



OPEN Maternal experience affects the behavioral response and the daily weight gain to weaning stress in lambs

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Sheep maternal experience is of great importance for the development and welfare of their offspring. This study aimed to compare the behavioral and physiological responses to weaning stress between lambs born and reared by multiparous (with maternal experience) and primiparous (without maternal experience) dams. Twenty-three singleton lambs born to Finnish-Landrace x Polwarth dams were used: twelve lambs were born and reared by multiparous dams (LMD) and eleven lambs by primiparous dams (LPD). Before and after weaning body weight, serum proteins and the behaviors were evaluated. While the LPD group increased daily weight gain, the LMD group experienced weight loss ($p = 0.0005$). Most of the differences observed between groups in behavioral responses were found in the first two days after weaning, where the LMD lambs displayed lower frequency of grazing and standing behaviors ($p < 0.05$), but greater frequency of vocalizing, pacing, and walking behaviors ($p < 0.001$). Using global positioning devices, weaning affected the circadian rhythm of the distance traveled by lambs, and maternal experience affected the circadian rhythm of distance and mean daily velocity ($p < 0.05$). In conclusion, considering all the behaviors assessed, as well as the daily weight gain, maternal experience affected lambs' response to weaning stress.

Keywords Body weight, Grazing, Pacing, Serum proteins, Sheep, Vocalization

Artificial weaning involves separating lambs from their dam at ages earlier than what occurs naturally, evokes an important stress response, which is evidenced by physiological and behavioral indicators^{1–5}. Various factors are involved in the response to weaning in lambs, including, for example, the separation from dam, changes in nutrition, lack of access to the udder and the cessation of suckling behavior, as well as other social and environmental changes^{2,4,6}. Among the different factors that contribute to the weaning stress response in lambs, maternal separation is of great importance, given the key role it plays in the behavioral response⁴. The importance of maternal separation was demonstrated by comparing the stress response at weaning between artificially reared lambs and those reared by their dam, with the latter displaying greater frequency of pacing, vocalizing, and walking behaviors⁴.

Maternal experience in sheep is of great importance for the development and welfare of the offspring⁷. Unlike ewes with previous maternal experience (multiparous), ewes without previous maternal experience (primiparous) display poorer maternal behavior toward their offspring^{7,8}. Primiparous ewes are lighter and give birth to smaller lambs, their lambs take longer to get up and seek the udder and suckle, tend to be more aggressive towards the lamb, spend less time looking for them and being with them^{7,9–13}. Furthermore, using the Maternal Behavior Score (MBS), Madani et al.¹⁴ and Damián et al.¹⁵ reported that multiparous ewes are better

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dams than primiparous ewes on the day of lambing. These elements highlight the poor bond that exists between the primiparous dams and their offspring.

Therefore, given that the dam represents a central factor in the lambs' weaning stress response and that multiparous ewes generate a better dam-offspring bond, we hypothesized that weaning elicits greater behavioral and physiological responses in lambs from multiparous ewes compared to those from primiparous ewes. In this sense, the objective of this study was to compare the behavioral and physiological responses to weaning between lambs born and reared by multiparous and primiparous dams.

Methods

All the experimental procedures were approved by the Comisión de Ética en el Uso de Animales (CEUA) of the Instituto Nacional de Investigación Agropecuaria (INIA, No. 2023.17). All experiments were carried out in strict accordance with the relevant regulations (Uruguayan law number 18611) and with the ARRIVE guidelines.

Location, animals and weaning management

The study was conducted at the Experimental Sheep Unit, INIA La Estanzuela, Uruguay (34°19'S;57°40'E). Twenty-three singleton lambs (Finnish Landrace x Polwarth) were used in this study, of which twelve lambs (LMD) were born to multiparous dams (3.31 ± 0.84 years old) and eleven lambs (LPD) were born to primiparous dams (1.87 ± 0.01 years old). Lambings were recorded several times a day, and both dyad members (dams and lambs) were identified. Before and after lambing, all animals were managed in the same paddock and under the same conditions. The lambs and their dams were kept in the same paddock and pasture conditions (in a semi-extensive system, always under grazing conditions and throughout the day), but separated by a fence into two grazing areas (each 2,000 m²), with lambs of multiparous dams and their dams on one side, and lambs of primiparous dams and their dams on the other. They grazed improved pastures composed of *Bromus inermis* and *T. pratense*, or *Dactylis glomerata* and alfalfa (*Medicago sativa*), *Avena s.* and ryegrass (*Lolium m.*), and had water *ad libitum*.

Weaning was carried out when the lambs were 114 days (3.8 months) old. The day of weaning was defined as day 0. On day 0 at 7:00 a.m., the dams were separated from their lambs and moved to a paddock more than 500 m away from the lambs. After weaning, the lambs returned to the paddock and grazing areas corresponding to each group.

Physiological parameters

Body weight

Both groups of lambs were weighed three days before weaning (day -3) and on day 5 post-weaning.

Blood sample collection and serum protein determination

Blood samples were collected by jugular venipuncture three days before weaning (day -3) and day 5 post-weaning. The blood samples were centrifuged at 1500 rpm for 15 min, and serum was separated and stored at -20 °C until serum protein determinations. Serum protein concentrations (total proteins and albumin) were determined with commercial kits (Bio-System, Barcelona, Spain) at the Laboratorio de Bioquímica (Facultad de Veterinaria, Universidad de la República, Uruguay). The lower detection limits of these kits were 0.46 g/dL and 0.11 g/dL for total proteins and albumin, respectively. The concentration of globulins was estimated by the difference between total proteins and albumin¹⁶.

Behavioral recordings

Behavior recording through direct observation

On day -3, lambs were identified with numbers painted on both flanks. Two observers were stationed in the same position relative to and outside of the paddock, from where they recorded behavior. Individual behavior was assessed using instantaneous scan sampling every 10 minutes⁴. Behavior was evaluated from two days prior to weaning (day -2) to four days after weaning (day 4), for 8 h per day. Behavioral assessment was conducted in two daily periods, each lasting 4 h: (1) in the morning: from 08:00 to 12:00 h, and (2) in the afternoon: from 14:00 to 18:00 h.

The behaviors assessed and their definitions were as follows: Lying (lying in any resting position), Standing (maintaining an upright position on extended legs), Walking (moving all four legs with or without the head raised), Grazing (collecting or consuming grass, with the head near the ground, standing still or moving slowly), Pacing (brisk, repetitive walking parallel to the fence), and Vocalizations (emitting bleats). Vocalizations were recorded during 30 s every 10 min using 0/1 sampling¹⁷ as previously described in Damián et al.⁴.

Behavior recording using GPS

Global Positioning System (GPS) sensors (i-gotU GT-600, Mobile Action Technology Inc., Taiwan) were attached to a neck collar of the lambs¹⁸. Collars with these GPS devices were randomly assigned to nine animals in each group. Data were collected every 10 minutes^{19–21} from two days prior to weaning (day -2) to two days after weaning (day 2). The variables of distance traveled by the animal (m) and mean daily velocity (m/s) were recorded^{22–25}. For the analysis of distance (m) and mean daily velocity (m/s) data, the 24-hour day was divided into 4 periods of 6 h each: Period 1: from 7:00 a.m. to 1:00 p.m. (dawn-midday), Period 2: from 1:00 p.m. to 7:00 p.m. (midday-dusk), Period 3: 7:00 p.m. to 1:00 a.m. (dusk-midnight), and Period 4: from 1:00 a.m. to 7:00 a.m. (midnight-dawn). This criterion of schedules in periods during the 24 h of the day was carried out considering the moment of weaning, and based on what was reported by Rietema et al.²⁶ for the evaluation of circadian rhythms in sheep.

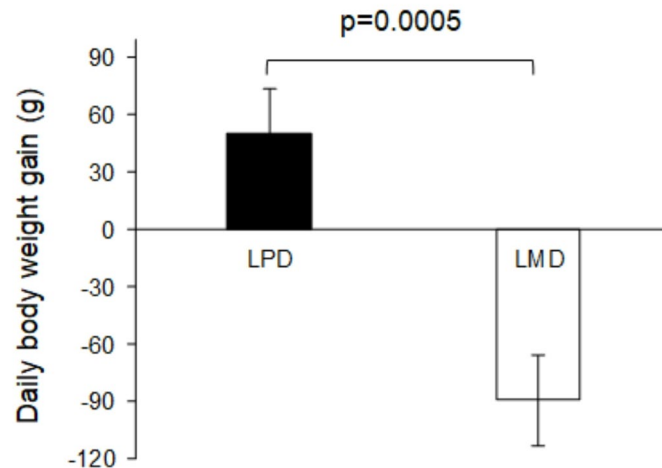


Fig. 1. Daily body weight gain ($\text{g} \pm \text{SEM}$) in lambs born and reared by primiparous dams (LPD: black bar) and lambs born and reared by multiparous dams (LMD: white bar) between day -3 and 5 weaning relative to weaning (day 0).

Variables	Group (G)		P value	Time (Ti)		P value	G*Ti
	LPD	LMD		Day -3	Day 5		
Total proteins	72.1 ± 1.49	72.6 ± 1.61	0.831	72.5 ± 1.29	72.2 ± 1.39	0.856	0.772
Albumin	31.6 ± 1.58	31.7 ± 1.71	0.953	29.6 ± 1.48	33.7 ± 1.59	0.066	0.572
Globulins	40.4 ± 2.55	40.8 ± 2.76	0.937	42.9 ± 2.33	38.3 ± 2.50	0.176	0.839

Table 1. Serum concentrations (g/L) of total proteins, albumin and globulins (mean $\pm$ SEM) of lambs that were born to multiparous dams (LMD) and of lambs that were born to primiparous dams (LPD) on day -3 and day 5 after weaning. G: Group (LMD vs. LPD); Ti: Time (days), G*Ti: interaction between Group and Time.

Statistical analysis

The difference in body weight between both groups on day -3 (before weaning), as well as the daily body weight gain (difference between body weight on day 5 post-weaning and day -3), were compared between groups using a Student's *t* test. A repeated measurements analysis of variance was performed on serum proteins concentrations and frequencies of each behavior (expressed as percentages of total observations) using the GLIMMIX procedure of SAS OnDemand for Academics (v. 3.1.0, SAS Institute Inc., Cary, NC, USA). The main effects studied were group (LPD vs. LMD), time (days) and the interaction between group and time as fixed effects, and the lamb as a random effect. Time was considered as a repeated measure in the model. For the distance traveled and mean daily velocity data, the model included group, time (days), period of the day, and their interactions as fixed effects, and animal as a random effect. Post hoc comparisons were performed with the Tukey-Kramer test. The co-variance structure used was autoregressive of the first order. Data are presented as mean $\pm$ SEM. Significance was considered with an alpha of ≤ 0.05 and a trend between 0.05 and 0.10.

Results

Physiological parameters

Body weight

At day -3 there was no significant difference in body weight between LPD and LMD lambs (24.6 ± 1.06 Kg vs. 26.6 ± 0.88 Kg, respectively, $p = 0.128$). However, daily body weight gain between day -3 and day 5 relative to weaning varied significantly between both groups ($p = 0.0005$), in which lambs in the LPD group showed an increase in daily gain, while those in the LMD group showed a loss in daily gain (Fig. 1).

Serum proteins concentrations

There was no significant effect of group, time or interaction between group and time in any of these variables (Table 1). There was only a trend in albumin concentration, which tended to increase from day -3 to day 5 (Table 1).

Behaviors

Behavior recording through direct observation

There was a significant effect of time ($p < 0.01$) and a significant interaction between group and time for all behaviors ($p < 0.0002$) (Table 2). Regarding the main effect of group, there were significant effects on the following behaviors: lying, vocalizations, pacing, and walking (Table 2). The behaviors of grazing and standing were not affected by group (Table 2).

Grazing

There was no significant group effect on grazing frequency (Table 2). The significant interaction between group and time ($p < 0.0001$) was evidenced by the lower frequency of grazing in LMD lambs on day 0 compared to LPD lambs ($p < 0.001$, Fig. 2A). For all lambs, grazing frequency was highest on days 1 and 2 (Fig. 2A).

Standing

There was no significant group effect on standing frequency (Table 2). The significant interaction between group and time ($p < 0.0001$) was evidenced by the lower frequency of standing in LMD lambs on day 0 and day 1 ($p < 0.05$) and greater frequency on day 4 ($p < 0.01$) compared to LPD (Fig. 2B). Standing frequency tended to decrease from the days before weaning (DBW) to day 3 ($p = 0.078$) and from DBW to day 4 ($p = 0.080$), and similarly also tended to decrease from day 0 to day 3 ($p = 0.082$) and from day 0 to day 4 ($p = 0.085$) (Fig. 2B). There was also a tendency for standing to decrease from day 1 to day 3 ($p = 0.056$) and from day 1 to day 4 ($p = 0.058$) (Fig. 2B).

Lying

LMD lambs were less frequently lying down than LPD lambs ($p = 0.023$, Table 2). The significant interaction between group and time ($p = 0.0002$) was evidenced by the lower frequency of lying in LMD lambs on day 4 ($p < 0.05$) compared to LPD (Fig. 2C). For all lambs, lying frequency was higher on day 4 (Fig. 2C).

Vocalizations

The frequency at which lambs were observed vocalizing was higher in LMD than in LPD lambs ($p = 0.0005$, Table 2). The significant interaction between group and time ($p < 0.0001$) was evidenced by the higher frequency at which LMD lambs were observed vocalizing than LPD lambs on day 0 ($p < 0.0001$, Fig. 3A). For all lambs, the frequency of vocalizations increased significantly from the DBW to day 0 ($p < 0.0001$), then returned to baseline levels by day 2 (Fig. 3A).

Pacing

The frequency at which lambs were observed pacing was greater in LMD than in LPD lambs ($p = 0.0002$, Table 2). The significant interaction between group and time ($p < 0.0001$) was evidenced by the higher frequency at which LMD lambs were observed pacing than LPD lambs on day 0 ($p < 0.0001$, Fig. 3B). For all lambs, the frequency of pacing increased significantly from DBW to day 0 ($p < 0.0001$), then returned to baseline levels by day 1 (Fig. 3B).

Walking

The frequency at which lambs were observed walking was greater in LMD than in LPD lambs ($p < 0.0001$, Table 2). The significant interaction between group and time ($p < 0.0001$) was evidenced by the higher frequency at which LMD lambs were observed walking than LPD lambs on day 0 and day 1 ($p < 0.0001$, Fig. 3B). For all lambs, the frequency of walking was highest on days 3 (Fig. 3B).

Behavior recording using GPS

Distance traveled

Distance traveled was not affected by group ($p = 0.198$), but there was a significant effect of day ($p = 0.012$): distance traveled increased from DBW to Day 0 ($p = 0.002$, Fig. 4) and no differences between these days and the days 1 and 2 post-weaning were observed ($p > 0.05$). There was no interaction between group and day for distance traveled ($p = 0.658$). There was a significant effect of period on distance traveled ($p = 0.0001$), with lambs traveling more distance in periods 1 and 2 compared to period 3 ($p < 0.05$), and in turn, during period 3 the lambs traveled more than during period 4 ($p = 0.0006$, Fig. 4).

Behavior	Group (G)		P value	Time (Ti)	G*Ti
	LPD	LMD		P value	P value
Grazing (%)	72.1 ± 11.64	71.3 ± 11.61	0.5222	< 0.0001	< 0.0001
Standing (%)	66.1 ± 8.98	64.9 ± 8.98	0.4420	0.0026	< 0.0001
Lying (%)	19.3 ± 9.23	15.5 ± 9.023	0.0238	0.0006	0.0002
Vocalizations (%)	2.72 ± 1.32	4.85 ± 1.32	0.0005	< 0.0001	< 0.0001
Pacing (%)	0.39 ± 0.31	1.68 ± 0.29	0.0002	< 0.0001	< 0.0001
Walking (%)	14.0 ± 0.65	19.6 ± 0.63	< 0.0001	0.0002	< 0.0001

Table 2. The percentage of behaviors before and after weaning displayed by lambs reared by primiparous dams (LPD) or multiparous dams (LMD).

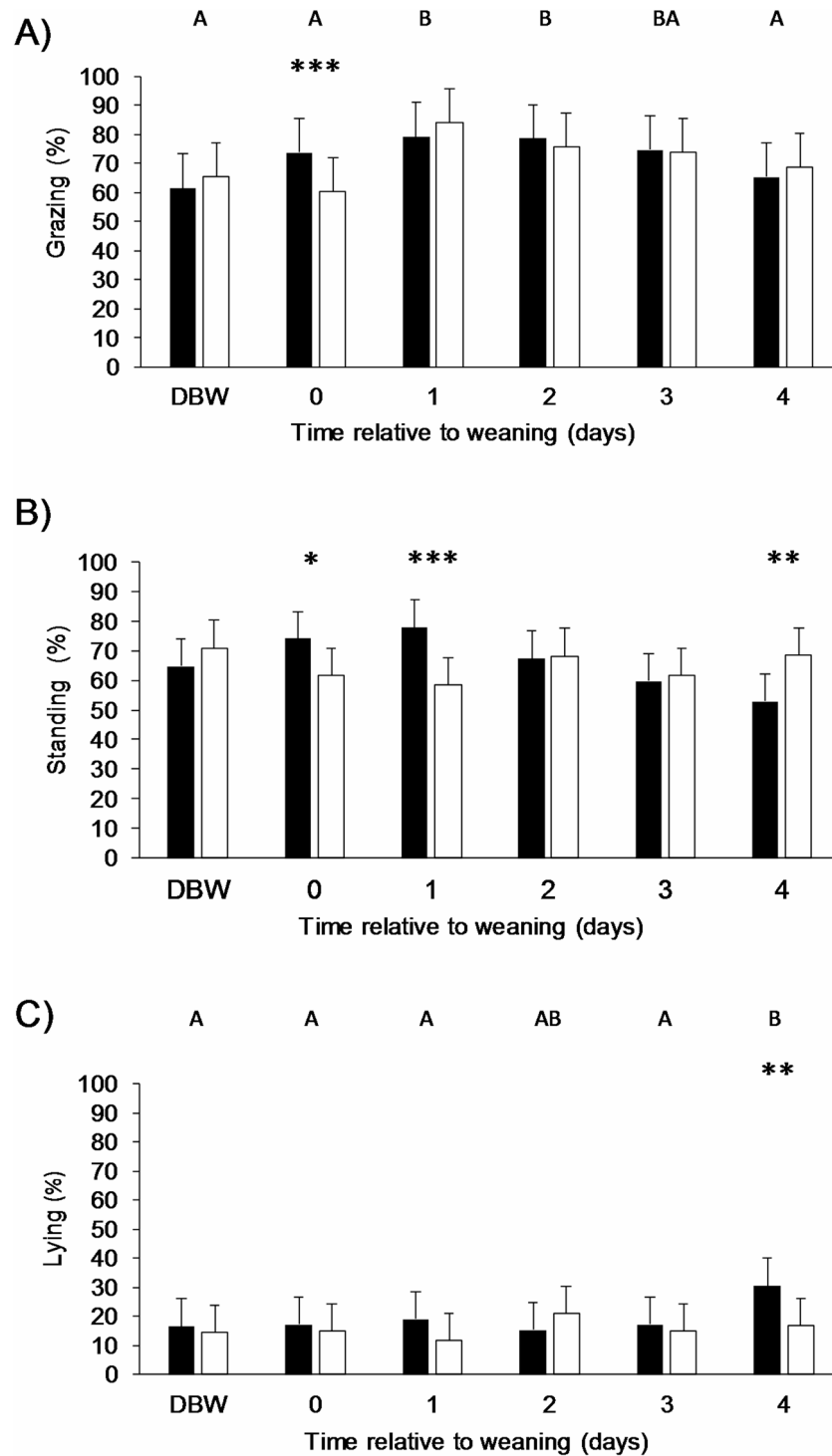


Fig. 2. Frequency (percentages of total observations) in which lambs were observed grazing (A), standing (B), and lying (C) in both groups: lambs born and reared by primiparous dams (LPD: black bars) and lambs born and reared by multiparous dams (LMD: white bars). DBW: mean data recorded during the 2 days before weaning. Differences between LPD and LMD in the same day are indicated as: * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$. Different letters indicate significant differences ($p < 0.05$) among days.

There was also an interaction between day and period on distance traveled ($p = 0.03$). During DBW, there was no significant difference among periods 1, 2, and 3 ($p > 0.05$). However, on the day of weaning (day 0), there was an increase in distance traveled in period 2 ($p = 0.01$), and there was no difference between periods 1 and 4 ($p > 0.05$, Fig. 4). The differences between periods 1 and 2 on day 0 disappeared on days 1 and 2 post-weaning ($p > 0.05$, Fig. 4). On the other hand, on day 1, the lambs traveled more distance in periods 1 and 2 than in

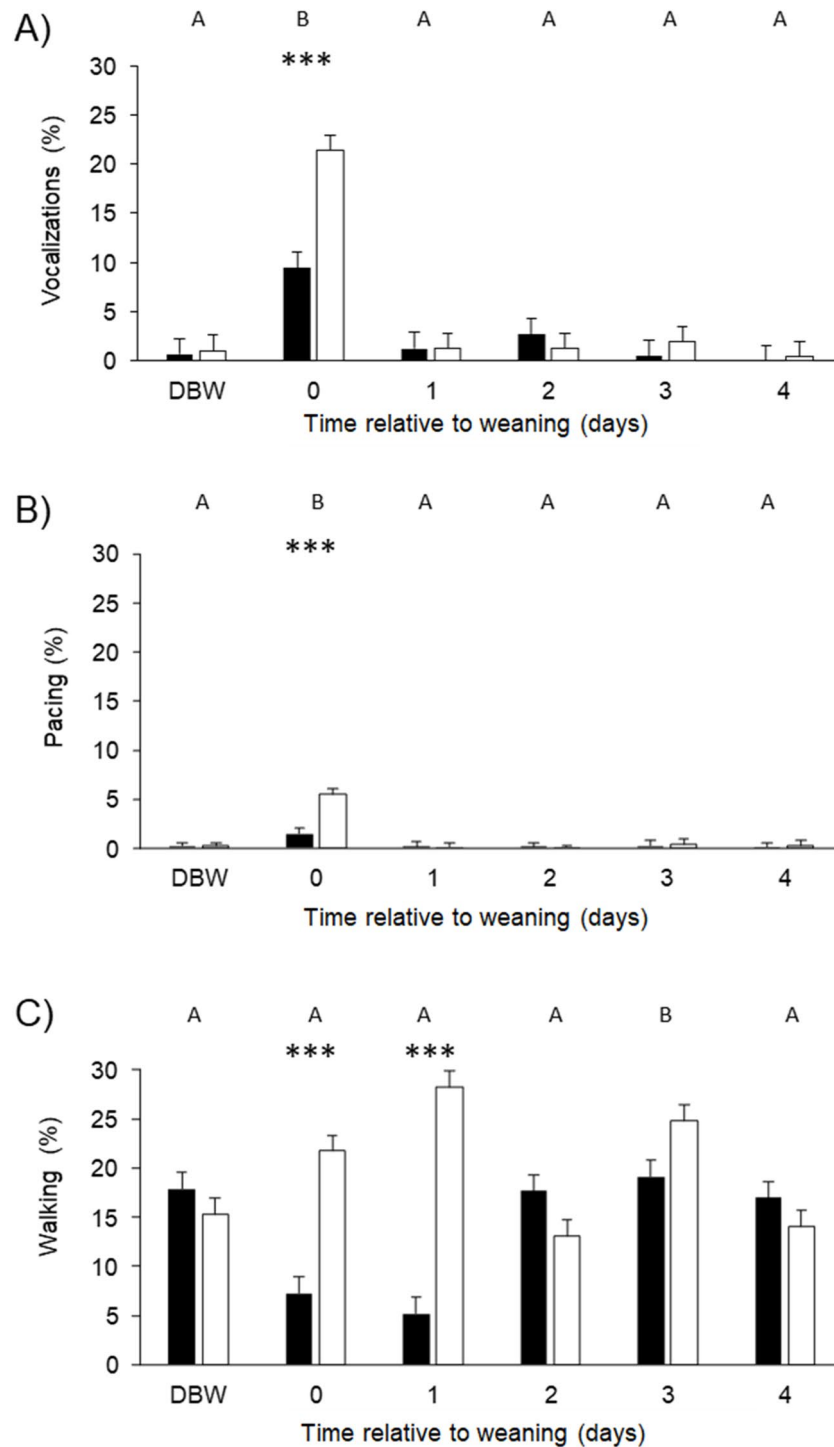


Fig. 3. Frequency (percentages of total observations) in which lambs were observed vocalizing (A), pacing (B), and walking (C) in both groups: lambs born and reared by primiparous dams (LPD: black bars) and lambs born and reared by multiparous dams (LMD: white bars). DBW: mean data recorded during the 2 days before weaning. Differences between LPD and LMD in the same day are indicated as: *** $p < 0.001$. Different letters indicate significant differences ($p < 0.05$) among days.

periods 3 and 4 ($p < 0.05$), but this pattern changed on day 2, where differences were evident between periods 1 and 3 ($p = 0.004$), and between periods 3 and 4 ($p = 0.03$, Fig. 4).

There was an interaction between group and period ($p = 0.002$). The groups behaved differently depending on the period, with the LMD group covering a greater distance than the LPD group in period 2 ($p = 0.02$), and the LMD group covering a shorter distance than the LPD group in period 4 ($p = 0.001$, Fig. 5A). The interaction is also explained by the different changes in distance covered within each group over the different periods,

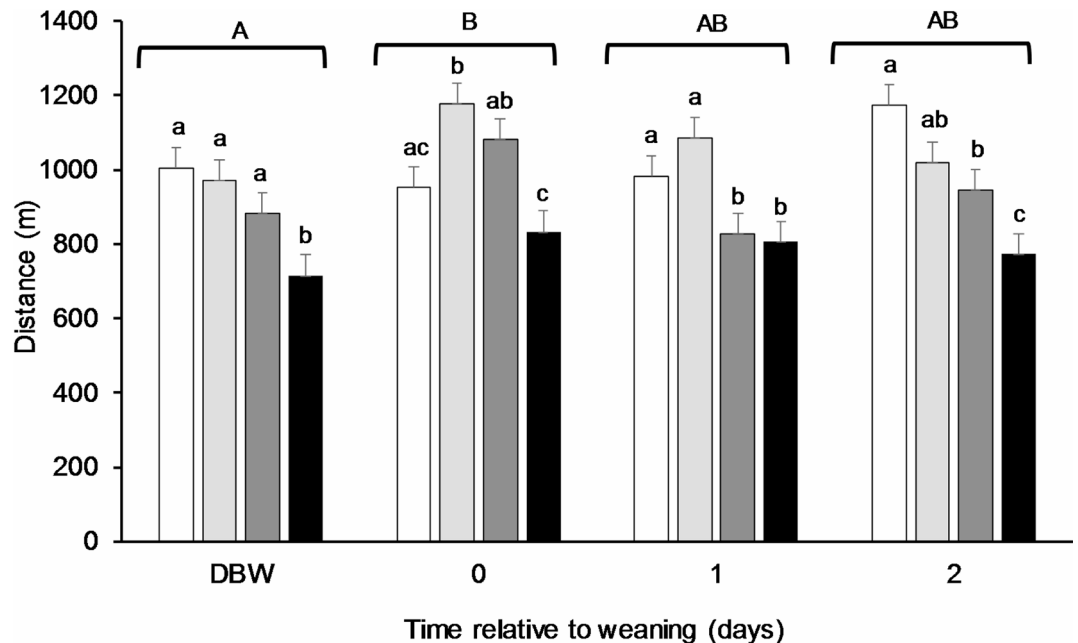


Fig. 4. Distance traveled (m) by lambs of both groups from data recorded 2 days before weaning (DBW) to Day 2 post-weaning and according to the daily period. Period 1: from 7:00 a.m. to 1:00 p.m. (dawn-midday: white bar), Period 2: from 1:00 p.m. to 7:00 p.m. (midday-dusk: light gray bar), Period 3: 7:00 p.m. to 1:00 a.m. (dusk-midnight: dark gray bar), and Period 4: from 1:00 a.m. to 7:00 a.m. (midnight-dawn: black bar). Differences among days for both groups together (LPD and LMD) are indicated with capital letters (A, B: $p < 0.05$). Differences among daily periods in the same day are indicated by different lowercase letters (a, b, c: $p < 0.05$).

with the LMD group covering the greatest distance in period 2, followed by periods 1 and 3 ($p < 0.05$), and the lowest values in period 4 ($p < 0.0001$). In the LPD group, differences were only observed between periods 1 and 2 compared to period 4 ($p < 0.05$, Fig. 5A). There was no significant interaction between group, day, and period ($p = 0.296$).

Mean daily velocity

There was no effect of group ($p = 0.356$), day ($p = 0.786$), or interaction between group and day ($p = 0.453$), day and period ($p = 0.662$), or interaction between group, day and period ($p = 0.102$) on mean daily velocity. However, there was an effect of period ($p = 0.036$) with higher velocity in period 2 than in period 1 ($p = 0.02$), and with no differences among periods 1, 3, and 4 ($p = 0.05$, Fig. 5B).

Since there was an interaction between group and period ($p = 0.046$), the groups behaved differently depending on the period, with the LMD group showing higher velocity than the LPD group in period 2 ($p = 0.03$, Fig. 5B). The interaction between group and period is also explained by the different changes in velocities within each group over the different periods, where the LMD group tended to present a higher velocity in period 2 than in period 1 ($p = 0.09$) and a higher velocity in period 2 compared to the rest of the periods ($p < 0.05$, Fig. 5B). Meanwhile, in the LPD group no differences were found between periods (Fig. 5B).

Discussion

This study showed that the behavioral response to weaning in lambs was affected by their dams' maternal experience. Based on all the behaviors assessed, as well as the physiological indicator of daily weight gain, it was evident that lambs reared by multiparous dams displayed a greater stress response at weaning than those reared by primiparous dams. Therefore, this study demonstrates the hypothesis that the degree of weaning stress in lambs may also represent the degree of dam-offspring bonding, confirming that the stronger the dam-offspring bond, the greater the stress response to separation during weaning.

The behavioral response pattern to weaning in lambs varied depending on whether they were born and reared by primiparous or multiparous dams. Most of the differences observed between groups in behavioral responses were found in the first two days of weaning, highlighting the acute nature of the stress⁴. In the first days of weaning, LMD lambs displayed lower frequency of grazing and standing, but greater frequency of vocalizing, pacing, and walking. The lower frequency of grazing on the day of weaning in the LMD group can be explained by a greater emotional response to the separation from their dams, including the cessation of suckling, as reported in sheep⁴ and cattle²⁷. Increased vocalization, pacing, and walking displayed by weaned lambs are behaviors performed to seek and to contact their dams²⁻⁵. The fact that lambs born and reared by multiparous dams (LMD) responded to weaning with increased frequency of vocalizing, pacing, and walking could indicate greater stress related to the separation from their dams than those lambs reared by primiparous dams (LPD).

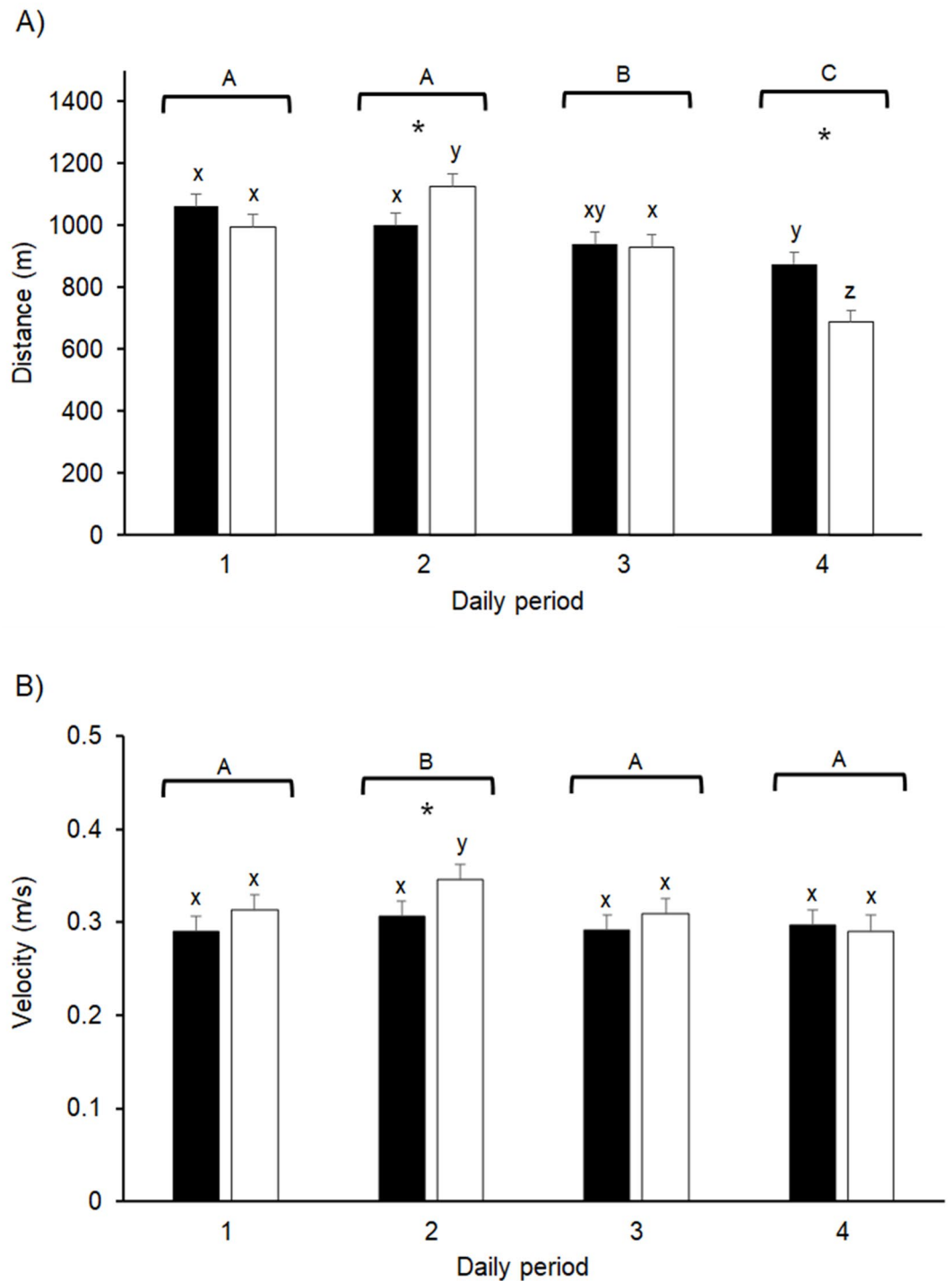


Fig. 5. (A) Distance traveled (m) and (B) velocity (m/s) of lambs born and reared by primiparous dams (LPD: black bars) and lambs born and reared by multiparous dams (LMD: white bars). Period 1: from 7:00 a.m. to 1:00 p.m. (dawn-midday), Period 2: from 1:00 p.m. to 7:00 p.m. (midday-dusk), Period 3: 7:00 p.m. to 1:00 a.m. (dusk-midnight), and Period 4: from 1:00 a.m. to 7:00 a.m. (midnight-dawn black bar). Differences among daily periods for both groups together (LPD and LMD) are indicated with capital letters (A, B: $p < 0.05$). Differences between LPD and LMD in the same period are indicated as: * $p < 0.05$. Differences among daily periods in the same group are indicated by different lowercase letters (x, y, z: $p < 0.05$).

Given that multiparous dams are maternally better and have a better bond with their offspring^{7,8,14,15}, it is possible that, at the time of weaning, the breaking of a stronger ewe-lamb bond induces a greater stress response in lambs. This was evidenced by the set of behaviors displayed by lambs from multiparous dams compared to those from primiparous dams.

The GPS-recorded behavioral pattern showed that weaning increased the distance traveled by the lambs, indicating a stress response to the separation from their dams^{2,4}. Furthermore, weaning also altered the circadian rhythm of the distance traveled by the lambs. Similar results were also reported in sheep using accelerometers²⁸. The greatest locomotor activity (distance) of the lambs occurred during daylight hours (periods 1 and 2) and the lowest activity at night (periods 3 and 4), which is in agreement with other reports^{22,29}. On the day of weaning, lambs traveled a greater distance in period 2 (midday-dusk), compared to period 1 (dawn-midday) and in relation to the days prior to weaning. It is interesting to observe how, when separated from their mothers, lambs increase their distance traveled more around midday and dusk than at other times of the day. Although we do not know the reasons for the increase in distance traveled by lambs around midday and dusk, it is possible that before weaning, as lambs are with their dams, the pattern of distance traveled in each period is partly determined by the presence of their dams. At weaning, as lambs no longer have this maternal reference, it is possible that the pattern of movement and distance traveled becomes more spontaneous. On the other hand, the LMD lambs exhibited different behavioral patterns based on their circadian rhythm compared to the LPD lambs. The LMD lambs covered a greater distance and displayed greater velocity in period 2 than the LPD lambs, reinforcing the possibility that this different behavioral profile could indicate a greater stress response to the separation from their dams in LMD lambs⁴. Furthermore, the distance covered in period 4 was shorter in the LMD group than in the LPD group. In this sense, it emphasized that maternal experience affected the circadian rhythm of their lambs. Period 4 is the final stage of the night and the beginning of dawn and is a critical moment due to the threat of possible predators, which possibly increases fear and lambs respond with less locomotor activity². Lambs reared by their mothers showed a greater increase in the frequency of resting in the shade during the first day of weaning than lambs reared artificially⁴. This behavioral strategy, triggered by the separation from the dam, could represent a search for protection from predators, such as reported in wild ruminants^{30,31}. Overall, it was evident that maternal experience affected the lambs' behavioral strategy at weaning in relation to their circadian behavioral rhythm. To our knowledge, there are no studies that have evaluated behavior using GPS to assess the stress response to weaning in sheep. In this sense, GPS technology can be used as a non-invasive tool to assess sheep welfare during the stress of weaning.

Regarding physiological parameters, significant changes between the two groups were only evident in daily gain, with no effects observed on any of the blood parameters. While LPD lambs showed daily weight gain between days -3 and 5 post-weaning, LMD lambs, on the other hand, lost body weight. The body weight loss at weaning highlights the negative impact of separation from their dams for lambs born and reared by multiparous ewes. In addition to the abrupt change in diet at weaning, given that lambs stop consuming milk^{2,3} the separation from their dams and the type of bond they had with them during lactation plays a key role in how lambs respond to weaning⁴. The difference in weight gain between the two groups can also be explained by the change in grazing behavior. On the day of weaning, lambs reared by multiparous dams grazed less frequently than those reared by primiparous dams, which is consistent with the differences in body weight. Another explanation for the difference in body weight gain is related to the differences in the activity patterns of lambs in both groups. LMD lambs engaged in vocalizing, pacing, and walking more frequently than LPD lambs, resulting in higher metabolic demands in LMD lambs. In this sense, and considering these results as a whole, it is clear that lambs born and reared by multiparous dams were more stressed than those born and reared by primiparous dams, spending more time on energy-intensive activities related to locomotion and less time on foraging. All these factors together may help explain why lambs born and reared by multiparous dams lost body weight, unlike those born and reared by primiparous dams. Beyond the previous explanations of possible factors involved in the difference in weight gain between the two groups, it is also possible that other factors more closely linked to feed intake could help explain these differences. Unfortunately, we do not have precise data on individual daily feed intake, in addition to observed grazing behavior. However, it is also important to mention that, before and after weaning, the lambs were kept on pasture at all times of the day. Besides, as body weight gain is also an indicator of animal welfare^{32,33} considering changes in daily body weight gain along with the displayed behaviors, this study also shows how maternal experience affects the welfare of their lambs at weaning.

Total proteins, albumin and globulin concentrations have been used as stress indicators in sheep^{16,34,35}. However, in this study, we have not found differences between groups in these indicators, nor significant effects over time, only a trend in the increase of the albumin concentration was observed from day -3 to day 5 post-weaning. These results are consistent with those reported by other studies on weaned lambs^{5,36,37} although not on the same days evaluated as in our study. Although we observed changes in all behaviors and in body weight gain due to weaning stress, these changes were not reflected in the blood protein indicators. The absence of changes in these indicators could be associated with the time points evaluated (day -3 and day 5), as changes may have occurred in the first few days post-weaning. In this sense, we prioritized behavioral indicators to avoid handling the lambs for blood sampling during the observation periods. Future studies are necessary to describe the daily changes in the blood protein profile of lambs after abrupt weaning.

In Uruguayan sheep production systems for meat and wool production weaning is done when lambs (e.g., Finnish Landrace x Polwarth or other crosses) are between 2.5 and 4.5 months old, and have a body weight between 15 and 30 kg^{38,39}. Weaning at this time allows farmers to obtain heavy lambs and, at the same time, gives their dams time to recover for the next year's breeding season, which occurs approximately three to five months later. In our study, lambs were weaned at 3.8 months of age, within the management range for the breed or crossbreed in Uruguay, which can be considered slightly late weaning. In this regard, it is important to mention that as the age of the lamb increases, the intensity of the ewe-lamb bond decreases². Therefore, it is possible

to hypothesize that our results on the response of lambs to weaning linked to maternal experience could be even more accentuated with early weaning. Future studies are needed to evaluate how the influence of maternal experience can affect the response of lambs at different weaning ages.

In sheep, it is known that the breed affects maternal behavior^{10–13,40,41} just as within each breed, maternal experience also influences its behavior^{10,12,15,40,42}. However, to the best of our knowledge, we are not aware of any studies that have evaluated the stress response to weaning according to breed and their interaction with maternal experience. Therefore, given that breed affects maternal behavior in sheep, it is possible that the results shown in this study could vary according to breed. Further studies could help elucidate these interesting interactions between breed and maternal experience at weaning.

Conclusions

Based on the behaviors assessed, as well as the physiological indicator of daily body weight gain, we conclude that maternal experience affected the lambs' responses to weaning. The lambs born and reared by multiparous dams exhibited a greater stress response at weaning than those born and reared by primiparous dams. Overall, maternal experience can be considered a key factor in the welfare of their lambs at weaning.

Data availability

Data is available upon reasonable request to the corresponding author.

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Author contributions

J.P.D. conceived and designed the study. J.P.D. and G.S. placed GPS collars on the animals. G.S. performed the GPS information capture. G.S. and M.C. participated in the provision of data and support from GPS. J.P.D. performed the blood biochemistry determinations. D.G., J.P.D. and G.S. participated in the handling and evaluation of the animals. G.B. and D.G. provided the conditions for conducting the study. J.P.D. conducted the statistical analysis and wrote the original draft. J.P.D. and E.V.L. discussed and interpreted results. J.P.D. and E.V.L. reviewed and adjusted the format and style of the manuscript. J.P.D. and G.B. project administration. All authors edited, reviewed and approved the final manuscript.

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Ethics declarations

Competing interests

The authors declare no competing interests.

Ethical consideration: All the experimental procedures were approved by the Comisión de Ética en el Uso de Animales (CEUA) of the Instituto Nacional de Investigación Agropecuaria (INIA, No. 2023.17).

Additional information

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