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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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26 **Abstract**

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

51 this study show that maternal care, lamb behavior and vigor vary lightly according to  
52 seasons, albeit not in a consistent manner. In conclusion, a season is no more  
53 detrimental than another for the onset of mother-young relationships.

54 **Keywords:** parturition, maternal behavior, neonate behavior, bonding, glycaemia

55

### 56 **Implications**

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

64

65

66 **Introduction**

67 In mammals giving birth to precocial young, maternal care from birth to weaning  
68 leads to a strong bond between the mother and her offspring. This bond is essential  
69 not only for the survival of the young but also for the establishment of its emotional  
70 behaviors and fitness (Dwyer, 2014). In sheep, behaviors displayed around and after  
71 lambing have a major impact on lamb survival but also on its growth rate and  
72 weaning weight, and consequently on global productivity (O'Connor et al., 1985).  
73 Lamb survival is crucially dependent on the ability to surmount transition difficulties  
74 from intra- to extra-uterine existence. This ability depends on the quality of the  
75 interactions with the mother especially in large litters. Maternal efforts to maintain  
76 proximity and to provide an adequate social environment will also strengthen the  
77 mother-young bond by providing juveniles with the abilities to cope with stressful  
78 situations (Nowak et al., 2000). Consequently, the expression of maternal care and  
79 the establishment of maternal-filial bond throughout lactation are dependent on the  
80 initial onset of maternal behavior (Pickup and Dwyer, 2011). Early maternal care,  
81 which appears abruptly around parturition, consists of grooming and nursing activities  
82 to provide the newborn with colostrum to satisfy its metabolic needs, and protection  
83 to face various elements of the environment (Nowak et al., 2000). Grooming the birth  
84 fluids limits heat loss by drying the coat, and prevents suffocation by removal of the  
85 membranes from the lamb's face. Grooming other parts of the body such as the rump  
86 stimulates the newborn to stand up, helps it to focus on the maternal body and  
87 guides the young toward her mammary region as the mother adopts a specific  
88 nursing posture which makes the teats easily accessible (Nowak, 2006). Moreover,  
89 during grooming, the ewe learns the smell of her newborn and an olfactory memory  
90 takes place, which is a crucial step for maternal bonding (Lévy et al., 2004). Nursing,

91 on the other hand, calms and comforts the lamb, and plays a reinforcing role in the  
92 development of the mother young bond (Nowak et al., 2000).

93 Infant behavior plays also an important part in the establishment of the mutual  
94 mother-young bond and survival (Nowak and Lindsay, 1992). Thermoregulation,  
95 which depends on birth weight and milk availability, is the most vital goals to be  
96 reached after birth (Dwyer, 2008). In cold and windy weather, neonates lose heat  
97 quickly as they have to surmount the abrupt drop in ambient temperature.

98 Maintenance of body temperature relies on a balance between heat loss and  
99 production (Dwyer et al., 2015). Therefore, success or failure to surmount cold  
100 conditions will depend on the lambs' capacity to suckle and ingest colostrum as soon  
101 as possible after birth.

102 Lamb mortality still remains a major problem both for economic and animal welfare  
103 issues as the highest rate occurs within the first three post-natal days (Dwyer, 2008;  
104 2014; Dwyer et al., 2015). It is particularly important amongst lambs born in large  
105 litters and with low birth weights (Chniter et al., 2011). Lambs with low birth weight  
106 have lower rectal temperature and glucose sera concentration than lambs with  
107 heavier birth weight (Chniter et al., 2013). Therefore, they are behaviorally slow and  
108 are more inclined to suffer from hypothermia (Dwyer et al., 2005). This difficulty to  
109 thermoregulate impairs their ability to suckle rapidly and to bond with their mothers  
110 (Chniter et al., 2017). Under intensive lambing conditions, Chniter et al. (2011)  
111 observed that multiple born D'man lambs had a higher mortality rate in winter  
112 especially within the first 72 h. This cannot be due to low milk production because  
113 D'man ewes produce more milk in winter and autumn than in summer (Dhaoui et al.,  
114 2018). If we exclude milk as a causal factor, a possible explanation could be found in  
115 the early mother-young interactions that may be influenced by season. There is no

116 evidence in the literature on this issue in sheep but it has been shown in Mountain  
117 goats that maternal characteristics affect winter survival of kids (Théoret-Gosselin  
118 and Hamel, 2015). In sheep, maternal care, neonatal behavior, and lamb survival are  
119 intimately linked (Dwyer et al., 2015). Should lambing take place under harsh  
120 weather conditions, neonatal behavior is penalized and lamb losses increase in the  
121 following 3 days. Therefore, we hypothesized that season of lambing affects the  
122 onset of maternal care and neonatal behavior in D'man sheep which would be  
123 weakened by winter conditions. Characterization of mother-young interactions at birth  
124 over different seasonal conditions should help us understand the increased winter  
125 loss reported by Chniter et al. (2011) and lead to more adequate management  
126 strategies during lambing.

127

## 128 **Material and methods**

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

136

137 **Insert table 1 here**

138

139

140

141 *Animals*

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

145

146 **Insert table 2 here**

147

148 Animals were reared under intensive production system, kept as a single flock in a  
149 communal yard for each season (4.5 m<sup>2</sup>/ewe), and managed as previously indicated  
150 in Dhaoui et al. (2018). Briefly, the yard had a North-East to South-West orientation  
151 and possessed a roof to protect the animals from adverse weather conditions  
152 providing more light in winter and more shade in summer. Feeding regimes and  
153 management procedures were adapted to the physiological stage of the ewes. They  
154 received a fixed diet based on Lucerne hay (*Medicago sativa*) and commercial  
155 concentrate mixture. Animals had access to fresh water and trace-mineral salt blocks  
156 *ad-libitum*.

157 Lambing periods spread from mid-November 2014 to January 2015 for winter (22  
158 ewes weighing 35.6 ± 6.7 kg; 38 lambs weighing 2.9 ± 0.9 kg), September to October  
159 2015 for autumn (25 ewes weighing 40.9 ± 6.8 kg; 51 lambs weighing 2.7 ± 0.8 kg),  
160 July to September 2016 for summer (31 ewes weighing 45.8 ± 6.8 kg; 65 lambs  
161 weighing 2.6 ± 0.8 kg) and March to May 2017 for spring season (33 ewes weighing  
162 43.6 ± 9.1 kg; 59 lambs weighing 3.1 ± 0.5 kg).

163 Once born, lambs were identified, ear-tagged and weighted. Newborn lambs were  
164 allowed 2 h to stand and suck successfully unaided. At 3 h, the ewe and her litter  
165 were transferred from the communal yard into a 1.5 m<sup>2</sup> individual pen and stayed in it

166 until the behavioral tests were terminated. Any lamb that had not sucked by 2 h after  
167 birth was assisted and excluded from the study.

168

169 *Behaviors on birth site*

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

177

178 **Insert table 3 here**

179

180 *Two-choice tests of preference*

181 Mother-young preference was assessed during a 5-min test approximately 48 h after  
182 parturition. The procedures and testing pens were similar as previously described by  
183 Chniter et al. (2017). For both lambs and ewes, the location of the testing pen was in  
184 an isolated indoor room. The pen consisted of an equilateral triangular arena (6 m ×  
185 6 m × 6 m). One corner ended with a starting pen for the subject to be tested and the  
186 two others consisted of individual pens for stimulus subjects (2 m × 1 m). These were  
187 separated by an empty pen (2 m × 1 m) which prevented subjects from being too  
188 close to each other. A 50-cm-wide zone next to the stimulus subjects was considered  
189 as a contact zone.

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

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

201 Both for young or mothers, the location of stimulus subjects was balanced so that  
202 own and alien conspecifics were allocated to left and right corners equally. The  
203 following data were taken: latency to reach the stimulus corners (both forelegs in the  
204 contact zone), time spent in the contact zones near the own or alien partner during  
205 the 1<sup>st</sup> min, and the 5 min of the test, and the number of bleats during the 5 min of the  
206 test. In addition, ewes and lambs were considered to make a correct choice if they  
207 spent more than 60 % of the time near the two stimulus subjects and at least two-  
208 thirds of this time near its familiar partner (Chniter et al., 2017).

209

#### 210 *Rectal temperature and glycaemia*

211 Rectal temperature (RT, °C) was recorded at 1 h and 48 h of age in an area  
212 unexposed to solar radiation, using a digital probe thermometer (range: 32.00 ~  
213 43.00°C, accuracy: +/- 0.10°C (35.50°C ~ 42.00°C). Blood samples were taken from  
214 190 lambs by jugular venipuncture into hemolysis vacutainer tubes for glucose

215 measurement. A fully trained person, who intervened calmly, caught the lamb,  
216 restrained it on its side, and collected the rectal temperature and the blood sample.  
217 Human intervention had no detrimental effect on subsequent ewe-lamb interactions.  
218 After the blood samples were centrifuged (3000 × g, 4°C) for 20 min, the separated  
219 sera were stored at - 20 °C until analysis of glucose using a colorimetric method (Kit  
220 Ref 20121, Biomaghreb, Tunisia).

221

## 222 **Statistical analyses**

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

233

234

## 235 **Results**

236 *Behaviors on the birth site*

237 *Lambing process and maternal care.* Season of lambing did not affect (Kruskal-Wallis  
238 = 4.94; P = 0.18) litter expulsion time. However, the latency required to groom each  
239 neonate was affected by season of lambing (Kruskal-Wallis =8.81; P = 0.03; Figure

240 1a). It was shorter in winter compared to lambing in spring (Mann-Whitney U = 243.0;  
241 P = 0.01) and summer (Mann-Whitney U = 309.5; P = 0.01). In autumn, ewes had an  
242 intermediate value.

243

244 **Insert Figure1 here**

245

246 During the first 30 min following birth, ewes lambing in winter spent significantly more  
247 time grooming their lambs than ewes lambing in spring (Mann-Whitney U = 89.0; P  
248 = 0.01) and in summer (Mann-Whitney U = 69.0; P = 0.04). Ewes lambing in autumn  
249 spent an intermediate value grooming their lambs (Mann-Whitney U = 97.0; P =  
250 0.16) (Figure 1b).

251 *Neonatal behavior.* Immediate postnatal behavior was affected significantly by  
252 season of lambing. Lambs born in spring pushed upon their knees (Kruskal-Wallis =  
253 10.36; P = 0.01; Figure 2a), stood up (Kruskal-Wallis = 8.26; P = 0.04; Figure 2b) and  
254 reached the udder earlier (Kruskal-Wallis = 8.15; P = 0.04; Figure 2c) than those  
255 born in the other seasons.

256

257 **Insert Figure 2 here**

258

259 *Preference for own lamb at 48 h postpartum*

260 All the ewes reached the contact zone before the end of the test without any effect of  
261 the season, and 78/111 made a correct choice. Ewes reached their own lamb's side  
262 significantly more rapidly than the side of the alien lamb in all four seasons (Wilcoxon  
263 tests = Spring: 5.0 s [4.0-14.0] vs 43.0 s [14.0-300.0], Z = 3.86, P < 0.001; Summer:  
264 4.0 s [3.0-8.0] vs 49.0 s [18.0-112.0] Z = 4.06, P < 0.001; Autumn: 7.0 s [3.0-6.0] vs

265 39.0 s [11.0-222.0],  $Z = 3.41$ ,  $P < 0.001$ ; Winter: 9.0 s [4.0-17.0] vs 25.5 s [5.0-74.0],  
266  $Z = 2.07$ ,  $P = 0.04$ ). They also spent significantly more time near their own than near  
267 the alien lamb during the first minute of the test (Wilcoxon tests = Spring: 42.0 s  
268 [17.0-50.0] vs 3.0 s [0.0-8.0],  $Z = 3.85$ ,  $P < 0.001$ ; Summer: 46.0 s [34.0-51.0] vs 2.0  
269 s [0.0-6.0],  $Z = 4.84$ ,  $P < 0.0001$ ; Autumn: 44.0 s [35.0-48.0] vs 1.0 s [0.0-4.0],  $Z =$   
270 4.37,  $P < 0.0001$ ; Winter : 25.5 s [18.0-42.0] vs 2.0 s [0.0-10.0],  $Z = 3.04$ ,  $P = 0.002$ ).  
271 These differences were confirmed for the whole duration of the test (Wilcoxon tests =  
272 Spring: 224.0 s [95.0-278.0] vs 10.0 s [0.0-29.0],  $Z = 4.37$ ,  $P < 0.0001$ ; Summer:  
273 211.0 s [184.0-270.0] vs 11.0 s [2.0-39.0],  $Z = 4.62$ ,  $P < 0.0001$ ; Autumn: 233.0  
274 [148.0-271.0] vs 9.0 s [1.0-16.0],  $Z = 4.34$ ,  $P < 0.0001$ ; Winter: 175.0 s [82.0-228]  
275 [6.0-39.0] vs 16.0 s,  $Z = 3.45$ ,  $P < 0.001$ ).

276 During the first minute, ewes lambing in winter spent significantly less time near their  
277 own lamb than those lambing in autumn (25.5 s [18.0-42.0] vs 44.0 s [35.0-48.0],  
278 Mann-Whitney  $U = 156$ ;  $P = 0.01$ ) or in summer (25.5 s [18.0-42.0] vs 46.0 s [34.0-  
279 51.0], Mann-Whitney  $U = 186.5$ ;  $P = 0.01$ ). This effect was lost over the whole  
280 duration of the test even though winter lambing ewes still had the lowest median  
281 value. There was no difference between seasons on the latency to reach the contact  
282 zone next to a lamb, on the time spent near the alien lamb during the first minute or  
283 during the whole duration of the test, nor on the number of bleats emitted by ewe.

284

#### 285 *Preference for the mother at 48 h of age*

286 Lambs reached more rapidly their own mothers than the alien ewes (Wilcoxon tests =  
287 Spring: 30.0 s [10.0-82.0] vs 78.0 s [33.0-176.0],  $Z = 2.66$ ,  $P < 0.01$ ; Summer: 43.0 s  
288 [19.0-136.0] vs 147.0 s [41.0-300.0],  $Z = 3.30$ ,  $P < 0.001$ ; Autumn: 31.0 s [12.0-  
289 126.0] vs 83.0 s [26.0-238.0],  $Z = 2.08$ ,  $P = 0.03$ ; Winter: 22.0 s [12.0-88.0] vs 90.5 s

290 [20.0-147.0],  $Z = 1.57$ ,  $P = 0.01$ ). Latencies to reach a ewe were not affected by the  
291 season. They spent more time near their own mothers than alien ewes during the  
292 first minute (Wilcoxon tests = Spring: 26.0 s [0.0-42.0] vs 0.0 s [0.0-13.0],  $Z = 3.23$ ,  $P$   
293  $< 0.01$ ; Summer: 8.0 s [0.0-39.0] vs 0.0 s [0.0-8.0],  $Z = 3.39$ ,  $P < 0.001$ ; Autumn: 21.0  
294 s [0.0-40.0] vs 0.0 s [0.0-15.0],  $Z = 2.95$ ,  $P = 0.003$ ; Winter: 29.5 s [0.0-42.0] vs 0.0 s  
295 [0.0-14.0],  $Z = 2.24$ ,  $P = 0.02$ ) and for the whole duration of the test (Wilcoxon tests =  
296 Spring: 175.0 s [95.0-241.0] vs 50.0 s [9.0-113.0],  $Z = 4.25$ ,  $P < 0.0001$ ; Summer:  
297 151.0 s [10.0-238.0] vs 24.0 s [0.0-101.0],  $Z = 3.74$ ,  $P < 0.001$ ; Autumn: 145.0 s  
298 [85.0-217.0] vs 52.0 s [14.0-98.0],  $Z = 3.94$ ,  $P < 0.0001$ ; Winter: 159.5 s [116.0-  
299 210.0] vs 47.0 s [17.0-118.0],  $Z = 3.16$ ,  $P < 0.01$ ).

300 Overall, season of birth did not affect the capacity of lambs to discriminate between  
301 their own and alien mothers at 48 h of age. However, lambs born in winter emitted  
302 significantly less bleats (13.5) than lambs born in autumn (13.5 [9.0-25.0] vs 21.0  
303 [12.0-33.0], Mann-Whitney  $U = 711$ ;  $P = 0.03$ ), in summer (13.5 [9.0-25.0] vs 23.0  
304 [16.0-40.0], Mann-Whitney  $U = 833.5$ ;  $P = 0.01$ ) and in spring (13.5 [9.0-25.0] vs 26.0  
305 [14.0-41.0], Mann-Whitney  $U = 671.5$ ;  $P = 0.001$ ).

### 306 *Birth weight, rectal temperature and glycaemia*

307 Lambs born in summer were lighter than those born in autumn (Mann-Whitney  $U =$   
308 527.0;  $P = 0.04$ ) and spring (Mann-Whitney  $U = 1210$ ;  $P = 0.001$ ; Figure 3). Winter  
309 born lambs had an intermediate birth weight.

310

311 **Insert here Figure 3.**

312

313 One hour after birth, the median rectal temperature of D'man lamb was 38.7 °C  
314 [38.40 – 39.08]. It rose to 39.20 °C [39.00 – 39.60] at 48 h of age with a median

315 difference of + 0.6 °C. Season of birth did not affect the temperatures recorded  
316 around birth but it did 48 h later (Kruskal-Wallis = 19.74;  $P < 0.001$ ; Figure 4a and  
317 4b). It was higher in summer than in winter (Mann-Whitney U = 763.5;  $P = 0.001$ ) and  
318 in spring born lambs (Mann-Whitney U = 1064;  $P < 0.0001$ ). Autumn born lambs had  
319 an intermediate value. Season of birth had a significant effect on rectal temperature  
320 variation from 1 to 48 h of age (Kruskal-Wallis = 12.67;  $P = 0.01$ ; Figure 4c). Spring  
321 born lambs had a lower rectal temperature increase than winter (Mann-Whitney U =  
322 735;  $P = 0.01$ ), autumn (Mann-Whitney U = 1041;  $P = 0.01$ ) and summer born lambs  
323 (Mann-Whitney U = 1260;  $P = 0.001$ ).

324

325

**Insert here Figure 4.**

326

327 Median glycaemia was 2.72 mmol / L [2.24 – 3.12] within the hour following birth and  
328 reached 4.63 mmol / L [3.92 – 6.34] 48 h later with a rise of 1.67 mmol / L [1.17 –  
329 4.08]. Season of birth had a significant effect both at 1 h (Kruskal-Wallis = 11.09;  $P =$   
330 0.01; Figure 4a) and 48 h of age (Kruskal-Wallis = 54.63;  $P < 0.0001$ ; Figure 4b), and  
331 also on the increase between 1 h and 48 h of age (Kruskal-Wallis = 48.65;  $P <$   
332 0.0001; Figure 4c). At birth, summer born lambs had significantly lower glycaemia  
333 compared to spring born lambs (Mann-Whitney U = 293;  $P = 0.0001$ ). Winter and  
334 autumn born lambs had an intermediate value. At 48 h age, spring born lambs had  
335 the lowest concentrations compared to winter (Mann-Whitney U = 35;  $P < 0.0001$ ),  
336 autumn (Mann-Whitney U = 46;  $P < 0.0001$ ) and summer born lambs (Mann-Whitney  
337 U = 571;  $P < 0.0001$ ). In fact, glycaemia increased significantly less in spring born  
338 lambs than in winter (Mann-Whitney U = 25;  $P < 0.0001$ ), autumn (Mann-Whitney U =  
339 29.5;  $P < 0.0001$ ) and summer born lambs (Mann-Whitney U = 281.5;  $P < 0.0001$ ).

340

341 **Discussion**

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

365 that the yard was enclosed, possessed a roof, and the animals were put in individual  
366 pens could have protected them from adverse conditions.

367 D'man ewes started to groom their neonates sooner and for longer during the first 30  
368 min postpartum in winter than in spring and summer. Although smaller lambs lead to  
369 an easier birth (Grommers et al., 1985) and an easy birth is associated with better  
370 maternal care (Poindron et al., 1984), neither lambing process nor litter size and  
371 lambs weight are satisfactory explanations for the difference in the expression of  
372 grooming behavior. Maternal experience on the other hand, instead of season, is a  
373 more likely causal factor. The number of primiparous ewes was lower in winter than  
374 in spring and in summer and parity, and not just age, is known to influence the  
375 expression of maternal care (Alexander et al 1993). It appears quite logical that the  
376 grooming activity reflecting maternal motivation should be more pronounced in winter  
377 lambing ewes. Some authors report that maternal attention stimulates neonatal  
378 behavior progression (Alexander and William, 1964; McGlone and Stobart, 1986;  
379 O'Connor et al., 1992) while others suggest that maternal attention slows down  
380 neonatal behavior progression (Vince, 1993; Dwyer and Lawrence, 1999). Our  
381 results are more in favor of the second findings since winter born lambs were the  
382 least active, while it was in spring and summer that lambs were the quickest to stand  
383 up and reach the udder. According to Vince (1993) grooming the head and  
384 shoulders, which is where the mother usually starts from, has soothing properties.  
385 Some studies also report that the lamb's neonatal activity is impaired in winter  
386 (Scotland: Dwyer and Morgan, 2006; Brazil: Fonsêca et al., 2014) but this does not  
387 seem an unanimous fact (Wassmuth et al. 2001). Local conditions as well as breed  
388 differences may account for the differences reported between authors.

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

402 The fact that summer born lambs had a higher body temperature than winter born  
403 lambs at 48 h is quite logical as they were born during the warmest season, a  
404 difference that had already been found by Chniter et al. (2013). Our results on rectal  
405 temperature and glycaemia suggest that overall D'man neonates were not  
406 susceptible to oases climatic conditions and could maintain body temperature within  
407 normal range through two mechanisms. The first is mobilization of brown adipose  
408 tissue, a mechanism that helps newborn lambs adapt to cold immediately after being  
409 born (Dwyer and Morgan, 2006; Dwyer et al., 2015). The second is based on glucose  
410 plasma levels. Glucose is an important source of energy originating from colostrum  
411 and is required to maintain homeothermy beyond the neonatal period (Thompson et  
412 al., 2006; Miller et al., 2010). After birth, D'man lambs rapidly found the udder and

413 sucked successfully making glycaemia increased as a consequence which has also  
414 been reported by Chniter et al. (2016).

415 While in the present study, the overall rise in glycaemia and body temperature is a  
416 clear sign of the newborns' ability to adapt to their rearing conditions, it does not  
417 imply that D'man lambs are unsusceptible to hypothermia. Chniter et al. (2011; 2013)  
418 had shown that winter born lambs were more fragile and suffered a higher mortality  
419 rate. However, in the present work we only took into account the lambs that survived  
420 until the time of the preference tests, excluding the weakest subjects more prone to  
421 suffer from hypothermia or postnatal trauma. Under such experimental conditions,  
422 confirming differences in neonatal physiology like in the studies by Chniter et al.  
423 (2011; 2013) was a more arduous task. Spring born lambs appeared anyhow to be  
424 the most vigorous at birth; they were also amongst the heaviest. The most important  
425 birth weight and vigor in spring born D'man lambs could reflect optimal climatic  
426 conditions during gestation with enhancing fetal growth in late pregnancy as  
427 suggested by Chniter et al. (2011). Their lesser glycaemic variability and their lower  
428 postnatal rise in body temperature suggest easier adaptation to the moderate spring  
429 weather conditions. Spring born lambs did not have to metabolize as much energy as  
430 autumn and winter born lambs, and could thermoregulate better than summer born  
431 lambs.

432 While our data indicate that lambing season affects some aspects of maternal and  
433 neonatal behavior in D'man sheep as well as neonatal physiology, we cannot  
434 conclude that one season has more impact than another does. Spring born lambs  
435 appeared the most vigorous at birth but mothers showed more intense maternal care  
436 in winter. Unsurprisingly, born lambs had a higher body temperature in summer but it  
437 was in spring that physiological factors were the least variable suggesting better

438 neonatal adaptation to mild climatic conditions. Some behavioral aspects involved in  
439 the identification of mother or young were suboptimal in winter but overall the ewe-  
440 lamb bond was clearly established in all four seasons by 48 h postpartum. Should  
441 lamb survival be at more at risk in winter as reported by Chniter et al. (2011), there is  
442 no reason to think that early mother-young interactions and bonding can be hold  
443 responsible for it in the conditions of the study.

444

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451 INRA-PRC Val de Loire-France” and gave birth to the present manuscript. The first  
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453

#### 454 **Declaration of interest**

455 The authors declare that they have no conflicts of interest.

#### 456 **Ethical statement**

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

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463 **Software and data repository resources**

464 None of the data were deposited in an official repository.

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- 558

559 **Table 1** Mean monthly temperature values and rainfall during lambing seasons of D'man  
560 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    |

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

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| Season | Parturition period |       | Maternal experience |             | Litter size |      |           |
|--------|--------------------|-------|---------------------|-------------|-------------|------|-----------|
|        | 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        |

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

574 sheep

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**Ewes**

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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.

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**Lambs**

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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 4 feet for at least 5 s.

Attempts to suckle(s) Lamb with head under the ewe's udder region.

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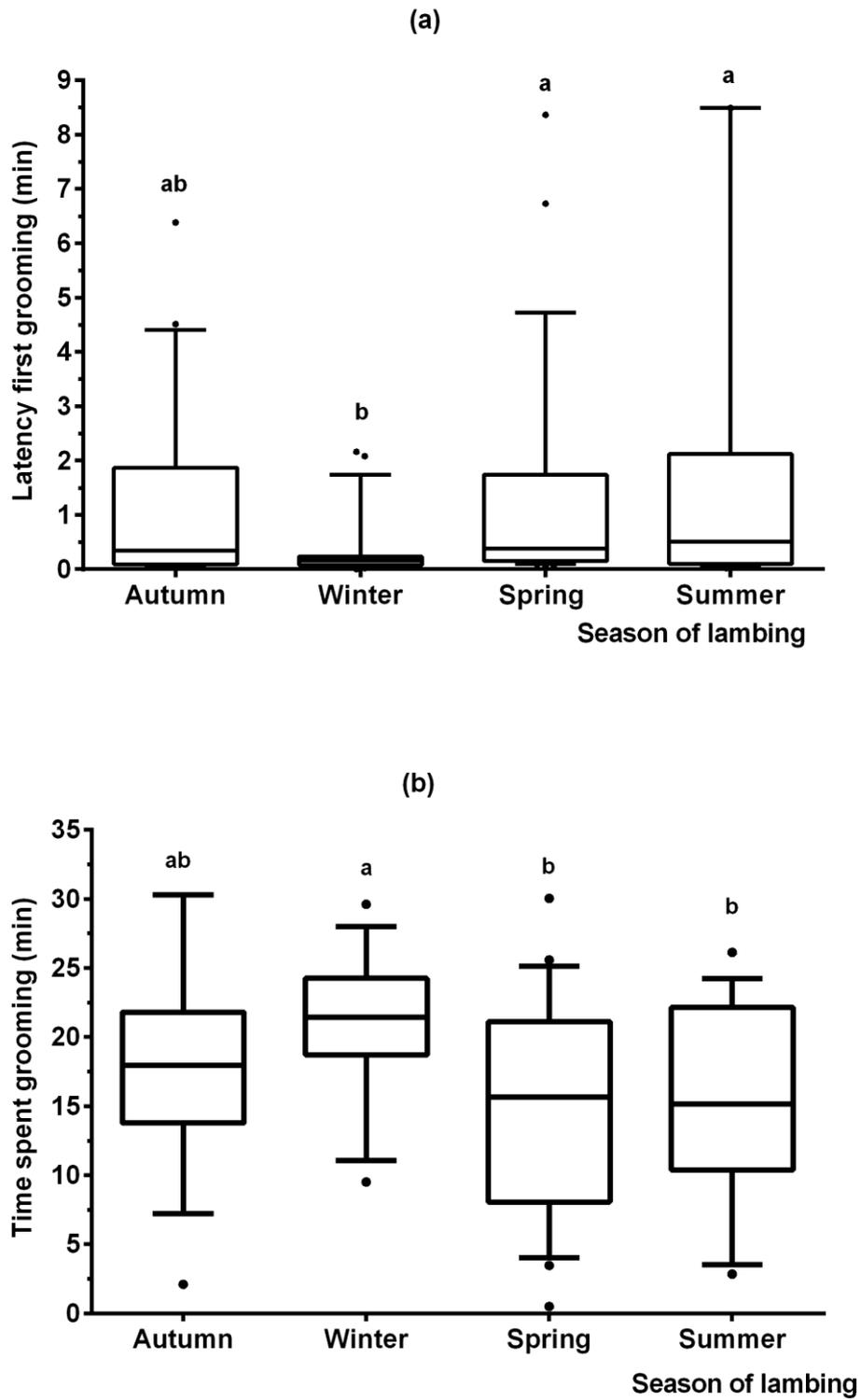
578 **Figure 1** Postnatal grooming activity of D'man ewes according to lambing season: (a) time  
579 interval between full expulsion of each lamb and first grooming given each of them;(b) and total  
580 duration of grooming during the first 30 min after giving birth to the first lamb. Values are 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

583 **Figure 2** D'man lambs'neonatal behavior according to season of birth: (a) latency to get up on  
584 forelegs, (b) latency to stand up, (c) latency to reach the udder. Values are medians (bar within  
585 the box) and upper and lower quartiles (boarders of box) with 10 and 90 percentile. a,b :  $P <$   
586 0.05

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

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

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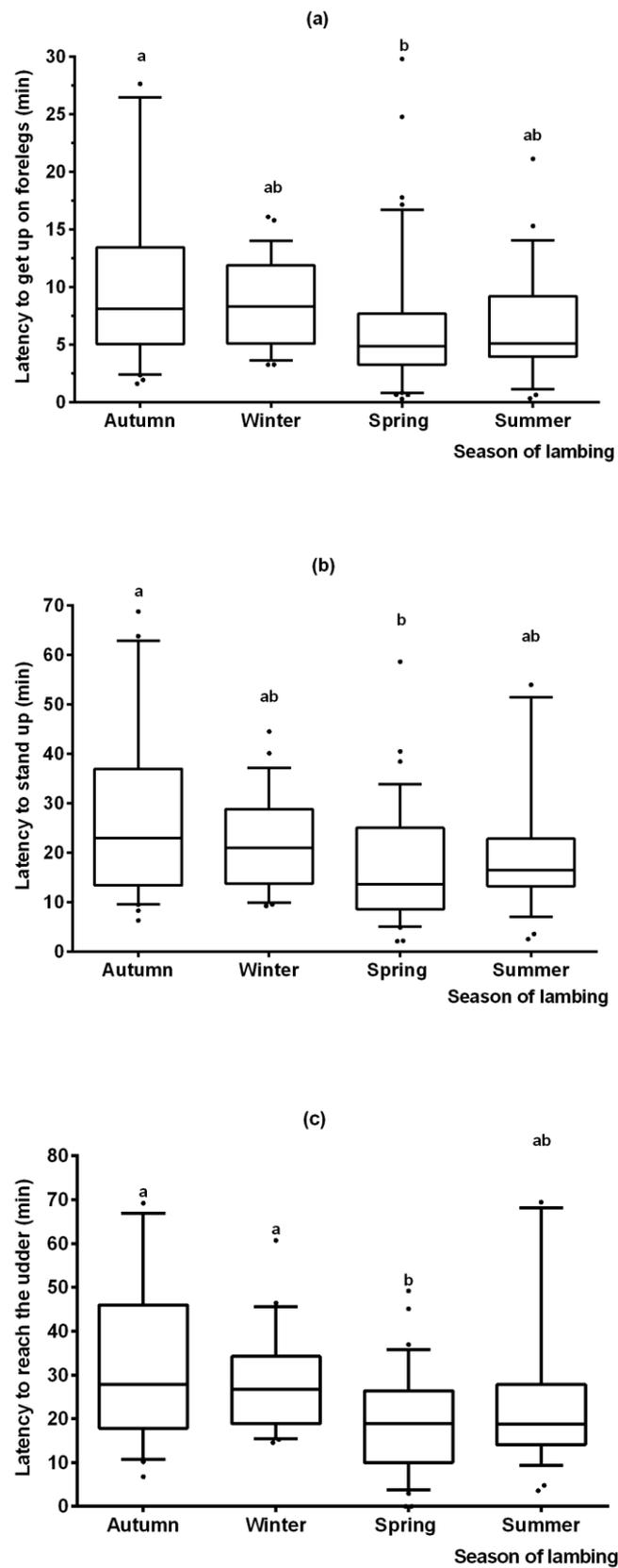


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Figure 1

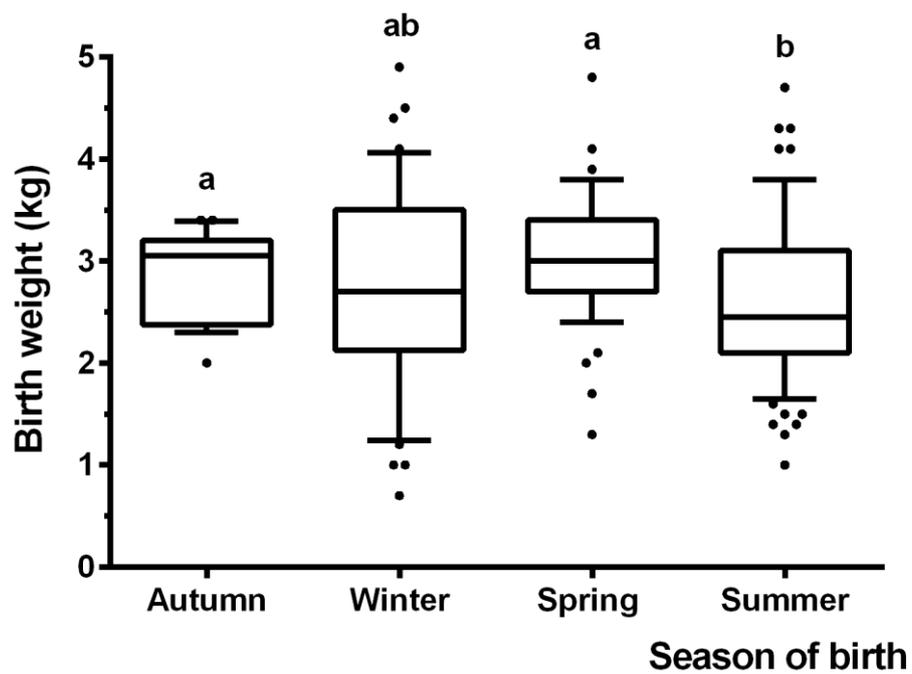
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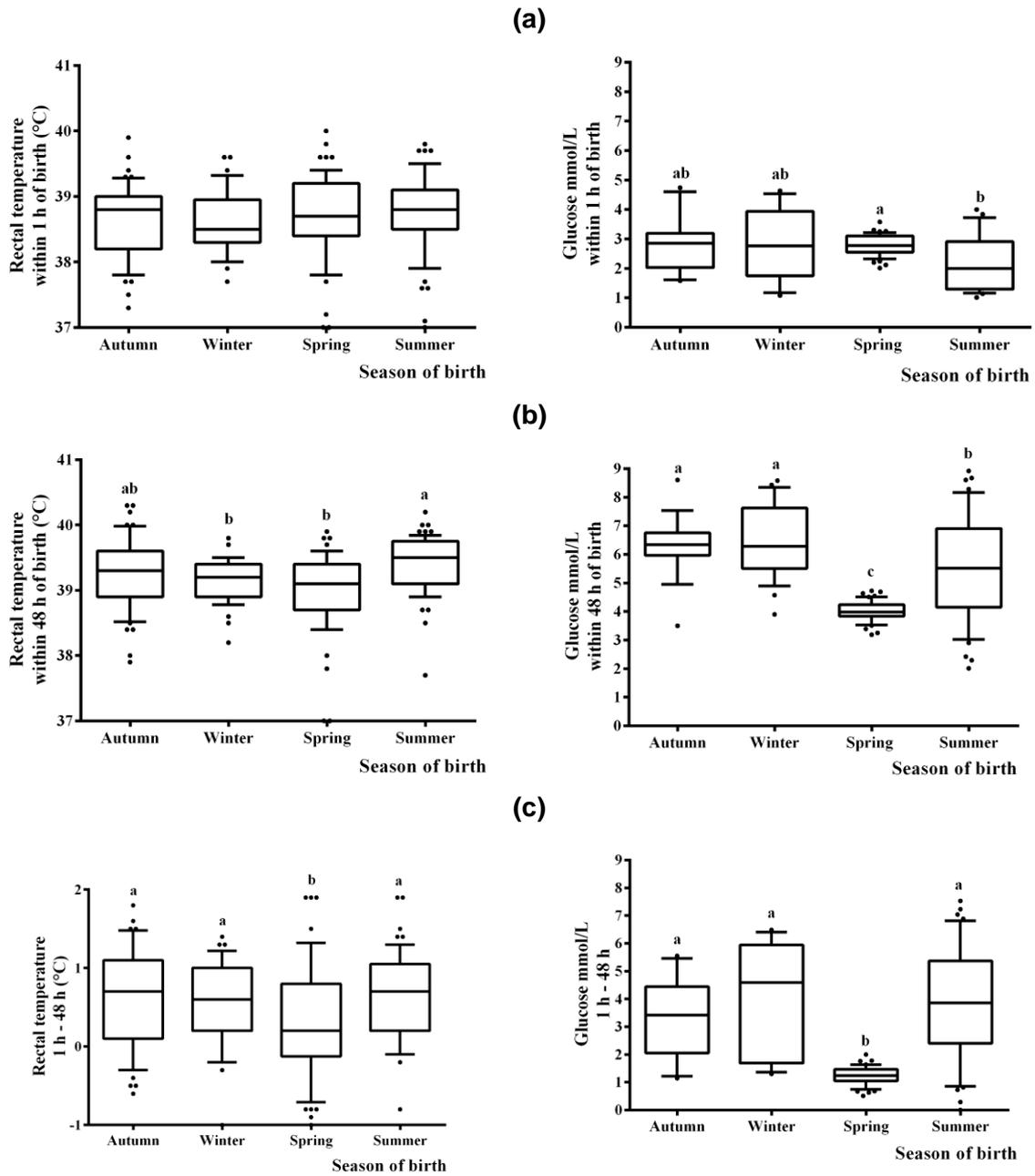
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Figure 2



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602 **Figure 3**

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**Figure 4**