Primiparous and Multiparous Ewes Undernourished During Gestation: A Study on Mother-Lamb Bond at Birth and at 3 Months of Age

Aline Freitas de Melo (✉ alinefreitasdemelo@hotmail.com)

Universidad de la República, Facultad de Veterinaria

Raquel Pérez-Clariget

Universidad de la República, Facultad de Agronomía

Angélica Terrazas

Universidad Nacional Autónoma de México

Rodolfo Ungerfeld

Universidad de la República, Facultad de Veterinaria

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**Primiparous and multiparous ewes undernourished during
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of age**

Aline Freitas-de-Melo^{1,*}, Raquel Pérez-Clariget², Angélica Terrazas³ & Rodolfo Ungerfeld¹

¹Departamento de Biociencias Veterinarias, Universidad de la República, Lasplaces 1620,
Montevideo 11600, Uruguay

²Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Universidad de la
República, Garzón 780, CP 12 400, Montevideo, Uruguay

³Departamento de Ciencias Pecuarias, Facultad de Estudios Superiores Cuautitlán,
Universidad Nacional Autónoma de México, km 2.5 Cuautitlán-Teoloyucan San Sebastián
Xhala, Cuautitlán Izcalli, Edo. México, 547114, México

* Corresponding author: Tel: +598-26221195; Fax: +598-26221195; E-mail:
alinefreitasdemelo@hotmail.com (A. Freitas-de-Melo)

Abstract

The aims were to compare ewe-lamb behaviours between primiparous (PRI) and multiparous (MUL) undernourished grazing ewes at birth and at 3 months of age, and to determine if mothers' parity affects milk yield and composition, and lambs' body weight (BW). Food availability restricted the nutritional requirements from day 30 to day 143 of gestation. The PRI ewes had lower BW than MUL during gestation, and their lambs tended to vocalize less times until their first suckle. PRI ewes displayed a lower frequency of acceptance behaviours toward the alien lamb than toward their own lamb, but MUL did not. Whilst PRI ewes emitted a greater number of high-pitched bleats toward the alien lambs than toward their own lamb, MUL did not. PRI ewes produced less milk than the MUL ewes. The heart rate was greater in lambs reared by MUL ewes than by PRI. Although PRI ewes had a lower BW throughout the gestation, this difference was stable and did not affect the establishment of the ewe-lamb bond at birth or at 3 months postpartum. At least under nutritionally restricted conditions during gestation, inexperienced mothers appeared to have a shorter sensitivity period of maternal responsiveness than experienced mothers.

Keywords: filial attachment; milk yield; sheep; maternal separation; selectivity test

Introduction

Ewes establish a selective bond with their offspring after birth during a sensitive period of maternal responsiveness¹. Subsequently, ewes only display maternal care toward the offspring, whose odour was identified during that period, rejecting other lambs. During the first weeks of life, the mother is the main source of food for the lamb, providing heat together with protection against predators, increasing the probability of lambs' survival². As the postpartum period advances, nursing becomes less frequent³, as the lamb gradually gains nutritional and social independence⁴.

Many factors, including that of parity, breed and maternal nutrition, influence the establishment and the evolution of ewe-lamb bond^{5,6}. For instance, in extensive grazing sheep systems based on natural pastures, pregnant ewes undergo periods of undernutrition as gestation coincides with winter, during which time the lowest natural pastures quantity with the poorest quality are observed. Undernutrition affects the subsequent ewe-lamb relationship at birth and during postpartum period^{7,8}. However, the effect of undernutrition might be more greatly realised in multiparous (MUL) than in primiparous (PRI) ewes, as MUL ewes produce heavier lambs⁹. Thus, food restriction might impose greater limitations to the growth of foetus and lambs from MUL ewes, affecting the ewe-lamb behaviours at birth and the independence from the dam during lactation. In sheep production systems, where pregnant ewes are well fed, PRI ewes lick their lambs for the first time generally later, spend less time licking and, make more movements when lambs attempt to suckle than MUL ewes^{10,11}. Additionally, while MUL ewes prefer their lamb over an alien lamb 6 h after birth, PRI ewes do not¹². Lambs born from PRI mothers are lighter and thus, slower to stand up and suckle for the first time than lambs born from MUL mothers⁹. Even one day after birth, lambs born from PRI ewes have impaired ability to distinguish their mother from that of an alien one¹³, which is related with a greater mortality rate of their lambs¹⁴. However, to the best of our knowledge,

there is paucity on information on the evolution of the ewe-lamb bond throughout lactation according to the parity. In particular, PRI ewes and their lambs display a more intensive physical motion in a partial separation test than MUL ewes and their lambs at 2 months of age¹⁵. This might be a consequence of the greater reactivity of PRI ewes to a challenge situation¹⁶. Parity also affects the bond between the mother and its offspring in cattle, as MUL cows vocalize and pace more after early-weaning than PRI cows¹⁷.

The aim of this study was to compare ewe-lamb behaviours between PRI and MUL undernourished grazing ewes at birth and at 3 months of age. It has been demonstrated that PRI ewes produce less milk¹⁸, and therefore, their lambs grow slower than those from MUL ewes¹⁹, which may affect the nutritional independence from the mother and the strength of bond during lactation. A complementary aim was to determine if milk yield and composition, together with lambs' weight, differ according to their mothers' parity.

Results

Body weight, BCS and udder size of ewes during gestation

The MUL ewes were heavier and tended to have a greater BCS than PRI ewes during gestation (BW: 46.0 ± 0.4 kg vs 41.1 ± 0.5 kg; DF= 261; F= 51.73; $p < 0.0001$; BCS: 3.2 ± 0.04 vs 3.1 ± 0.04 , respectively; DF= 261; F= 3.35; $p=0.07$). The BW and BCS of ewes changed with time ($p < 0.0001$). From day 86 to day 108 of gestation, BW decreased ($p < 0.002$); and from day 108 to day 123 of gestation, it increased ($p < 0.0001$) (Fig. 1A and B). From day 69 to day 108 of gestation BCS decreased in both groups ($p < 0.0001$), remaining stable from day 108 to day 143 of gestation. There was no interaction between group and time in BW and BCS.

The volume of the udder did not differ between MUL and PRI ewes on day 143 of gestation (936.03 ± 103.4 cm³ vs 794.9 ± 116.9 cm³, respectively).

Serum biochemical variables

The MUL ewes had greater concentrations of total serum protein (6.3 ± 0.06 g/dL vs 6.0 ± 0.07 g/dL; DF= 91; F= 6.38; $p = 0.01$) and globulins (3.1 ± 0.04 g/dL vs 2.9 ± 0.05 g/dL; DF= 90; F= 5.96; $p = 0.02$) than PRI ewes (Fig. 2A and 2C). There were no effects of parity in albumin, cholesterol or glucose concentrations (Figs. 2B, 2D and 2E). All the biochemical variables changed with days, decreasing from day 30 to day 100 of gestation ($p < 0.007$). There were no interactions between parity and time in any biochemical variable.

Behaviours, body weight and body temperature at birth

Duration of the first licking, latency of the lamb to stand up, latency to first suckling, duration of first suckling and number of vocalizations of the mother and lamb until first suckling did not differ between groups (Table 1). The frequency of vocalizations of the lambs from birth

until first suckling (number of vocalizations of the lamb until first suckling/the time required to suckle for the first time) tended to be lower in lambs born from MUL ewes than in the lambs born from PRI ewes (Table 1; $P=0.06$). The parity did not affect lamb BW at birth, rectal and surface temperatures. Birth weight was 4.0 ± 0.1 kg vs 3.8 ± 0.2 kg for MUL and PRI ewes. Surface temperatures on the neck area were 24.1 ± 0.8 °C and 22.9 ± 0.8 °C in MUL (n=12) and PRI (n=8) ewes, respectively. Surface temperature on the hips area were 25.2 ± 1.0 °C and 24.7 ± 1.0 °C in MUL (n=12) and PRI ewes (n=8) respectively; and rectal temperature were 39.0 ± 0.7 °C and 37.9 ± 0.8 °C in MUL (n=11) and PRI (n=8), respectively.

Behaviours during the selectivity test

The results of this test are presented in Table 2. While PRI ewes emitted a lower number of low-pitched bleats toward the alien lamb ($p=0.04$; $W=39.5$) and allowed these lambs to stay near the udder for a shorter time than their own lamb ($p=0.04$; $W=33.0$), MUL ewes did not. However, both, MUL ($p=0.07$) and PRI ($p=0.1$) ewes tended to accept more frequently their own lamb than the alien lamb at the udder.

In relation to the rejecting behaviours, while PRI ewes emitted a greater number of high-pitched bleats toward the alien lambs than toward their own lamb ($W=39.0$; $p=0.01$), MUL ewes did not. The MUL ($W=45.0$; $p=0.006$) and PRI ($W=17.0$; $p=0.02$) ewes rejected more frequently the alien lamb than their lamb at the udder. Lastly, both MUL ($W=45.0$; $p=0.006$) and PRI ($W=21.0$; $p=0.05$) ewes displayed a greater number of aggressions toward the alien lamb than toward their own lambs.

Body weight and BCS of ewes and lambs during the postpartum period

The MUL ewes tended to be heavier than PRI ewes also during this period (41.3 ± 0.8 kg vs 39.0 ± 0.1 kg; DF= 58; F=2.94; $p < 0.09$), but BCS did not differ between groups (MUL: 2.5 ± 0.08 vs PRI: 2.7 ± 0.08). The BCS did not change with time, but BW of ewes decreased from day 45 (41.6 ± 1.0 kg) until day 91 (38.7 ± 1.0 kg; DF= 58; F= 4.29; $p=0.04$), independently of the parity. There were no significant interactions between groups and time in ewes' BW or BCS.

Lambs born from MUL ewes tended to be heavier than those born from PRI ewes (15.0 ± 0.4 kg vs 13.8 ± 0.5 kg; respectively; DF= 58; F= 3.57; $p < 0.06$). The BW of lambs increased from day 45 (12.3 ± 0.5 kg) until day 91 (16.5 ± 0.5 kg; DF= 58; F= 41.93; $p < 0.0001$) postpartum. There were no interactions between groups and time in BW and BCS of the lambs.

Short-term maternal separation test

Parity did not affect any behaviour recorded during the test (Table 3) or the rectal temperature (MUL: 40.6 ± 0.07 °C vs PRI: 40.8 ± 0.09 °C). The heart rate was greater in lambs born from MUL than from PRI ewes (148.8 ± 3.1 beats/min vs 138.2 ± 3.9 beats/min; DF= 57; F= 4.57; $p = 0.04$, respectively), but heart rate did not change with days, and there was no interaction between group and days.

Independently of the parity, the lambs walked longer when their mother was in the other side of the fence than when they were alone (time walking: from 29.4 ± 0.6 s to 49.3 ± 0.4 s; DF= 58; F= 4.84; $p = 0.03$). The rectal temperature also increased after the second part of the test, from 40.5 ± 0.08 °C to 40.9 ± 0.08 °C (DF= 57; F= 7.42; $p = 0.009$).

141 *Milk yield and milk composition*

142 MUL ewes produced more milk/day than the PRI ewes at 91 days postpartum (DF= 28;
143 F= 4.59; $p = 0.04$), without differences in fat, protein and lactose milk content (Table 4). The
144 total milk solid content tended to be greater in MUL than in PRI ewes (Table 4; $p = 0.07$). The
145 quantity in grams of milk fat, protein and lactose were calculated considering the
146 concentration (%), the total volume produced per ewe, and the density of ewe milk.

Discussion

In general, parity did not affect ewe-lamb behaviours immediately after birth, as did happen in other previous studies^{2,10,25}. Additionally, parity did not influence lambs' birth weight, different to that published in others studies of the same breed, in which MUL ewes produce heavier lambs than PRI ewes²⁶. This is consistent with the difference in BW of PRI and MUL ewes from conception until lambing, indicating that PRI ewes invested proportionally more energy than MUL ewes in the development of their foetus rather than investing in maintaining their growth rate. Although pregnancy is a greater metabolic challenge for PRI than for MUL ewes¹³, it would appear that in these study conditions, PRI ewes prioritized the foetus at the cost of their own growth. The PRI ewes carried a greater foetal burden through gestation than MUL ewes in relation to the maternal BW, which is consistent with lower blood protein concentration in PRI ewes, a consequence of higher protein expenditure for foetal growth. In this experiment, it should also be considered that the level of nutrition did increase from day 100 of gestation until lambing, and thus, was sufficient for PRI foetuses to reach a birth weight within the range of the Corriedale breed²⁷. Ewes from both groups accessed to the same amount of pasture and supplementation despite MUL ewes were heavier during gestation. Thus, food might have had greater impact in PRI than MUL ewes. An alternative interpretation for this result could be that the undernourishment restricted the potential of MUL ewes to produce heavier lambs at birth. In this case, foetuses from MUL ewes may have suffered more with the food being restricted during gestation than those from PRI ewes. This also coincides with the tendency of the lambs from MUL ewes to vocalize less at birth than the lambs from PRI ewes, which suggests that the former lambs were less vital and/or had less energy reserves. It should also be considered that this study was performed in a flock in which maternal behaviour score²⁸ was

171 considered during many years for selecting the ewes that remain for breeding⁷, probably
172 improving their maternal behaviour.

173 The present results also provide inputs on possible differences on the effects of parity
174 on the length of the sensitive period of maternal responsiveness, as PRI and MUL ewes
175 differed in their selective maternal behaviour as early as 2 h after birth. In general, while
176 inexperienced mothers accepted more their own lamb than the alien, experienced mothers
177 accepted similarly both lambs at that time. This indicates that PRI ewes ended their sensitive
178 period of maternal responsiveness earlier than MUL ewes, expanding previous suggestions in
179 sheep²⁹ and information available also in goats³⁰. Probably the greater incidence of lamb
180 rejection and mortality rates of lambs born from PRI ewes^{14,31} can be explained not only by
181 their less intensive maternal behaviour²⁵, but also by a shorter sensitive period after which
182 they would not recognize any lamb²⁹. Additionally, the main lambs' behaviour, and lambs'
183 BW and body temperature at birth did not differ between groups, likely due to birth weight
184 and lamb's body temperature, both of which are related to the lambs' behaviour around birth
185 in the same Uruguayan extensive sheep production system³².

186 Lambs born from PRI and MUL ewes did not differ in their birth weight, but those
187 born from MUL ewes tended to be heavier than those born from PRI ewes during the
188 lactation period. This might be explained by the greater milk yield produced by MUL ewes,
189 and the tendency to provide access to more solid contents. The greater milk production in
190 MUL than PRI ewes agrees with previous reports in sheep^{33,34}, and might be related to a
191 better energy balance in the former along the lactation period^{35,36}, coinciding with the spring
192 in which ewes accessed better pastures²⁰ and received supplementation. On the other hand,
193 ewes' parity did not influence the behavioural response of their lambs during the short-term
194 maternal separation test, indicating that the lambs remained with similar attachments even at 3
195 months of age. This result reinforces the initial interpretation indicating that in these

conditions MUL and PRI ewes established similar bond strength. Moreover, it also fits with the interpretation of Freitas-de-Melo et al.²⁰, who suggested that the milk yield is not the main factor determining the strength of the ewe-lamb bond during the lactation period, as proposed by Arnold et al.³⁷.

Lambs born from MUL ewes had a greater heart rate than those born from PRI during the separation and subsequent reunion of lambs and their mothers. However, it should be considered that the initial heart rate was recorded while they remained with other lambs from the flock, but separated from their mother for 30-60 min. Therefore, the general difference in heart rate was consequence of the general stressful situation, without basal recordings. The difference in heart rate could be considered as even greater as lambs reared from MUL ewes tended to be heavier than those reared from PRI ewes during lactation. In this sense, lighter and younger lambs have greater heart rates than heavier and older lambs^{38,39} due to their bigger relative costs of maintenance. Another complementary and non-opposed explanation is that lambs reared by MUL ewes were more stressed by the loss of their mother as she produced more milk, as previously reported that happens in lambs at weaning⁸. On the other hand, during the short-term maternal separation test, all lambs increased their time walking after including the mother in the pen, which indicates that the presence of their mothers on the other side of the fence generated greater anxiety in all the lambs. Therefore, it appears that although the behavioural response between groups did not differ, this indicate a comparable motivation to get together back with their mothers.

In conclusion, it seems that although PRI ewes had a lower BW and a poor metabolic status throughout the gestation, this difference was stable and did not affect the establishment of the ewe-lamb bond at birth, and this lack of differences was maintained until 3 months postpartum. At least under nutritionally restricted conditions during gestation, inexperienced

220 mothers appear to have a shorter sensitivity period of maternal responsiveness than
221 experienced mothers.

Methods

Location and animal management

All the procedures were approved by the Comisión de Ética en el Uso de Animales, Facultad de Agronomía (Universidad de la República, Uruguay), in accordance with national guidelines and regulations, and with the ARRIVE guidelines. The experiment was carried out at the Estación Experimental Bernardo Rosengurtt, Facultad de Agronomía, Universidad de la República (Cerro Largo, Uruguay; 32° S) using PRI and MUL Corriedale ewes. Oestrous cycles of all the ewes were synchronized with an intravaginal sponge impregnated with medroxyprogesterone acetate (60 mg, Syntex, Buenos Aires, Argentina) during 6 days plus a dose of a PGF₂α analogue (10 mg, Dinoprost tromethamine, Lutalyse, Pfizer, Kalamazoo, MI, USA) and 200 IU of eCG (Novormon, Syntex, Buenos Aires, Argentina) at sponge withdrawal. Ewes were joined with marking vasectomized rams (1 male:10 ewes ratio). Marks on the rumps of the ewes were recorded twice daily, and marked ewes were inseminated with fresh semen 12 h after oestrus detection (Day 0). Pregnancy status and foetal number were determined on Day 30 with transrectal ultrasound, and only ewes carrying a single foetus were included in the study (14 PRI ewes: 5 carrying female and 9 male foetuses; 21 ME ewes: 14 carrying female and 7 male foetuses).

From 30 to day 143 of gestation, all ewes remained grazing natural pastures (pasture allowance: 6 to 10 kg of dry matter (DM) /100 kg of body weight (BW)/day; crude protein: 6.4 to 6.7 % and metabolizable energy: 2.0 to 2.1 Mcal/kg of dry matter) on three paddocks of approximately 3 ha separated by electric fences and with free access to water. With this pasture allowance, during the aforementioned gestation period, pregnant ewes suffered a restriction up to 30% of their energy requirements and 60% of their protein requirements²⁰. From day 100 of gestation until lambing, ewes were daily supplemented with 300 g/animal of

rice brain provided collectively (88% DM, 14% crude protein, 9% acid detergent fibre and 24% neutral detergent fibre). Despite of supplementation from day 100 of gestation, at day 143 of gestation, ewes were still in negative protein balance of 12%, but achieved a positive energetic balance of 56%. From day 143 of gestation until parturition, all ewes were kept in a paddock of approximately 2.5 ha, grazing native pasture during daylight. During the night, they were moved to a 40 m X 20 m paddock located close to the former paddock, with artificial dim light to allow observations.

From one week after parturition until the end of the study, all ewes and their lambs grazed in a paddock of approximately 2 ha on native pasture. During this period, all animals were daily supplemented with 300 g/animal of rice brain provided collectively. All ewes were shorn 30 days after lambing.

Recordings during gestation

Ewes' body weight (BW) and body condition score (BCS) were recorded in the morning of days 0, 30, 55, 69, 86, 108, 123 and 143 of gestation. Blood samples were collected from all ewes by jugular venipuncture in the morning of days 30, 100 and 143 of gestation. Samples were placed in tubes without anticoagulant and centrifuged for 10 min at 1500 g within 30 min after collection. Serum samples were separated and frozen at -20 °C. Glycaemia and cholesterol, serum total protein and albumin concentrations were measured by colorimetry using commercial kits (Bio-Systems, Barcelona, Spain). Serum globulin concentration was estimated by subtracting the serum albumin concentration from the serum total protein concentration.

269 On the morning of day 143 of gestation (from 08:00 h to 10:00 h), the following udder
270 measures were recorded: circumference (circumference of the udder near the belly), width
271 (from left insertion of the udder to right insertion) and depth (from insertion of the udder to
272 the inferior extremity). Each semiaxis and udder volume was calculated assuming that the
273 udder was a semi-sphere: $V=2/3\pi.R1.R2.R3$

274 Where: each R corresponds to each semiaxis (horizontal, depth and width).

275

276 *Recordings during the first hours after birth*

277 All ewes were continuously observed during the 24 h period by six trained observers,
278 who remained 5 to 7 m from each ewe when behaviours indicative of the proximity of
279 parturition began (a distance enough to avoid disturbing the normal behaviour of the animals)
280 until lambing occurred. Then, the following data were recorded according to Freitas-de-Melo
281 et al.²⁰: duration of the first licking (the total time spent by the ewe licking its lambs for the
282 first time), latency of the lamb from parturition to standing up (the time required by the lamb
283 to maintain an upright position on extended legs for at least 5 continuous seconds), latency
284 from parturition to first suckling (the time taken by the lamb to access its mothers' teat and
285 suckle for at least 5 continuous seconds), duration of first suckling (total time spent by the
286 lambs sucking for the first time) and number of vocalizations of the mother and the lamb from
287 birth until the first suckling occurred. The frequency of vocalizations of ewes and lambs were
288 calculated as the number of vocalizations/latency from parturition until the first suckling
289 occurred. After recording the first suckling ended, the rectal temperature, and the surface
290 temperatures on the skin of the interscapular zone and of the hips of the lambs were measured
291 with the aid of laser thermometer (IR-102 Infrared Thermometer, Super Elec. Equip. Co,
292 China). Thereafter, lambs were tagged and weighed, and their sex was registered. Lambing
293 assistance was provided to ten ewes (five MUL and five PRI ewes), as the second stage of

parturition (from appearance of foetal front or rear legs to complete lamb expulsion) took more than 30 min with no progress and/or with evidence of meconium in the amniotic fluid^{21,22}. Suckling assistance was provided when the latency from parturition to first suckling took more than 2 h (one lamb born from MUL and two born PRI ewes). In these cases the latency to suckle for the first time was considered as 120 min.

A maternal selectivity test was performed approximately 2 h after birth in a 2 m × 1 m pen placed near the pen where the ewes lambed. Each dam was subjected to two successive tests, that lasted 3 min each: one with its' lamb and the other with an alien lamb of similar age and physical appearance (colour and size). The selection and the order of presentation of their own lamb and the alien lamb were randomized between ewes, within each group. The number of the following behaviours was counted according to Poindron et al.²³: (1) low-pitched bleats (number of vocalization emitted with the mouth closed), (2) acceptances at the udder (number of times that the lamb engaged the head in the inguinal region of the ewe, without being rejected), (3) the time near the udder (time spent by the lamb at the udder, with its head engaged in the inguinal region of the ewe during at least 5 continuous seconds), (4) refusals at the udder (number of times that the lamb engaged the head in the inguinal region of the ewe, it was interrupted within less than 5 s by the ewe moving away and showing back leg movement or any aggressive behaviour), (5) aggressive behaviours towards the lamb (head butts and threats), (6) high-pitched bleats (number of vocalizations emitted with mouth open, indicating displeasure). The first three behaviours indicate acceptance of the lamb, whereas the last three indicate rejection. At the end of the test, ewes and their lambs were moved to a larger pen where they remained together with other mothers and lambs from the flock.

317 *Recordings during lactation*

318 *Mother-lamb short-term separation test*

319 Three months after lambing, lambs were subjected to a temporal separation test when they
320 were 3-mo old. The test was similar to that described by Barnard et al.¹⁵ and Freitas-de-Melo
321 et al.²⁰, in a pen that was divided in two similar areas of 3.0 m x 2.5 m by a wire mesh plastic
322 fence. A reference line was drawn 80 cm parallel to the fence on the lamb side of the pen. All
323 lambs from each group were separated from their mother 30 to 60 min before testing and
324 allocated all together in a pen (6.0 m x 5.0 m) 30 m away from that in which their mothers
325 remained, where they cannot see or smell each other. Each lamb was introduced alone during
326 5 min to one area of the testing pen, registering the total time that the lamb spent walking and
327 the number of vocalizations emitted. Thereafter, the mother was introduced in the other side
328 of the fence, and the number of times that the lamb crossed the line, entering to the area (< 80
329 cm from the fence) was also registered during 5 min. In addition, the time each lamb spent
330 into this area, the total time walking, the number of vocalizations emitted, and the number of
331 attempts to reunite with its' mother hitting against the fence with any part of the body were
332 also recorded. Lambs' heart frequency and rectal temperature were measured immediately
333 before and after the tests.

334

335 *Milk yield and composition, body weight and body condition score of ewes and their lambs*

336 On days 45 and 91 after lambing, ewes were weighed and their BCS registered, and their
337 lambs were weighed. On day 91 postpartum, all ewes were milked to estimate milk yield
338 according to Freitas-de-Melo et al.²⁴. Briefly, lambs were separated from their mothers, which
339 received 5 IU of oxytocin, and were milked completely. Six hours later, ewes were milked
340 with the same protocol, and daily milk production was calculated based in the 6-h production.

Milk fat, protein and lactose concentrations were measured as described by Freitas-de-Melo et al.²⁴.

Statistical analyses

Not all the data could be recorded in all the animals, so the exact number of animals is included in the results when data were not recorded in all. All the variables were tested for normal distribution with the Shapiro-Wilk test. The behaviours and body temperature recorded at birth, and the parameters recorded after including the mother in the short-term maternal separation test were not normally distributed, and thus, were compared between groups with the Mann-Whitney test. The behaviours displayed by each ewe in the selectivity test toward its own lamb or the alien lamb were compared with the Wilcoxon test into each group. The aforementioned variables were analysed using the software Past 2.02, and the remained data were analysed using the SAS University Edition (SAS Institute, Cary, NC, USA). Biochemical measurements, BW and BCS were compared in two separately analysis, during gestation and during postpartum. The aforementioned variables, heart rate, rectal temperature, the number of vocalizations and the square root of the time walking during the short-term maternal separation test were compared between groups with a mixed model (proc mixed), including the time as the repeated data. The model included the groups, the time, and the interaction between groups and time as fixed effects. The animal and the sex were included as a random effect into the model. The udder volume and the milk yield and composition were also compared with the proc mixed, including the group as the main effect and the ewe as random effect into each group. Post-hoc comparisons were done with the pdiff function of the proc mixed.

Results were considered significantly different when $p \leq 0.05$, and a tendency when $0.05 < p \leq 0.1$. Data are presented as LSmean $\pm$ sem.

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Authors' contributions

All the authors participated in the design of the study. AFM, AT and RPC organized the field work and collected the data. AFM analysed the data. AFM wrote the original draft, and all the authors reviewed and edited the manuscript. AFM, RPC and RU provided the resources and funding acquisition. The submitted version was approved by all the authors.

Competing interests

The authors declare no competing interests.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figure legends

Figure 1. Body weight (A) and body condition score (B) of multiparous (-▲-) and primiparous (-▲-) ewes. Body weight was greater and body condition score tended to be greater in multiparous than in primiparous ewes ($p < 0.0001$ and $p=0.07$, respectively). Different letters indicate significant differences between gestation days ($P < 0.0001$). The light grey area represents the period when all ewes were supplemented with 300 g/animal/day of rice bran.

Figure 2. Total protein (A), albumin (B), globulin (C), cholesterol (D) concentrations and glycaemia (E) (LS mean $\pm$ s.e.m.) of multiparous (-▲-) and primiparous (-▲-) ewes. Total protein and globulin concentrations were greater in multiparous than in primiparous ewes ($p < 0.02$). Different letters indicate significant differences between the days ($P < 0.0009$). The light grey area represents the period when all ewes were supplemented with 300 g/animal/day of rice bran.

Figure 1

Figure 2

526 **Table 1.** Behaviours [LS mean $\pm$ sem (min-max values)] of multiparous and primiparous ewes and their lambs recorded at birth.

	Multiparous	<i>N</i>	Primiparous	<i>N</i>	P
Ewes' behaviours					
Duration of the first licking (min)	9.5 $\pm$ 2.5 (1-32)	15	9.9 $\pm$ 3.5 (1-35)	9	ns
Number of vocalizations until first suckling	73.9 $\pm$ 17.7 (0-207)	14	98.7 $\pm$ 25.2 (3-268)	10	ns
Vocalizations until first suckling (number/min)	1.5 $\pm$ 0.4 (0-4.4)	13	2.4 $\pm$ 0.7 (0.02-7.1)	9	ns
Lambs' behaviours					
Time to first stand (min)	23.4 $\pm$ 3.7 (6-57)	15	23.4 $\pm$ 9.6 (6 -101)	9	ns
Time to first suckling (min)	46.1 $\pm$ 8.1 (16-120)	14	60.7 $\pm$ 11.6 (31-120)	9	ns
Duration of first suckling (s)	87.6 $\pm$ 12.9 (15-180)	12	97.1 $\pm$ 17.1 (60-180)	7	ns
Number of vocalizations until first suckling	50.6 $\pm$ 14.0 (4-124)	13	83.4 $\pm$ 20.6 (13-169)	9	ns
Vocalizations until first suckling (number/min)	1.0 $\pm$ 0.3 (0.1-3.8)	13	1.9 $\pm$ 0.4 (0.4-4.4)	8	0.06

527

528

529 **Table 2.** Behaviours (mean $\pm$ sem) toward the alien or the mother's lambs during a selectivity test carried out approximately two hours after
530 lambing, in multiparous (n= 12) or primiparous (n= 9) ewes.
531

Behaviours	Multiparous		Primiparous	
	Own	Alien	Own	Alien
Number of low-pitched bleats	8.2 $\pm$ 1.6 ^a	4.2 $\pm$ 1.2 ^a	10.5 $\pm$ 3.0 ^a	2.2 $\pm$ 0.8 ^b
Number of high-pitched bleats	4.5 $\pm$ 2.8 ^a	17.7 $\pm$ 5.2 ^a	2.9 $\pm$ 1.0 ^a	20.8 $\pm$ 3.7 ^b
Time near the udder (s)	37.2 $\pm$ 12.7 ^a	19.7 $\pm$ 14.0 ^a	27.7 $\pm$ 10.3 ^a	4.9 $\pm$ 2.3 ^b
Number of udder rejection	0.6 $\pm$ 0.5 ^a	2.3 $\pm$ 0.7 ^b	0.1 $\pm$ 0.1 ^a	1.0 $\pm$ 0.3 ^b
Number of udder acceptance	1.7 $\pm$ 0.5 ^x	0.4 $\pm$ 0.2 ^y	1.7 $\pm$ 0.5 ^x	0.4 $\pm$ 0.2 ^y
Number of aggressive behaviours	0.08 $\pm$ 0.08 ^a	3.4 $\pm$ 1.2 ^b	0.0 $\pm$ 0.0 ^a	1.0 $\pm$ 0.3 ^b

532 Means within the same row bearing different letters (a and b) differ significantly ($P \leq 0.04$) or
533 tented to differ (x and y; $0.07 \leq p \leq 0.1$).

Table 3. Behaviours during the ewe-lamb short-term maternal separation test (LS mean $\pm$ sem) in lambs at 3 months of age reared by multiparous (n = 19) or primiparous (n = 12) ewes.

	Multiparous	Primiparous
Behaviours while the lamb remained isolated		
Time walking (s)	24.5 $\pm$ 4.4	37.2 $\pm$ 10.7
Number of vocalizations	35.2 $\pm$ 4.0	30.4 $\pm$ 5.0
Behaviours after including the mother in the test		
Time walking (s)	46.6 $\pm$ 7.9	48.7 $\pm$ 14.9
Number of vocalizations	30.2 $\pm$ 4.0	26.2 $\pm$ 5.0
Number of times that the lambs crossed the line	5.6 $\pm$ 0.9	5.7 $\pm$ 1.2
Time in the zone near fence (s)	203.5 $\pm$ 17.3	230.6 $\pm$ 19.2
Number of contact attempts	10.0 $\pm$ 2.5	10.7 $\pm$ 1.8

There were no significant differences between groups.

559 **Table 4.** Milk composition and milk yield (LS mean $\pm$ sem) at 91 days postpartum in
560 multiparous (n=19) or primiparous (n=12) ewes.

Milk yield and content	Multiparous	Primiparous	P
Milk yield (kg)	0.4 $\pm$ 0.03 ^b	0.3 $\pm$ 0.04 ^a	0.04
Fat milk (g)	29.9 $\pm$ 2.4	24.8 $\pm$ 3.2	ns
Protein (g)	19.7 $\pm$ 1.5	15.7 $\pm$ 2.1	ns
Lactose (g)	20.9 $\pm$ 1.7	16.7 $\pm$ 2.3	ns
Total solids (g)	52.0 $\pm$ 7.7	70.5 $\pm$ 5.9	0.07

Figures

Figure 1

Body weight (A) and body condition score (B) of multiparous (—) and primiparous (—) ewes. Body weight was greater and body condition score tended to be greater in multiparous than in primiparous ewes ($p < 0.0001$ and $p=0.07$, respectively). Different letters indicate significant differences between gestation days

($P < 0.0001$). The light grey area represents the period when all ewes were supplemented with 300 g/animal/day of rice bran.

Figure 2

Total protein (A), albumin (B), globulin (C), cholesterol (D) concentrations and glycaemia (E) (LS mean $\pm$ s.e.m.) of multiparous (—) and primiparous (—) ewes. Total protein and globulin concentrations were greater in multiparous than in primiparous ewes ($p < 0.02$). Different letters indicate significant

differences between the days ($P < 0.0009$). The light grey area represents the period when all ewes were supplemented with 300 g/animal/day of rice bran.