed (block) effects in the model, length of labour and the interval between littermates were fitted as covariates. For the analysis of lamb behaviours treatment, litter size, lamb sex and the degree of assistance required were fitted as fixed effects, and ewe and sire identity were fitted as random effects. As lamb birth-weight did not have a linear effect on behaviours, lamb birth-weight was divided into categories based on the number of standard deviations above and below the mean birth weight (ranging from −3 to +3SD) and this was fitted as a fixed effect. No behavioural data were collected for lambs weighing ≥ −3SD below mean birth weight as all these lambs were stillborn. Length of labour was fitted as covariates in this analysis. The GenStat 5 statistics package (GenStat 5 Committee, 1993; Lawes Agricultural Trust, Rothamstead Experimental Station, UK) was used throughout. Where the data could not be normalised by transformation significant effects were determined by Kruskal-Wallis ANOVA.

For data collected by scan sampling over the first 3 d after birth, there were no significant trends with time; therefore, all data were pooled by ewe (ewe behaviours) or by lamb (lamb behaviours) for analysis. The effects of treatment, litter size and lamb sex were analysed by Residual Maximum Likelihood procedures. In the analysis of lamb behaviour, dam and sire identity were fitted as random effects.

Maternal plasma oestradiol, progesterone and β-hydroxybutyrate concentrations were analysed at each time point using Residual Maximum Likelihood statistics and fitting litter size and treatment as fixed effects.

Results

Effects on ewe weight and condition score

There was no significant difference between ewes in mean pre-conception body weight (H 50·37 (SE 0·84), L 49·29 (SE 0·99) kg) or mean condition score (H 3·59 (SE 0·04), L 3·60 (SE 0·06)). H ewes gained weight throughout their pregnancy (Fig. 1) and had a net (conceptus-free) increase in weight of 2·84 kg at parturition. L ewes maintained body weight for most of their pregnancy, showing an increase in weight only over the last 2 weeks, and had a net loss of 3·39 kg during gestation. H ewes were significantly heavier than L ewes at week 14 of gestation (Fig. 1, $P<0·05$) and remained so for the rest of gestation ($P<0·001$). Litter size significantly affected mean ewe weight only at week 20 (single 54·09 (SE 1·98), twin 57·87 (SE 1·19), multiple 62·80 (SE 2·95) kg, $P<0·01$). Ewes in both treatment groups lost condition during their pregnancy; however, the loss was greater in L ewes from week 10 of gestation (condition score loss: H −0·15 (SE 0·06), L −0·31 (SE 0·05), $P<0·05$) until lambing time (condition score loss at lambing: H −0·77 (SE 0·087), L −1·33 (SE 0·065), $P<0·001$).

Ewe physiology during gestation

The changes in ewe plasma oestradiol and progesterone concentration during gestation are shown in Fig. 2. There was no difference in pre-conception oestradiol concentration in ewes of either nutritional group until mid-gestation. L ewes had significantly higher plasma oestradiol at weeks 14 (Fig. 2(a), $P<0·05$) and 16 ($P<0·05$) of gestation but not at other times. Plasma progesterone was elevated in

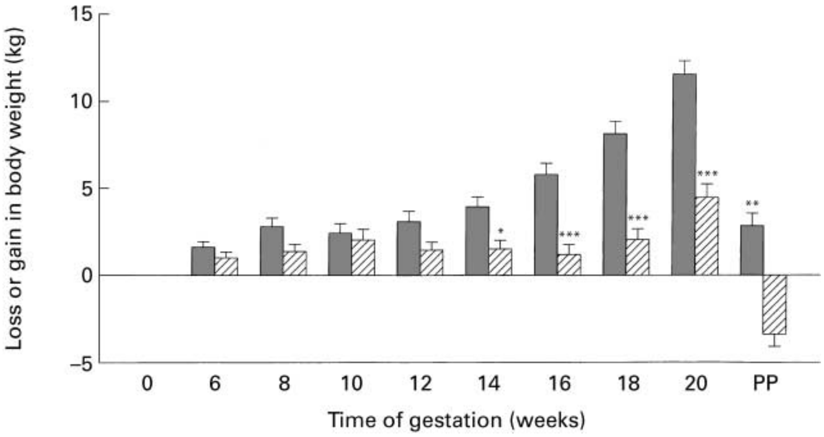

Fig. 1. Effect of high (■, n 32) or low (▨, n 29) food intake in ewes on gravid maternal body weight over gestation and on conceptus-free maternal body weight at 72 h post-partum (PP; full term was 145 d). Low-intake ewes were fed 65 % of the intake of high-intake ewes. For details of diets and procedures, see Table 1 and p. 124. Values are means with their standard errors shown by vertical bars. Mean values were significantly different from those of the high-intake ewes at each time of gestation: $^{*}P<0·05$, $^{***}P<0·001$.

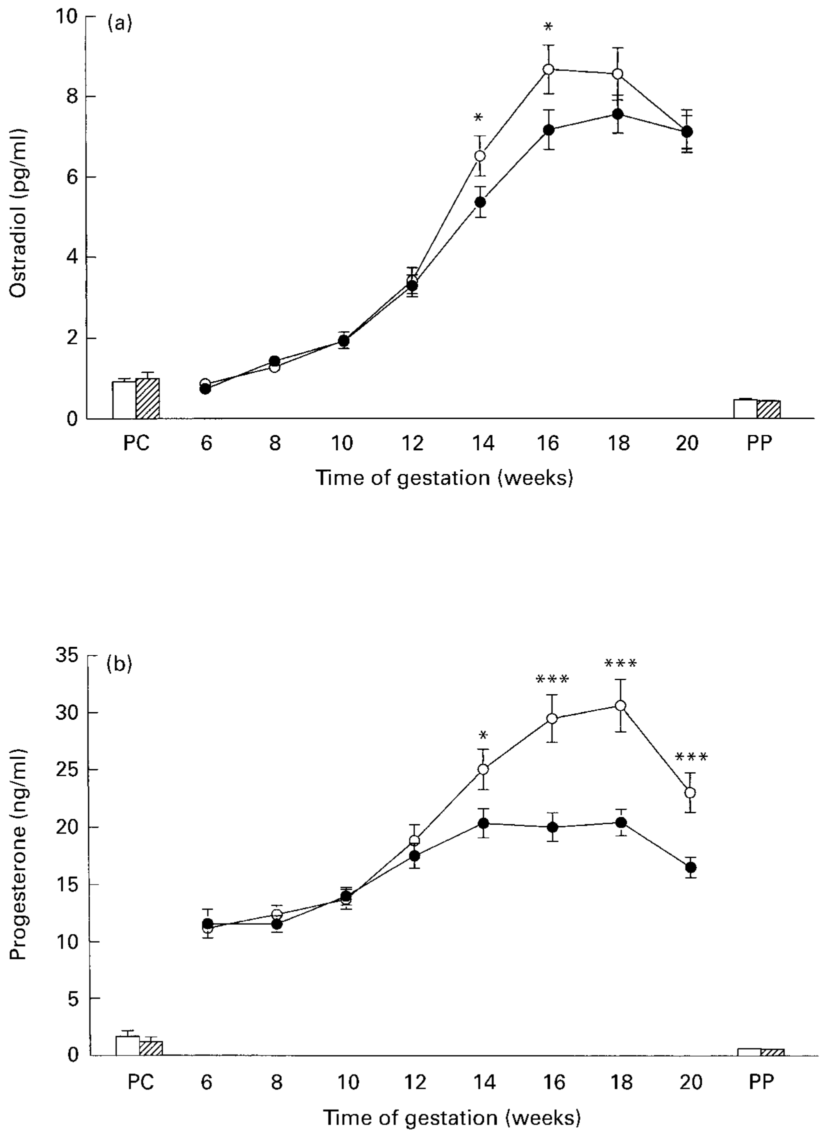

Fig. 2. Effect of high (■, ●; *n* 32) or low (▨, ○; *n* 29) food intake in ewes on (a), plasma oestradiol and (b), plasma progesterone and values pre-conception (PC) and 72 h postpartum (PP; full term was 145 d). For details of diets and procedures, see Table 1 and p. 124. Values are means with their standard errors shown by vertical bars. Mean values were significantly different from those of the high-intake ewes at each time of gestation: *$P<0.05$, ***$P<0.001$.

both groups at week 6 of gestation and increased in L ewes until week 18. This increase was smaller in H ewes resulting in a significantly higher progesterone concentration in L ewes at week 14 (Fig. 2(b), $P<0.05$) and this was maintained for the remainder of gestation ($P<0.001$). As the temporal relationship between oestrogen and progesterone concentration may be important for the expression of maternal behaviour, we also calculated the value of this ratio at each time point. The value of the oestradiol: progesterone concentration ratio increased throughout gestation in both treatment groups. There were no significant differences between treatment groups until week 20 of gestation when the mean value of the ratio was higher in H ewes than L ewes (H 0·456, L 0·352, SED 0·05, $P<0.05$).

The plasma concentration of β-hydroxybutyrate increased between weeks 15 and 18 in both treatment groups, and was maintained over the last 3 weeks of gestation. β-Hydroxybutyrate was significantly affected by litter size (single 0·299, twin 0·563, multiple 0·516 mmol/l, SED 0·095, $P<0.001$) and by treatment group (H 0·351, L 0·569 mmol/l, SED 0·04, $P<0.001$).

Parturition results and lamb survival

The effects of nutritional treatments on gestation length, length of labour, degree of birth difficulty and assistance required at delivery, lamb presentation and lamb birthweight are shown in Table 2. L ewes tended to have a longer gestation than H ewes by about 1 d. There was no

Table 2. Effects of nutritional treatments on the progress of parturition and lamb birth-weight*
(Mean values)

	High-intake ewes	Low-intake ewes	SED	Statistical significance of effect: P
Ewes (n)	32	29		
Lambs (n)	63	54		
Gestation length (d)	143·5	144·3	0·42	0·074
Interval between twins (min)	15·1	39·56	9·37	$<0·05$
Lambs requiring assistance at delivery (%)	18·8	32·7		0·08 (χ^2 3·07, df 1)
Lambs malpresented (%)	13·11	28·8		$<0·05$ (χ^2 4·29, df 1)
Lamb birth-weight (kg)	3·31	3·00	0·14	$<0·05$
Birth weights by litter:				
Single	4·22	3·71		
Twin	3·20	2·90		
Triplet	2·51	2·40		

* For details of diets and procedures, see Table 1 and p. 124.

significant difference between the two groups in the mean duration of labour (to the birth of the first lamb); however, L ewes had a significantly longer interval between the birth of the first and second lamb in a litter (Table 2, $P<0·05$). Lambs born to L ewes were more likely to be malpresented than lambs born to H ewes ($P<0·05$), and tended to require more intervention to be delivered ($P=0·08$). This was despite having a lower birth-weight than H lambs (Table 2, $P<0·05$). Lamb presentation was affected by birth weight, as lambs born with one or both forelegs retracted were heavier, and breech or backwards presentations lighter, than correctly presented lambs (one leg back 4·13 (SE 0·23), two legs back 3·28 (SE 0·16), breech or backwards 2·42 (SE 0·25), correct 2·95 (SE 0·08) kg, $P<0·001$).

In total, 82·4 % of lambs survived until weaning at 3 months, 12·6 % of lambs died within 3 d of birth (before moving outside to the paddock) and a further 5·0 % of lambs survived the postnatal period but died before weaning. Lambs that died within 3 d of birth were significantly lighter than lambs that survived ($P<0·01$); however, there was no significant difference in birth weight between lambs that died outside and lambs that survived (died within 3 d of birth 2·1 (SE 0·17), survived to 3 d but died before weaning 2·89 (SE 0·27), survived 3·16 (SE 0·08) kg.

Ewe behaviour at parturition

Ewe grooming behaviour. There was no effect of nutritional treatment on the time to start grooming the lamb after delivery (H 1·53 (95 % CI 1·13, 2·06), L 2·12 (95 % CI 1·57, 2·87) min, $P=0·58$). There was no effect of litter size or lamb sex on grooming behaviour, but latency to groom was affected by birth difficulty. An increase in the amount of intervention required to deliver the lamb resulted in an increase in the time taken for the ewe to start to groom the lamb (no assistance 0·99 (95 % CI 0·58, 1·66), partial assistance 1·52 (95 % CI 0·90, 2·55), manual delivery 3·91 (95 % CI 2·32, 6·60) min, $P<0·001$).

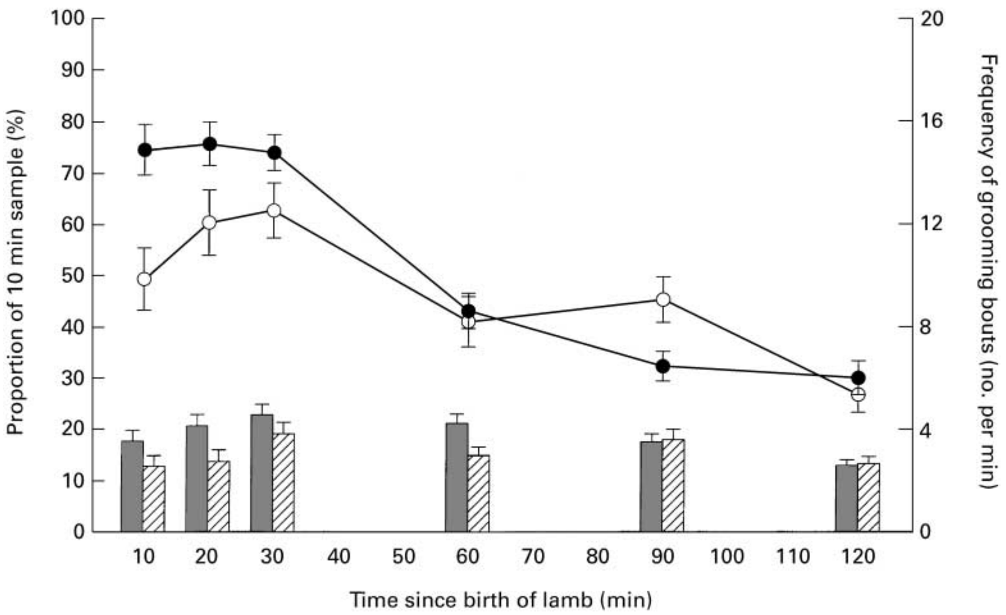

Fig. 3. Effect of high (n 32) or low (n 29) food intake in ewes on the proportion of each 10 min behaviour sample spent grooming the lamb (–●–, high intake; –○–, low intake) and frequency of grooming bouts in the first 2 h after parturition (■, high intake; ▨, low intake). For details of diets and procedures, see Table 1 and p. 124. For details of significant effects, see p. 124.

H ewes spent significantly more of the first 30 min after lamb birth grooming the lamb than L ewes (Fig. 3; H 74·67, L 57·39 min, SED 6·20, $P<0·01$). This appeared to be due to an increased number of grooming bouts by H ewes (Fig. 3; grooming frequency per min: H 4·06, L 3·03, SED 0·75, $P<0·05$) as the mean bout length did not differ between treatments. There was no significant difference between treatments in the proportion of time spent grooming (H 33·03, L 37·51 %, SED 3·42, $P=0·19$) over the last 90 min of the recording period (Fig. 3).

Ewe grooming behaviour was also affected by the length of labour, the interval between twins and by litter size. The proportion of time spent grooming in the first 30 min was reduced by a long labour (e.g. a 10 min increase in labour led to a 1·8 % reduction in time spent grooming, $P<0·01$), which appeared to be due to a reduction in both frequency ($P=0·058$) and mean bout length ($P<0·05$). Although labour had no effect on grooming behaviour in the later period, a long interval between twins did reduce the proportion of time spent grooming (e.g. a 10 min increase in twin interval resulted in a 2·00 % decrease in maternal grooming, $P<0·001$) by affecting both frequency ($P<0·05$) and length of bouts ($P<0·01$). An increase in litter size had no effect on the total amount of grooming behaviour expressed by the ewe, but there was a significant increase in the frequency of grooming bouts at both 30 min after birth (singles 1·89, twins 3·03, multiples 5·72 grooming bouts per min, SED 0·75, $P<0·001$) and in the subsequent 90 min (singles 2·06, twins 3·04, multiples 4·98 grooming bouts per min, SED 0·58, $P<0·001$). This was associated with a corresponding decrease in bout length with increase in litter size (singles 7·3 (95 % CI 6·1, 8·8), twins 6·2 (95 % CI 5·1, 7·4), multiples 5·0 (95 % CI 4·1, 6·0) s per bout, $P<0·05$).

Maternal rejection behaviours. There was no outright maternal rejection of lambs by any ewe. In total, 50·0 % of H ewes and 55·2 % of L ewes expressed some rejection behaviours (withdrawal or avoidance, pushing, butting) in the first 30 min following lamb delivery, but only 6·7 % of H ewes and 17·2 % of L ewes continued to express these behaviours after 30 min. There was no significant effect of treatment group on the number of ewes performing any lamb rejection behaviours, or the frequency that these behaviours were expressed. However, there was a tendency for L ewes to butt their lambs more frequently in the 90 min after delivery (H 0·00 (95 % CI 0·00, 0·01), L 0·05 (95 % CI 0·00, 0·26) butts per h, $P = 0·1$).

Ewe feeding behaviour. L ewes tended to be more likely to eat during the first 30 min after the birth of their lambs (L 42·9, H 22·6 % ewes seen eating, χ^2 2·77, df 1, $P=0·096$). L ewes also spent significantly more time eating in the first hour after the birth of their lambs than H ewes (median values: H 0·30 (interquartile range 0·00–3·58), L 2·56 (interquartile range 0·64–17·46) % time spent eating, χ^2 Kruskal-Wallis H 5·95, df 1, $P<0·05$).

Neonatal lamb behavioural progress

There were no direct effects of nutritional treatment on the time taken by the lamb to reach any of the developmental

Table 3. Effects of birth weight on neonatal lamb behavioural progress*

(Mean values and 95 % confidence intervals)

Weight code† …	−2SD		−1SD		+1SD		+2SD		+3SD		Statistical significance of effect:
	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	*P*
Lambs (*n*)	13		41		35		14		4		
Shakes head (s)	88·5	62·5, 125·3	48·7	34·4, 68·9	33·8	23·9, 47·9	42·1	29·8, 59·7	28·4	20, 40·2	<0·05
To knees (min)	5·25	3·80, 7·24	3·41	2·47, 4·70	2·92	2·11, 4·02	2·91	2·11, 4·01	2·99	2·17, 4·12	NS
Stand attempt (min)	13·84	10·38, 18·46	7·69	5·76, 10·25	5·82	4·36, 7·76	6·53	4·90, 8·71	7·83	5·87, 10·44	<0·01
Stands (min)	39·51	28·98, 53·89	23·17	16·99, 31·59	17·16	12·58, 23·40	16·03	11·76, 21·86	17·95	13·16, 24·48	<0·05
To udder (min)	49·29	35·37, 68·68	28·04	20·12, 39·07	20·81	14·94, 29·00	17·39	12·48, 24·23	19·88	14·27, 27·70	<0·05
Suck attempt (min)	68·21	48·73, 95·48	40·35	28·83, 56·48	28·29	20·21, 39·61	22·91	16·37, 32·07	26·67	19·06, 37·34	<0·01
Sucks (min)	153·66	127·78, 181·93	100·00	79·35, 123·04	88·72	69·34, 110·49	89·33	69·88, 111·17	82·23	63·61, 103·23	<0·05
Suck in 2 h (%)	66·7		78·6		62·9		85·7		50		NS (χ^2 4·81, df 4)
Play in 2 h (%)	10·0		60·0		42·7		42·9		25·0		<0·005 (χ^2 14·76, df 4)

* For details of diets and procedures, see Table 1 and p. 124.

† Weight codes are based on the number of standard deviations above and below the mean birth weight. All lambs weighing ≥ − 3SD below mean birth weight were stillborn.

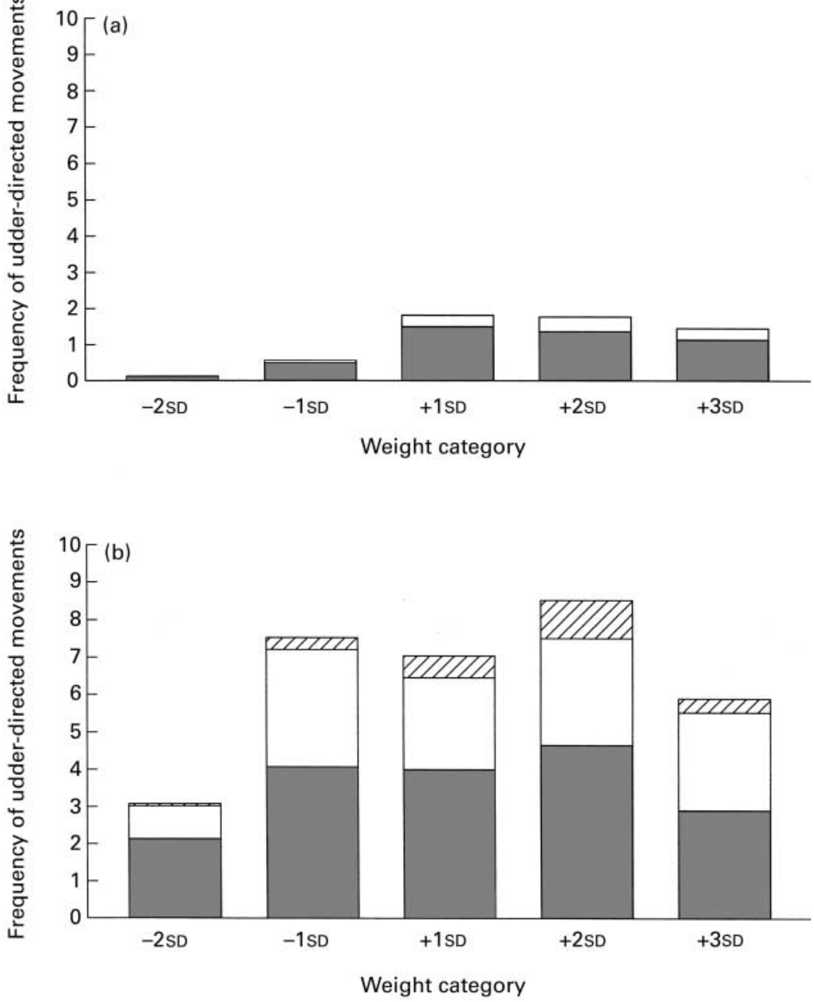

Fig. 4. Effect of weight category of lambs on frequency of udder-directed movements and the types of movements made by lambs in (a), the first 30 min after birth and (b), the subsequent 90 min. ■, Lamb reaches udder; □, lamb attempts to suck; ▨, lamb sucks successfully. For details of diets and procedures, see Table 1 and p. 124. For division of lambs into birth weight categories see p. 124–125. All lambs weighing ≥ − 3SD below mean birth-weight were stillborn.

milestones. However, lamb birth-weight had a significant effect on the latency to perform nearly all neonatal behaviours (Table 3). In general, very-low-birth-weight lambs (≥2SD below the mean) were slower than all other lambs. Low-birth-weight lambs (≥1SD below the mean birth weight) were slower than lambs that weighed greater than the mean value. The amount of assistance required to deliver the lamb also had a significant effect on the early behaviours of the lamb but not thereafter (latency to reach knees: no assistance 2·33 (95 % CI 1·83, 2·96), partial assistance 3·54 (95 % CI 2·78, 4·50), manual delivery 3·90 (95 % CI 3·06, 4·96) min, $P<0.05$). There were no significant effects of lamb sex or litter size on lamb behavioural development.

Lamb sucking behaviour. There were no direct effects of nutritional treatment on the sucking behaviour of the lamb. The frequency of lamb udder-directed movements (at the udder, attempting to suck and sucking successfully) is shown in Fig. 4. Heavier lambs had a significantly greater percentage of udder-directed movements that were sucking attempts than lighter lambs (−2SD 3·5, −1SD 7·8, +1SD 15·4, +2SD 20·9, +3SD 20·4 %, $P<0.05$). In the subsequent 90 min period (Fig. 4(b)), lamb weight had a significant effect on all movements of the lamb to the udder ($P<0.05$). Body weight of the lamb also had a significant effect on the proportion of udder-directed movements that resulted in successful sucking (−2SD 1·9, −1SD 3·8, +1SD 7·6, +2SD 11·3, +3SD 6·0 %, $P<0.01$). There were no significant differences between treatment groups in ewe responses to lamb sucking behaviours.

Ewe and lamb vocalisations

Ewe vocalisations were classified as ‘low-pitched’ (the specific lambing vocalisation made by ewes to their lambs) or ‘high-pitched’ (characteristically distress vocalisations). There were no direct effects of nutritional treatment on ewe high- or low-pitched vocalisations. Litter

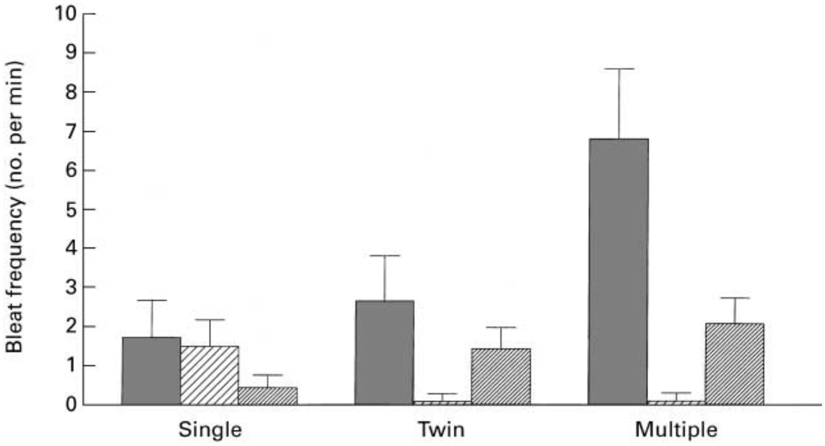

Fig. 5. Effect of lamb litter size on bleat frequency for single, twin and multiple litters. ■, Ewe low-pitched bleats; ▨, ewe high-pitched bleats; ▨, lamb bleats. For details of diets and procedures, see Table 1 and p. 124.

size and the amount of assistance required at delivery were the main effects on both ewe vocalisations. An increase in litter size caused a corresponding increase in low-pitched bleating ($P<0{\cdot}01$), but a decrease in high-pitched bleating by the ewe (Fig. 5, $P<0{\cdot}001$). Ewes that required assistance at delivery had a lower frequency of low-pitched bleating than unassisted ewes (no assistance 5·34 (95 % CI 4·09, 6·75), partial assistance 3·32 (95 % CI 2·36, 4·46), manual delivery 2·02 (95 % CI 1·28, 2·92) bleats per min, $P<0{\cdot}01$).

Lamb bleats were unaffected by maternal nutritional treatment. Lamb vocalisations were affected only by litter size: an increase in litter size caused an increase in lamb bleat frequency (Fig. 5, $P<0{\cdot}01$).

Behaviour to 3 d postnatal

There was no effect of nutritional treatment on the mean distance between the ewe and lamb, or on the proportion of observations where the ewe or lamb was standing. The main difference between the two treatments was in the time spent eating in the first 3 d of lactation, as L ewes were seen eating significantly more often than H ewes (H 9·11, L 25·31% observations of SED 1·66, $P<0{\cdot}001$). H ewes were more vocal than L ewes and this was significant for ewe low-pitched bleating (H 4·50, L 2·61% observations of SED 0·84, $P<0{\cdot}05$). By contrast, H lambs tended to be less vocal than L lambs over the same period (H 1·55 (95 % CI 1·05, 2·15), L 2·57 (95 % CI 1·91, 3·33) percent observations, $P=0{\cdot}11$). There was no effect of maternal gestational treatment on the frequency with which lambs were seen either sucking or playing. However, L lambs had a significantly greater frequency of performing exploratory behaviours (of both the pen fixtures and other animals) than H lambs (L 3·90, H 2·56 % observations, SED 0·60, $P<0{\cdot}05$).

Tests of maternal attachment

Maternal behaviour score. H ewes were significantly more likely to receive high maternal behaviour scores (score 6: H 41·3, L 21·4 % ewes) and less likely to receive low scores (score 2 (no ewes received a score of 1): H 3·5, L 14·3 %, χ^2 9·74, df 4, $P<0{\cdot}05$) than L ewes.

Maternal attachment score. H ewes were less likely to be assigned a low score (score 1 or 2: H 3·2, L 20·7 %) than L ewes and more likely to receive a high score (score 4: H 61·3, L 37·9 %, χ^2 5·69, df 2, $P=0{\cdot}058$).

Ewes that scored highly on the first test of maternal attachment (maternal behaviour score) also received high scores for the maternal attachment score (r 0·34, n 56, $P<0{\cdot}05$).

Discussion

Poor lamb survival following maternal undernutrition during pregnancy is a probable consequence of a low lamb birth-weight, leading to impaired thermoregulatory ability and reduced maternal lactation. The present study demonstrates that even a moderate level of maternal undernutrition in pregnancy can have deleterious consequences for the expression of maternal behaviour in the sheep, which may compound the reduced survivability of the lamb. Furthermore, the effect of maternal undernutrition on lamb birth-weight may also impair lamb neonatal behaviours leading to a delay in sucking.

Maternal response at parturition to undernutrition in pregnancy

The level of undernutrition used in the present study reduced maternal weight-gain during pregnancy and resulted in a net loss of 6·7 % maternal body weight in the L ewes. This is considerably less than values of up to 25 % weight loss reported in Cheviot sheep wintered outdoors (Thomson & Thomson, 1949). However, this level of nutritional restriction did require the ewes in our present study to mobilise body reserves as shown by both their loss of backfat over pregnancy and the increased β-hydroxybutyrate concentration in the L ewes from week 16 of gestation onwards.

Maternal nutritional restriction also had an effect on plasma steroid hormone concentrations in the later stages of pregnancy. Although the effect of undernutrition on plasma oestradiol was relatively short-lived, L ewes had elevated plasma progesterone concentrations over the last third of gestation in comparison with the H ewes. A similar inverse relationship between nutrition level and plasma progesterone concentration has been reported previously in sheep and cattle (Gauthier *et al.* 1983; Vincent *et al.* 1985; Hall *et al.* 1992; O'Doherty & Crosby, 1996). A low feed intake is also associated with a delay in the postpartum decline in plasma progesterone (Mellor *et al.* 1987). Plasma progesterone concentration is negatively correlated with colostrum and milk yield (Hall *et al.* 1992; O'Doherty & Crosby, 1996) and postpartum delay in lactogenesis in under-fed ewes is associated with a delayed decline in progesterone (Mellor *et al.* 1987). These results suggest that, in our present study, the L ewes with elevated plasma progesterone are likely to have had impaired colostrum and milk production.

In addition to their roles in preparing the maternal body for birth and lactation, the steroid hormones also play an important function in the onset of maternal behaviour. In sheep, oestradiol and progesterone play an essential priming role in the onset of maternal behaviour (for reviews, see Keverne, 1988; Keverne & Kendrick, 1994). In the rat, the onset of maternal behaviour is also related to temporal changes in the relative concentrations of oestrogen and progesterone, essentially an increase in oestradiol and a decline in progesterone (Doerr *et al.* 1981; Rosenblatt, 1994). These temporal effects have not been investigated in sheep. In both rats and sheep, maternal behaviour is then induced by the central release of oxytocin (Fahrbach *et al.* 1984; Keverne & Kendrick, 1992). In sheep, both progesterone and oestrogen promote the synthesis of oxytocin mRNA in areas of the brain implicated in maternal behaviour (Broad *et al.* 1993). In behavioural studies in sheep and rats, high oestradiol (Pryce *et al.* 1988; Shipka & Ford, 1991; Dwyer *et al.* 1999) and a high oestradiol: progesterone value (Pryce *et al.* 1993; Dwyer *et al.* 1999) in late gestation is correlated with the expression of maternal behaviour. The effects of undernutrition in elevating plasma progesterone in late gestation and reducing the oestradiol: progesterone value, seen in the present study, may be responsible for differences in maternal behaviour between the L and H ewes.

Despite the relatively mild nutritional restriction in comparison with what ewes may experience during the winter, L ewes did show deficits in maternal care in comparison with the H ewes. There was no apparent difference in the timing of onset of maternal care; however L ewes did not spend as much time grooming the lamb as H ewes immediately after the birth of their lambs. This may have been due to the competing motivations to eat and to care for their lambs, as the L ewes spent more time eating after the birth of their lambs than H ewes. In addition to the direct effects of undernutrition on maternal care, there were several effects of nutrition level on the parturition process that have responses correlated with reduced expression of maternal care. L ewes had a longer interval between the birth of the first and second twins, as has been reported previously (Thomson & Thomson, 1949), which is known to reduce total grooming attention in ewes (Dwyer & Lawrence, 1998) as was the case in the present study. In addition, L ewes had a higher incidence of malpresented lambs and required more assistance with lamb delivery. Assistance at delivery was shown to reduce ewe low-pitched bleats, a specific vocalisation associated with maternal care (Shillito, 1972; Dwyer *et al.* 1998) that is thought to play a role in ewe–lamb bond formation (Nowak, 1996). Thus, undernutrition affected the expression of maternal care directly through a reduction in maternal grooming behaviour, and indirectly by prolonging the parturition process.

Effects of maternal undernutrition on neonate behaviours

Maternal nutritional restriction during pregnancy caused a small but significant ($P<0{\cdot}05$) reduction in mean lamb birth-weight of 9 % when compared with well-fed ewes. Although there were no direct effects of maternal nutritional treatment on lamb behaviour, lamb birth-weight was shown to have a significant influence on neonatal lamb behavioural development in the first few hours after birth. In general, low-birth-weight lambs had a slower progression to standing and sucking than heavier lambs. Standing and sucking quickly have been shown to be related to improved survival rates in lambs (Owens *et al.* 1985; Cloete, 1993; Dwyer *et al.* 2001). This is related not only to the nutritional and immunological benefits of early colostrum ingestion, but also to the effects of sucking on lamb bonding to the ewe (Nowak *et al.* 1997). In addition to their slower development, low-birth-weight lambs appeared to lack co-ordination or the ability to accomplish successful sucking when compared with heavier lambs, as shown by the lower proportion of sucking attempts that resulted in successful sucking. Impaired neuromotor development has been demonstrated in prenatally undernourished young in other species (e.g. Gramsbergen & Westerga, 1992), suggesting that undernutrition impairs both vigour and the ability to carry out complex behaviours. This may be due to the effects of undernutrition on the development and complexity of brain structures, leading to reductions in cell numbers and myelination of axons (for reviews, see Morgane *et al.* 1993; Rees *et al.* 1998; Dauncey & Bicknell, 1999; Mallard *et al.* 2000).

Lambs born to L mothers had a higher incidence of malpresentation than H lambs, and tended to require greater assistance at delivery, despite their lower birth-weight. This supports our previous conclusions that birth difficulty is not simply related to lamb birth-weight (Dwyer *et al.* 1996), but may reflect events occurring prenatally, particularly those that may restrict fetal movement *in utero* such as restrictions in uterine growth or the volume of amniotic fluid. Requiring assistance at delivery caused a delay in the expression of early lamb behaviours in the present study and previously (Dwyer *et al.* 1996), and reduced the number of udder-directed movements made by the lambs. Thus, maternal undernutrition during gestation had an indirect effect on the early expression of neonatal lamb behaviours as well as effects on lamb birth-weight.

Effects on the development of the ewe–lamb relationship

Over the first 3 d of the developing ewe–lamb relationship there were few behavioural differences between H and L ewes although H ewes made low-pitched vocalisations to their lambs more frequently than L ewes. As low-pitched bleating is an expression of maternal care or concern (Dwyer *et al.* 1998), this suggests that the effects of nutrition on the initial expression of maternal behaviour may persist over the early postnatal period. L ewes were also found to spend significantly more time eating than H ewes. Lactation is an energetically expensive process (e.g. Reynolds & Tyrrell, 1990); thus, L ewes may have needed to spend more time eating to meet the requirements of lactation as they had less body reserves to draw on than the H ewes. In addition, L ewes appeared to be more food-motivated than H ewes. This increased food-motivation is a possible explanation for the increased likelihood of undernourished Merino ewes moving away from the birth-site soon after birth (Putu *et al.* 1988).

There were also few behavioural differences between H and L lambs in the first 3 d of postnatal life, although L lambs were seen performing investigatory behaviours more often than H lambs. In addition, L lambs tended to bleat more frequently than H lambs. As suggested earlier, although not measured in the present study, it is likely that the L ewes had reduced udder development and lactation rates in comparison with the H ewes. The increased investigatory behaviour of the L lambs, particularly of other ewes, may be a consequence of lower intake or poorer quality of milk ingested by these lambs. Neonate vocal behaviour has been suggested to function as an expression of degree of need (Brunelli *et al.* 1994; Weary & Fraser, 1995) and, in lambs, bleat rate is inversely related to the amount of maternal care received (Dwyer *et al.* 1998). Increased lamb vocalisations in the L lambs may, therefore, be an expression of reduced maternal care by the L ewes.

In tests of ewe attachment to her lamb at both 24 and 72 h after birth, L ewes were more likely to receive low scores (reflecting maternal disinterest in her lambs) than H ewes. This may be a result of inadequate bond formation between the ewe and her lambs at the initiation of maternal behaviour, mediated by reduced grooming behaviour. However, as sucking and milk ingestion play a fundamental role in lamb bonding to the ewe (Nowak *et al.* 1997) and in the maintenance of maternal behaviour (Rosenblatt & Siegel, 1981), lower milk production in the L ewes may influence the quality of the relationship formed by the lamb to the ewe, and interfere with the maintenance of maternal behaviour.

Conclusions and implications

This present study has demonstrated that even a moderate reduction in maternal nutritional intake in primiparous ewes can result in a quantitative reduction in the expression of maternal behaviour at parturition. This suggests that more extreme undernutrition, as may be experienced by hill ewes under normal farm conditions, is likely to cause larger deficits in maternal behaviour. Tests of ewe–lamb bonding demonstrated a weaker relationship between ewe and lamb in undernourished mothers, although whether this was related to the onset or maintenance of maternal behaviour could not be disentangled in the present study. As lamb survival is crucially dependent on the adequate expression of maternal care from the ewe (for review, see Nowak *et al.* 2000), any impairment in maternal care will increase the probability that the lamb will not survive, in addition to compromised lamb survival through low birth-weight or poor maternal lactation. A possible mechanism by which maternal undernutrition can influence the expression of maternal behaviour is the elevation of plasma progesterone in mid to late gestation, and the lower oestradiol: progesterone value in L ewes in comparison with H animals.

These present studies were carried out in primiparous ewes, which may be more susceptible to nutritional influences on maternal care than multiparous ewes. Primiparous ewes are known to perform less well as mothers in comparison with multiparous ewes (O'Connor *et al.* 1992; Dwyer & Lawrence, 2000) and are more sensitive to manipulations of both external and internal cues than multiparous ewes (e.g. presence of olfactory cues: Lévy & Poindron, 1987; Lévy *et al.* 1995; effects of progesterone, oestrogen, opioids and oxytocin: Kendrick & Keverne, 1991; Keverne & Kendrick, 1991). Thus, multiparous ewes may have a more robust maternal response to moderate undernutrition than primiparous ewes. However, these studies were carried out on hill sheep, which have been selected primarily for their ability to survive and reproduce under unfavourable conditions. It is likely that these animals may have a lesser response to undernutrition than breeds selected to perform under more favourable conditions, which are already known to show reduced maternal care under improved conditions (Dwyer & Lawrence, 1998).

Finally, although the present study did not show an effect of maternal nutritional treatment on the expression of neonatal lamb behaviours, the effect of treatment produced only a small average difference in birth weight. In the present study, however, lamb neonatal progress was affected by birth weight with low-birth-weight lambs taking longer to perform all behaviours than heavier lambs. This delayed development is likely to be exacerbated by more severe undernutrition and is known to be related to lamb survival (Owens *et al.* 1985; Cloete, 1993; Dwyer *et al.* 2001). Furthermore, as the present experiment was carried out indoors, the lambs were protected from the effects of precipitation and wind chill. In outdoor hill conditions, even small delays in standing and sucking may have more severe consequences for lamb survival.

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