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Does lambing season affect mother-young relationships and lamb vigor in D'man sheep reared in oases?

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*Although sheep are known to be seasonal breeders and give birth in winter, not all of them follow this trend. A few breeds can be mated and give birth all year round, meaning that mothers and neonates will have to face contrasting climatic conditions. The aim of this study was to test whether lambing season affects maternal and neonatal behaviors in D'man sheep. During four different lambing seasons (winter, autumn, summer and spring), periparturient ewes (*n *= 111) and their lambs (*n *= 213) were kept under 24-h-video surveillance in order to record* postpartum *behaviors. Mother-young preference was tested around 48 h after parturition. Lamb vigor was studied by the determination of birth weight, early postnatal behavior and rectal temperature at birth and 48 h later. Litter expulsion time was not affected by lambing season, but birth weight was biased against summer and winter born lambs. Ewes provided a higher intensity of care to their offspring in winter: latency for grooming was shorter and time spent grooming was longer compared to lambing in spring and summer (*P *= 0.01 in all cases). On the other hand, lambs were the most active in spring as they were faster to extend their hind legs (*P *= 0.01), stand up (*P *= 0.04) and reach the udder (*P *= 0.04). Rectal temperature at 48 h was affected by season of birth (*P *< 0.001) with higher values observed in summer. Glycemia variation between birth and 48 h was the lowest in spring born lambs and plasma levels increased less in spring born lambs than in winter (*P *< 0.0001), autumn (*P *< 0.0001) and summer born lambs (*P *< 0.0001). In the choice test, mothers clearly preferred their own young and no season effect was detected except that in the first minute of the test they spent less time near their own young in winter than in the other seasons (*P *= 0.04). Lambs also chose their mother successfully without any major effect of the season however, but winter born lambs were the least vocal (*P *= 0.01). Overall, this study show that maternal care, lamb behavior and vigor vary lightly according to seasons, albeit not in a consistent manner. In conclusion, a season is no more detrimental than another for the onset of mother-young relationships.*

Keywords: parturition, maternal behavior, neonate behavior, bonding, glycemia

Implications

D'man ewes give birth all year round in oases, which may have a major impact on mother-young interactions and lamb survival due to weather fluctuations (heat/cold), especially since mortality is reported to be higher in winter. This study showed that maternal care, lamb behavior and neonatal physiology may vary according to seasons but only mildly and not in a consistent manner. We concluded that winter is no more detrimental than another season for the onset of mother-young relationships in intensive rearing conditions providing shelter.

Introduction

In mammals giving birth to precocial young, maternal care from birth to weaning leads to a strong bond between the mother and her offspring. This bond is essential not only for the survival of the young but also for the establishment of its emotional behaviors and fitness (Dwyer, 2014). In sheep, behaviors displayed around and after lambing have a major impact not only on lamb survival but also on its growth rate and weaning weight, and consequently on global productivity (O'Connor *et al.*, 1985). Lamb survival is crucially dependent on the ability to surmount transition difficulties from intra- to extra-uterine existence. This ability depends on the quality of the interactions with the mother

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especially in large litters. Maternal efforts to maintain proximity and to provide an adequate social environment will also strengthen the mother-young bond by providing juveniles with the abilities to cope with stressful situations (Nowak *et al*., 2000). Consequently, the expression of maternal care and the establishment of maternal-filial bond throughout lactation are dependent on the initial onset of maternal behavior (Pickup and Dwyer, 2011). Early maternal care, which appears abruptly around parturition, consists of grooming and nursing activities to provide the newborn with colostrum to satisfy its metabolic needs, and protection to face various elements of the environment (Nowak *et al*., 2000). Grooming the birth fluids limits heat loss by drying the coat and prevents suffocation by removal of the membranes from the lamb's face. Grooming other parts of the body such as the rump stimulates the newborn to stand up, helps it to focus on the maternal body and guides the young toward her mammary region as the mother adopts a specific nursing posture which makes the teats easily accessible (Nowak, 2006). Moreover, during grooming, the ewe learns the smell of her newborn and an olfactory memory takes place, which is a crucial step for maternal bonding (Lévy *et al*., 2004). Nursing, on the other hand, calms and comforts the lamb, and plays a reinforcing role in the development of the mother-young bond (Nowak *et al*., 2000).

Infant behavior plays also an important part in the establishment of the mutual mother-young bond and survival (Nowak and Lindsay, 1992). Thermoregulation, which depends on birth weight and milk availability, is the most vital goals to be reached after birth (Dwyer, 2008). In cold and windy weather, neonates lose heat quickly as they have to surmount the abrupt drop in ambient temperature. Maintenance of body temperature relies on a balance between heat loss and production (Dwyer *et al*., 2015). Therefore, success or failure to surmount cold conditions will depend on the lambs' capacity to suckle and ingest colostrum as soon as possible after birth.

Lamb mortality still remains a major problem both for economic and animal welfare issues as the highest rate occurs within the first three postnatal days (Dwyer, 2008 and 2014; Dwyer *et al*., 2015). It is particularly important amongst lambs born in large litters and with low birth weights (Chniter *et al*., 2011). Lambs with low birth weight have lower rectal temperature (**RT**) and glucose sera concentration than lambs with heavier birth weight (Chniter *et al*., 2013). Therefore, they are behaviorally slow and are more inclined to suffer from hypothermia (Dwyer *et al*., 2005). This difficulty to thermoregulate impairs their ability to suckle rapidly and to bond with their mothers (Chniter *et al*., 2017). Under intensive lambing conditions, Chniter *et al*. (2011) observed that multiple born D'man lambs had a higher mortality rate in winter especially within the first 72 h. This cannot be due to low milk production because D'man ewes produce more milk in winter and autumn than in summer (Dhaoui *et al*., 2018). If we exclude milk as a causal factor, a possible explanation could be found in the early mother-young interactions that may be influenced by season. There is no evidence in the literature on this issue in sheep, but it has been shown in mountain goats that maternal characteristics affect winter survival of kids (Théoret-gosselin et al., 2015). In sheep, maternal care, neonatal behavior and lamb survival are intimately linked (Dwyer *et al*., 2015). Should lambing take place under harsh weather conditions, neonatal behavior is penalized and lamb losses increase in the following 3 days. Therefore, we hypothesized that season of lambing affects the onset of maternal care and neonatal behavior in D'man sheep which would be weakened by winter conditions. Characterization of mother-young interactions at birth over different seasonal conditions should help us understand the increased winter loss reported by Chniter *et al*. (2011) and lead to more adequate management strategies during lambing.

Material and methods

This study was carried out in the Experimental Station of the Institut des Régions Arides located in Chenchou (Gabès), approximately 20 km West of Gabès (N33°53′ E9°53′, Tunisia). This region is characterized by a low arid Mediterranean climate with an irregular rainfall over the year (Dhaou *et al*., 2018). An automatic weather station (CR510 Campbell Scientific data logger) provided average ambient temperatures, minima and maxima, and rainfall data throughout the study. Meteorological data are summarized in Table 1.

Animals

A total of 111 D'man ewes (80 multiparous and 31 primiparous) and their 213 lambs were used in this study. Details about maternal experience and litter size are presented in Table 2.

Animals were reared under intensive production system, kept as a single flock in a communal yard for each season (4.5 m^2/ewe) and managed as previously indicated in Dhaoui *et al*. (2018). Briefly, the yard had a North-East to South-West orientation and possessed a roof to protect the animals from adverse weather conditions providing more light in winter and more shade in summer. Feeding regimes and management procedures were adapted to the physiological stage of the ewes. They received a fixed diet based on Lucerne hay (*Medicago sativa*) and commercial

Table 1 *Mean monthly temperature values and rainfall during lambing seasons of D'man ewes*

	Autumn	Winter	Spring	Summer
Minimum temperature (°C)	8.5	2	5.9	15.6
Average temperature (°C)	19.5	11.8	18.9	28.4
Maximum temperature (°C)	32.9	23.4	35.4	46.7
Rainfall (mm)	17.3	8.3	1.2	0.1

Table 2 *Number of ewes giving birth at day or night time, maternal experience and number of lambs involved in the study according to lambing seasons*

	Parturition period		Maternal experience		Litter size		
Season	Day	Night	Multiparous	Primiparous	Single	Twin	Triplet +
Autumn	13	12	21	4	9	15	14
Winter	13	9	20	2	7	25	19
Spring	20	13	20	13	8	29	28
Summer	17	14	19	12	16	20	23

concentrate mixture. Animals had access to fresh water and trace-mineral salt blocks *ad libitum*.

Lambing periods spread from mid-November 2014 to January 2015 for winter (22 ewes weighing 35.6 ± 6.7 kg; 38 lambs weighing 2.9 ± 0.9 kg), September to October 2015 for autumn (25 ewes weighing 40.9 ± 6.8 kg; 51 lambs weighing 2.7 ± 0.8 kg), July to September 2016 for summer (31 ewes weighing 45.8 ± 6.8 kg; 65 lambs weighing 2.6 ± 0.8 kg) and March to May 2017 for spring season (33 ewes weighing 43.6 ± 9.1 kg; 59 lambs weighing 3.1 ± 0.5 kg).

Once born, lambs were identified, ear-tagged and weighted. Newborn lambs were allowed 2 h to stand and suck successfully unaided. At 3 h, the ewe and her litter were transferred from the communal yard into a 1.5 m^2 individual pen and stayed in it until the behavioral tests were terminated. Any lamb that had not sucked by 2 h after birth was assisted and excluded from the study.

Behaviors on birth site

Ewes were kept under 24-h-video surveillance (Xenyum Pro Surveillance System), and 2 h video sessions were chosen to assess maternal and neonatal behaviors on the birth site based on Dwyer *et al.* (2005). An ethogram was defined for coding the duration of litter expulsion, latency of first grooming of each neonate and time spent grooming during the 30 min following first lamb expulsion (Table 3). Behavioral data were videotaped and analyzed by one expert person using Boris recording software (Friard and Gamba, 2016).

Two-choice tests of preference

Mother-young preference was assessed during a 5-min test approximately 48 h after parturition. The procedures and testing pens were similar as previously described by Chniter *et al.* (2017). For both lambs and ewes, the location of the testing pen was in an isolated indoor room. The pen consisted of an equilateral triangular arena (6 m × 6 m × 6 m). One corner ended with a starting pen for the subject to be tested and the two others consisted of individual pens for stimulus subjects (2 m × 1 m). These were separated by an empty pen (2 m × 1 m) which prevented subjects from being too close to each other. A 50-cm-wide zone next to the stimulus subjects was considered as a contact zone.

Table 3 *Behaviors of mother and young recorded during the first 2 h* postpartum *in D'man sheep*

Ewes	
Litter expulsion time(s)	Interval between appearance of the first lamb at the vulva and expulsion of the last one
First grooming (s)	Time interval between full expulsion of each lamb and first grooming given to each of them
Grooming (s)	Time spent grooming during the first 30 min after giving birth of the first lamb
Lambs	
Gets up on forelegs(s)	Lamb on chest, pushing up on knees, supporting part of body off the ground
Stands up(s)	Lamb supporting itself on all four feet for at least 5 s
Attempts to suckle(s)	Lamb with head under the ewe's udder region

The preference for the lamb by its mother was assessed first by placing the own and alien lambs in individual pens at the opposite stimulus corners and the ewe in the starting pen. In the case of large litters, only one lamb was used as a stimulus to minimize any bias due to differences in litter sizes.

The preference for the mother by the lamb was assessed immediately after. Mothers and alien recent parturient ewes (a maximum delay of 3 days after birth) were separately kept at the opposite stimulus corners of the testing arena. The lamb was carefully placed in the starting pen. Each ewe was used only twice: first with one of her own lamb and then as an alien mother in the following test before being released and returned to the individual pen with her litter. During the test, the siblings were kept away (15 m) from the testing arena to avoid vocal communication.

Both for young and mothers, the location of stimulus subjects was balanced so that own and alien conspecifics were allocated to left and right corners equally. The following data were taken: latency to reach the stimulus corners (both forelegs in the contact zone), time spent in the contact zones near the own or alien partner during the1st min, the 5 min of the test and the number of bleats during the 5 min of the test. In addition, ewes and lambs were considered to make a correct

choice if they spent more than 60% of the time near the two stimulus subjects and at least two-thirds of this time near its familiar partner (Chniter *et al.*, 2017).

Rectal temperature and glycemia

Rectal temperature (°C) was recorded at 1 and 48 h of age in an area unexposed to solar radiation, using a digital probe thermometer (range: 32.00 to 43.00°C; accuracy: ±0.10°C (35.50 to 42.00°C)). Blood samples were taken from 190 lambs by jugular venipuncture into hemolysis vacutainer tubes for glucose measurement. A fully trained person, who intervened calmly, caught the lamb, restrained it on its side and collected the RT and the blood sample. Human intervention had no detrimental effect on subsequent ewe-lamb interactions. After the blood samples were centrifuged (3000×**g**, 4°C) for 20 min, the separated sera were stored at −20°C until analysis of glucose using a colorimetric method (Kit Ref 20121; Biomaghreb, Tunis, Tunisia).

Statistical analyses

All the data were statistically analyzed and graphically represented using GraphPad Prism 6.0 software. Tests of normality (Shapiro-Wilk test) were performed and showed non-normal distributions. Consequently, two-tailed nonparametric tests were used to study the season effect on maternal and neonatal behavior, and on lamb vigor. Overall, the effect of the season was performed by Kruskal-Wallis test with Dunn's correction for multiple comparisons. Inter-season comparisons were performed using the Mann-Whitney test. Preference toward a partner (own *v.* alien) was analyzed by the Wilcoxon signed-rank test. In all cases, *P*-values less than 0.05 were considered as statistically significant. Results for behavioral traits and vigor are presented in terms of median and lower and upper quartiles (LQ-HQ).

Results

Behaviors on the birth site

Lambing process and maternal care. Season of lambing did not affect (Kruskal-Wallis = 4.94; $P = 0.18$) litter expulsion time. However, the latency required to groom each neonate was affected by season of lambing (Kruskal-Wallis = 8.81; $P = 0.03$; Figure 1a). It was shorter in winter compared to lambing in spring (Mann–Whitney $U = 243.0$; $P = 0.01$) and summer (Mann–Whitney $U = 309.5$; $P = 0.01$). In autumn, ewes had an intermediate value.

During the first 30 min following birth, ewes lambing in winter spent significantly more time grooming theirs lambs than ewes lambing in spring (Mann–Whitney $U = 89.0$; $P = 0.01$) and in summer (Mann–Whitney $U = 69.0$; $P = 0.04$). Ewes lambing in autumn spent an intermediate value grooming theirs lambs (Mann–Whitney $U = 97.0$; $P = 0.16$) (Figure 1b).

Neonatal behavior. Immediate postnatal behavior was affected significantly by season of lambing. Lambs born in spring pushed upon their knees (Kruskal-Wallis = 10.36; $P = 0.01$; Figure 2a), stood up (Kruskal-Wallis = 8.26; $P = 0.04$; Figure 2b) and reached the udder earlier (Kruskal-Wallis = 8.15; $P = 0.04$; Figure 2c) than those born in the other seasons.

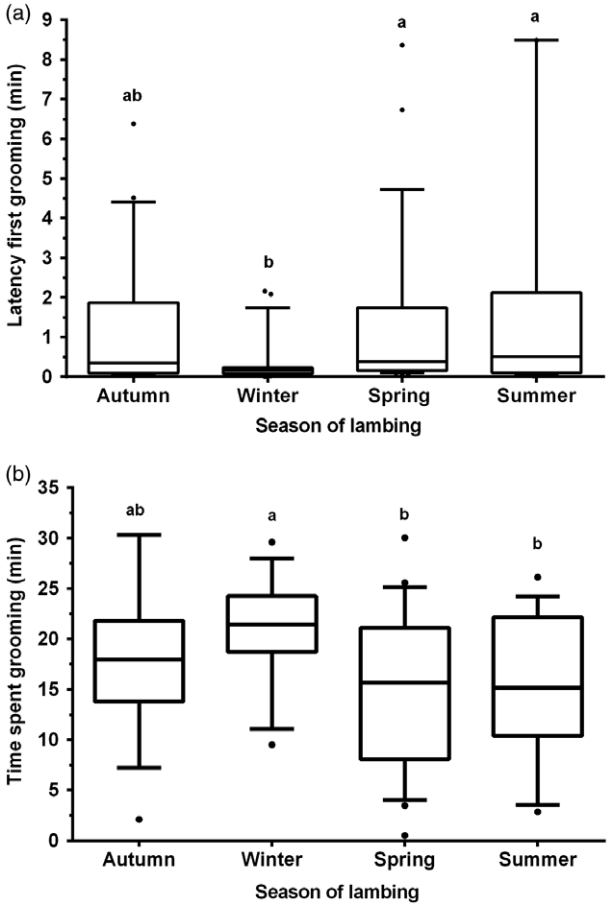

Figure 1 Postnatal grooming activity of D'man ewes according to lambing season: (a) time interval between full expulsion of each lamb and first grooming given each of them and (b) total duration of grooming during the first 30 min after giving birth to the first lamb. Values are medians (bar within the box) and upper and lower quartiles (boarders of box) with 10 and 90 percentile. a, b: $P < 0.05$.

Preference for own lamb at 48 h postpartum

All the ewes reached the contact zone before the end of the test without any effect of the season, and 78/111 made a correct choice. Ewes reached their own lamb's side significantly more rapidly than the side of the alien lamb in all four seasons (Wilcoxon tests = spring: 5.0 s (4.0 to 14.0) *v.* 43.0 s (14.0 to 300.0), $Z = 3.86$, $P < 0.001$; summer: 4.0 s (3.0 to 8.0) *v.* 49.0 s (18.0 to 112.0), $Z = 4.06$, $P < 0.001$; autumn: 7.0 s (3.0 to 6.0) *v.* 39.0 s (11.0 to 222.0), $Z = 3.41$, $P < 0.001$; winter: 9.0 s (4.0 to 17.0) *v.* 25.5 s (5.0 to 74.0), $Z = 2.07$, $P = 0.04$). They also spent significantly more time near their own than near the alien lamb during the first minute of the test (Wilcoxon tests = spring: 42.0 s (17.0 to 50.0) *v.* 3.0 s (0.0 to 8.0), $Z = 3.85$, $P < 0.001$; summer: 46.0 s (34.0 to 51.0) *v.* 2.0 s (0.0 to 6.0), $Z = 4.84$, $P < 0.0001$; autumn: 44.0 s (35.0 to 48.0)

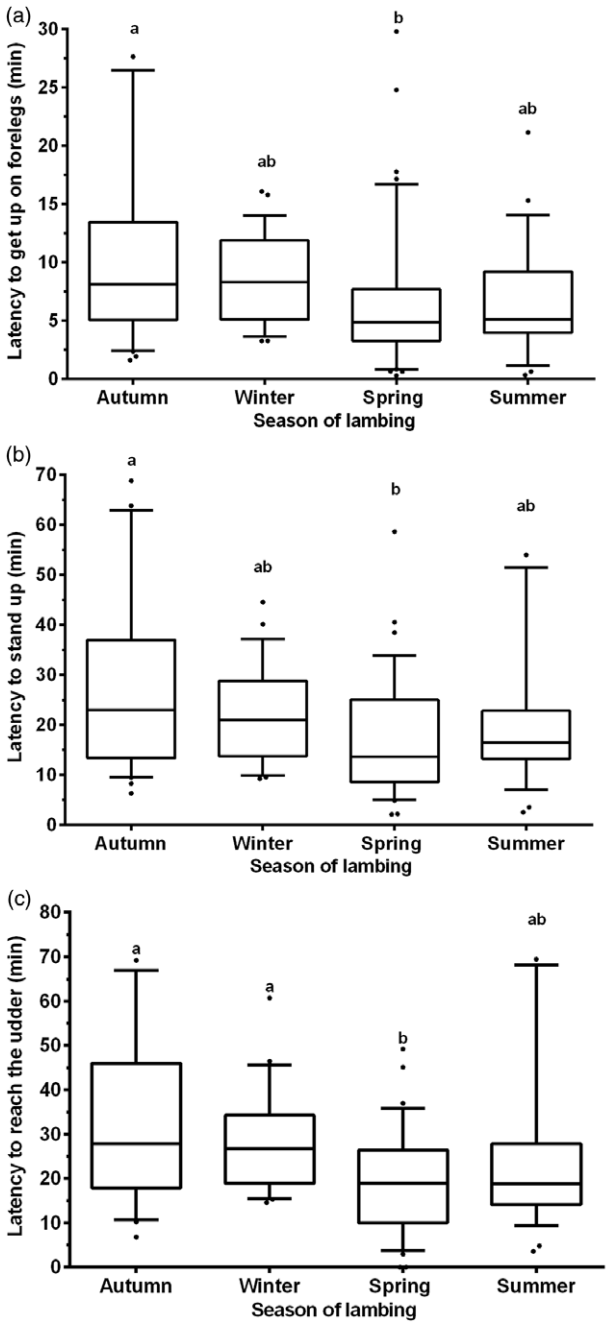

Figure 2 D'man lambs' neonatal behavior according to season of birth: (a) latency to get up on forelegs, (b) latency to stand up and (c) latency to reach the udder. Values are medians (bar within the box) and upper and lower quartiles (boarders of box) with 10 and 90 percentile. a, b: $P < 0.05$.

v. 1.0 s (0.0 to 4.0), $Z = 4.37$, $P < 0.0001$; winter : 25.5 s (18.0 to 42.0) *v.* 2.0 s (0.0 to 10.0), $Z = 3.04$, $P = 0.002$). These differences were confirmed for the whole duration of the test (Wilcoxon tests = spring: 224.0 s (95.0 to 278.0) *v.* 10.0 s (0.0 to 29.0), $Z = 4.37$, $P < 0.0001$; summer: 211.0 s (184.0 to 270.0) *v.* 11.0 s (2.0 to 39.0), $Z = 4.62$, $P < 0.0001$; autumn: 233.0 (148.0 to 271.0) *v.* 9.0 s (1.0 to 16.0), $Z = 4.34$, $P < 0.0001$; winter: 175.0 s (82.0 to 228) (6.0 to 39.0) *v.* 16.0 s, $Z = 3.45$, $P < 0.001$).

During the first minute, ewes lambing in winter spent significantly less time near their own lamb than those lambing in autumn (25.5 s (18.0 to 42.0) *v.* 44.0 s(35.0 to 48.0), Mann–Whitney $U = 156$; $P = 0.01$) or in summer (25.5 s (18.0 to 42.0) *v.* 46.0 s (34.0 to 51.0), Mann–Whitney $U = 186.5$; $P = 0.01$). This effect was lost over the whole duration of the test even though winter lambing ewes still had the lowest median value. There was no difference between seasons on the latency to reach the contact zone next to a lamb, on the time spent near the alien lamb during the first minute or during the whole duration of the test or on the number of bleats emitted by ewe.

Preference for the mother at 48 h of age

Lambs reached more rapidly their own mothers than the alien ewes (Wilcoxon tests = spring: 30.0 s (10.0 to 82.0) *v.* 78.0 s (33.0 to 176.0), $Z = 2.66$, $P < 0.01$; summer: 43.0 s (19.0 to 136.0) *v.* 147.0 s (41.0 to 300.0), $Z = 3.30$, $P < 0.001$; autumn: 31.0 s (12.0 to 126.0) *v.* 83.0 s (26.0 to 238.0), $Z = 2.08$, $P = 0.03$; winter: 22.0 s (12.0 to 88.0) *v.* 90.5 s (20.0 to 147.0), $Z = 1.57$, $P = 0.01$). Latencies to reach a ewe were not affected by the season. They spent more time near their own mothers than alien ewes during the first minute (Wilcoxon tests = spring: 26.0 s (0.0 to 42.0) *v.* 0.0 s (0.0 to 13.0), $Z = 3.23$, $P < 0.01$; summer: 8.0 s (0.0 to 39.0) *v.* 0.0 s (0.0 to 8.0), $Z = 3.39$, $P < 0.001$; autumn: 21.0 s (0.0 to 40.0) *v.* 0.0 s (0.0 to 15.0), $Z = 2.95$, $P = 0.003$; winter: 29.5 s (0.0 to 42.0) *v.* 0.0 s (0.0 to 14.0), $Z = 2.24$, $P = 0.02$) and for the whole duration of the test (Wilcoxon tests = spring: 175.0 s (95.0 to 241.0) *v.* 50.0 s (9.0 to 113.0), $Z = 4.25$, $P < 0.0001$; summer: 151.0 s (10.0 to 238.0) *v.* 24.0 s (0.0 to 101.0), $Z = 3.74$, $P < 0.001$; autumn: 145.0 s (85.0 to 217.0) *v.* 52.0 s (14.0 to 98.0), $Z = 3.94$, $P < 0.0001$; winter: 159.5 s (116.0 to 210.0) *v.* 47.0 s (17.0 to 118.0), $Z = 3.16$, $P < 0.01$).

Overall, season of birth did not affect the capacity of lambs to discriminate between their own and alien mothers at 48 h of age. However, lambs born in winter emitted significantly less bleats (13.5) than lambs born in autumn (13.5 (9.0 to 25.0) *v.* 21.0 (12.0 to 33.0), Mann–Whitney $U = 711$; $P = 0.03$), in summer (13.5 (9.0 to 25.0) *v.* 23.0 (16.0 to 40.0), Mann–Whitney $U = 833.5$; $P = 0.01$) and in spring (13.5 (9.0 to 25.0) *v.* 26.0 (14.0 to 41.0), Mann–Whitney $U = 671.5$; $P = 0.001$).

Birth weight, rectal temperature and glycemia

Lambs born in summer were lighter than those born in autumn (Mann–Whitney $U = 527.0$; $P = 0.04$) and spring (Mann–Whitney $U = 1210$; $P = 0.001$; Figure 3). Winter born lambs had an intermediate birth weight.

One hour after birth, the median RT of D'man lamb was 38.7°C (38.40 to 39.08). It rose to 39.20°C (39.00 to 39.60) at 48 h of age with a median difference of +0.6°C. Season of birth did not affect the temperatures recorded around birth but it did 48 h later (Kruskal–Wallis = 19.74; $P < 0.001$; Figure 4a and b). It was higher in summer than in winter

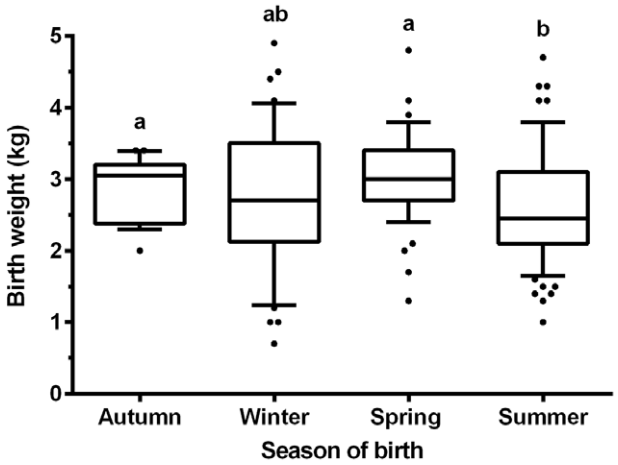

Figure 3 Birth weight of D'man lambs according to season of birth. Values are medians (bar within the box) and upper and lower quartiles (boarders of box) with 10 and 90 percentile. a, b: $P < 0.05$.

(Mann–Whitney $U = 763.5$; $P = 0.001$) and in spring born lambs (Mann–Whitney $U = 1064$; $P < 0.0001$). Autumn born lambs had an intermediate value. Season of birth had a significant effect on RT variation from 1 to 48 h of age (Kruskal-Wallis = 12.67; $P = 0.01$; Figure 4c). Spring born lambs had a lower RT increase than winter (Mann–Whitney $U = 735$; $P = 0.01$), autumn (Mann–Whitney $U = 1041$; $P = 0.01$) and summer born lambs (Mann–Whitney $U = 1260$; $P = 0.001$).

Median glycemia was 2.72 mmol/l (2.24 to 3.12) within the hour following birth and reached 4.63 mmol/l (3.92 to 6.34) 48 h later with a rise of 1.67 mmol/l (1.17 to 4.08). Season of birth had a significant effect both at 1 h (Kruskal-Wallis = 11.09; $P = 0.01$; Figure 4a) and 48 h of age (Kruskal-Wallis = 54.63; $P < 0.0001$; Figure 4b), and also on the increase between 1 and 48 h of age (Kruskal-Wallis = 48.65; $P < 0.0001$; Figure 4c). At birth, summer born lambs had significantly lower glycemia compared to spring born lambs (Mann–Whitney $U = 293$; $P = 0.0001$). Winter and autumn born lambs had an intermediate value. At 48 h of age, spring born lambs had the lowest concentrations compared to winter (Mann–Whitney $U = 35$; $P < 0.0001$), autumn (Mann–Whitney $U = 46$; $P < 0.0001$) and summer born lambs (Mann–Whitney $U = 571$; $P < 0.0001$). In fact, glycemia increased significantly less in spring born lambs than in winter (Mann–Whitney $U = 25$; $P < 0.0001$), autumn (Mann–Whitney $U = 29.5$; $P < 0.0001$) and summer born lambs (Mann–Whitney $U = 281.5$; $P < 0.0001$).

Discussion

In this study, immediately after parturition ewes provided a higher intensity of care to their offspring in winter, but lambs were the most active in spring getting up and reaching the udder more rapidly. Forty-eight hours later, season of lambing did not affect the partners' ability to express a preference for each other even though in winter ewes spent less time near their own lambs during the first minute of the test and their lambs were less vocal. In terms of neonatal physiology, body temperature and glycemia usually went up from birth to 48 h in all four seasons but spring born lambs had the lowest increase in both parameters, and summer born lambs reached the highest body temperature at 2 days of age. Clearly, while some behavioral and physiological parameters fluctuate according to seasons, the effects are minor and inconsistent. We cannot conclude that winter or any specific season (nor a temperature range) has detrimental consequences on mother-young relationships. The literature shows that lambs are at risk in winter when environmental conditions combine wind, rain and cold which prevents them from maintaining homeothermy and normal behavior (review: Pollard, 2006). This was not the case in our study even though we must keep in mind the limitations of the recording method concerning meteorological data. The database was based on average temperature, minima and maxima for each lambing season; it did not provide specific local conditions for each birth. We cannot exclude however that some very cold winter nights (or even very hot summer days) may have influenced the expression of early behavior patterns; this should be taken into account in future work. Nonetheless, our average recording does not reveal hostile winter climatic conditions especially in terms of rainfall and we cannot conclude that lambs were submitted to cold exposure. In addition, the fact that the yard was enclosed, possessed a roof and the animals were put in individual pens could have protected them from adverse conditions.

D'man ewes started to groom their neonates sooner and for longer during the first 30 min *postpartum* in winter than in spring and summer. Although smaller lambs lead to an easier birth (Grommers *et al.*, 1985) and an easy birth is associated with better maternal care (Poindron *et al.*, 1984), neither lambing process nor litter size and lambs weight are satisfactory explanations for the difference in the expression of grooming behavior. Maternal experience on the other hand, instead of season, is a more likely causal factor. The number of primiparous ewes was lower in winter than in spring and in summer and parity, and not just age, is known to influence the expression of maternal care (Alexander *et al.*, 1993). It appears quite logical that the grooming activity reflecting maternal motivation should be more pronounced in winter lambing ewes. Some authors report that maternal attention stimulates neonatal behavior progression (Alexander and Williams, 1964; McGlone and Stobart, 1986; O'Connor and Lawrence, 1992) while others suggest that maternal attention slows down neonatal behavior progression (Vince, 1993; Dwyer and Lawrence, 1999). Our results are more in favor of the second findings since winter born lambs were the least active, while it was in spring and summer that lambs were the quickest to stand up and reach the udder. According to Vince (1993) grooming the head and shoulders, which is where the mother usually starts from, has soothing properties. Some studies also report that the lamb's neonatal activity is impaired in winter (Scotland: Dwyer and Morgan,

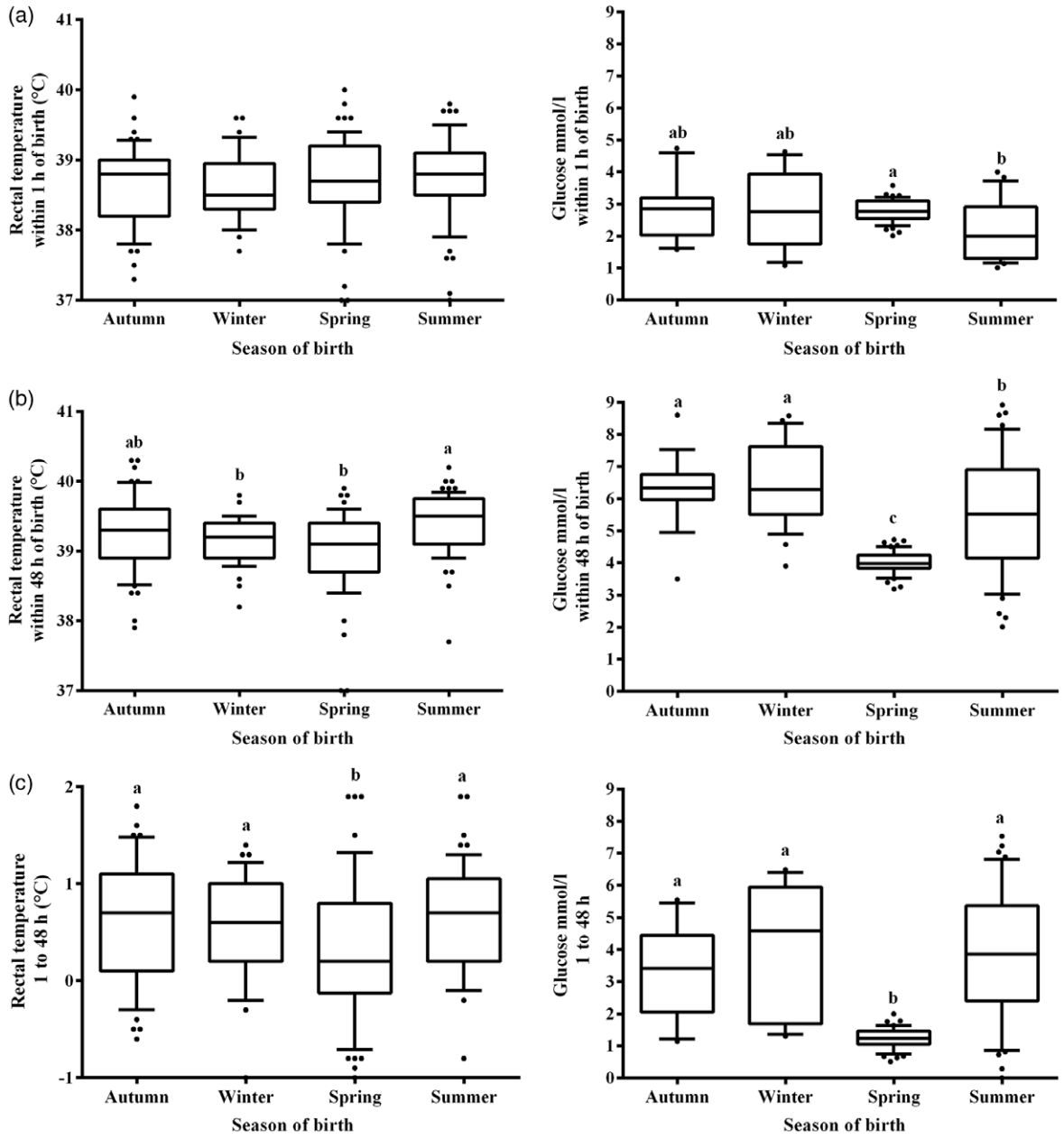

Figure 4 Rectal temperature (left panel) and glycemia (right panel) in D'man lambs according to season of birth: (a) within 1 h, (b) 48 h of birth and (c) variations between these two times. Values are medians (bar within the box) and upper and lower quartiles (boarders of box) with 10 and 90 percentile. a, b, c: $P < 0.05$.

2006; Brazil: Fonsêca *et al.*, 2014) but this does not seem an unanimous fact (Wassmuth *et al.* 2001). Local conditions as well as breed differences may account for the differences reported between authors.

Mother-young discrimination was well established at 48 h *postpartum* with no main season effect. All D'man ewes and lambs reached the contact zone and in most cases chose their own partner in comparison to an unfamiliar one. Nonetheless, the fact that winter parturient ewes spent less time next to their own young at the beginning of the test might suggest that their lambs were not initially as attractive or the mothers not as motivated to stay nearby. Limitation in the recording equipment did not allow us to analyze the behavioral interactions and find potential differences in animals' reactivity during the test. Acoustics in complement to olfactory cues from the lamb trigger maternal motivation and are important in the recognition of the young (Sèbe *et al.*, 2007; Morton *et al.*, 2018). This could have been valuable information especially because when lambs were submitted to the choice test it was found that winter born subjects were the least vocal. Whether a lesser vocal activity is a characteristic of lambs born in winter has to be confirmed.

The fact that summer born lambs had a higher body temperature than winter born lambs at 48 h is quite logical as they were born during the warmest season, a difference that had already been found by Chniter *et al.* (2013). Our results

on RT and glycemia suggest that overall D'man neonates were not susceptible to oases climatic conditions and could maintain body temperature within normal range through two mechanisms. The first is mobilization of brown adipose tissue, a mechanism that helps newborn lambs adapt to cold immediately after being born (Dwyer and Morgan, 2006; Dwyer *et al.*, 2015). The second is based on glucose plasma levels. Glucose is an important source of energy originating from colostrum and is required to maintain homeothermy beyond the neonatal period (Thompson *et al.*, 2006; Miller *et al.*, 2010). After birth, D'man lambs rapidly found the udder and sucked successfully making glycemia increased as a consequence which has also been reported by Chniter *et al.* (2016).

While, in the present study, the overall rise in glycemia and body temperature is a clear sign of the newborns' ability to adapt to their rearing conditions, it does not imply that D'man lambs are insusceptible to hypothermia. Chniter *et al.* (2011 and 2013) had shown that winter born lambs were more fragile and suffered a higher mortality rate. However, in the present work, we only took into account the lambs that survived until the time of the preference tests, excluding the weakest subjects more prone to suffer from hypothermia or postnatal trauma. Under such experimental conditions, confirming differences in neonatal physiology like in the studies by Chniter *et al.* (2011 and 2013) was a more arduous task. Spring born lambs appeared anyhow to be the most vigorous at birth; they were also amongst the heaviest. The most important birth weight and vigor in spring born D'man lambs could reflect optimal climatic conditions during gestation with enhancing fetal growth in late pregnancy as suggested by Chniter *et al.* (2011). Their lesser glycemic variability and their lower postnatal rise in body temperature suggest easier adaptation to the moderate spring weather conditions. Spring born lambs did not have to metabolize as much energy as autumn and winter born lambs and could thermoregulate better than summer born lambs.

While our data indicate that lambing season affects some aspects of maternal and neonatal behavior in D'man sheep as well as neonatal physiology, we cannot conclude that one season has more impact than another does. Spring born lambs appeared the most vigorous at birth but mothers showed more intense maternal care in winter. Unsurprisingly, born lambs had a higher body temperature in summer but it was in spring that physiological factors were the least variable suggesting better neonatal adaptation to mild climatic conditions. Some behavioral aspects involved in the identification of mother or young were suboptimal in winter but overall the ewe-lamb bond was clearly established in all four seasons by 48 h *postpartum*. Should lamb survival be at more at risk in winter as reported by Chniter *et al.* (2011), there is no reason to think that early mother-young interactions and bonding can be hold responsible for it in the conditions of the study.

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Declaration of interest

The authors declare that they have no conflicts of interest.

Ethical statement

The authors followed institutional and national guidelines for the care and use of laboratory animals. While there is no ethic committee in Tunisia, experimental conditions in the present work complied with the European Directive 2010/63/EU on the protection of animals used for scientific purposes.

Software and data repository resources

None of the data were deposited in an official repository.

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