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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 m²/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 m² 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 1st 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

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 4 feet for at least 5 s.

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

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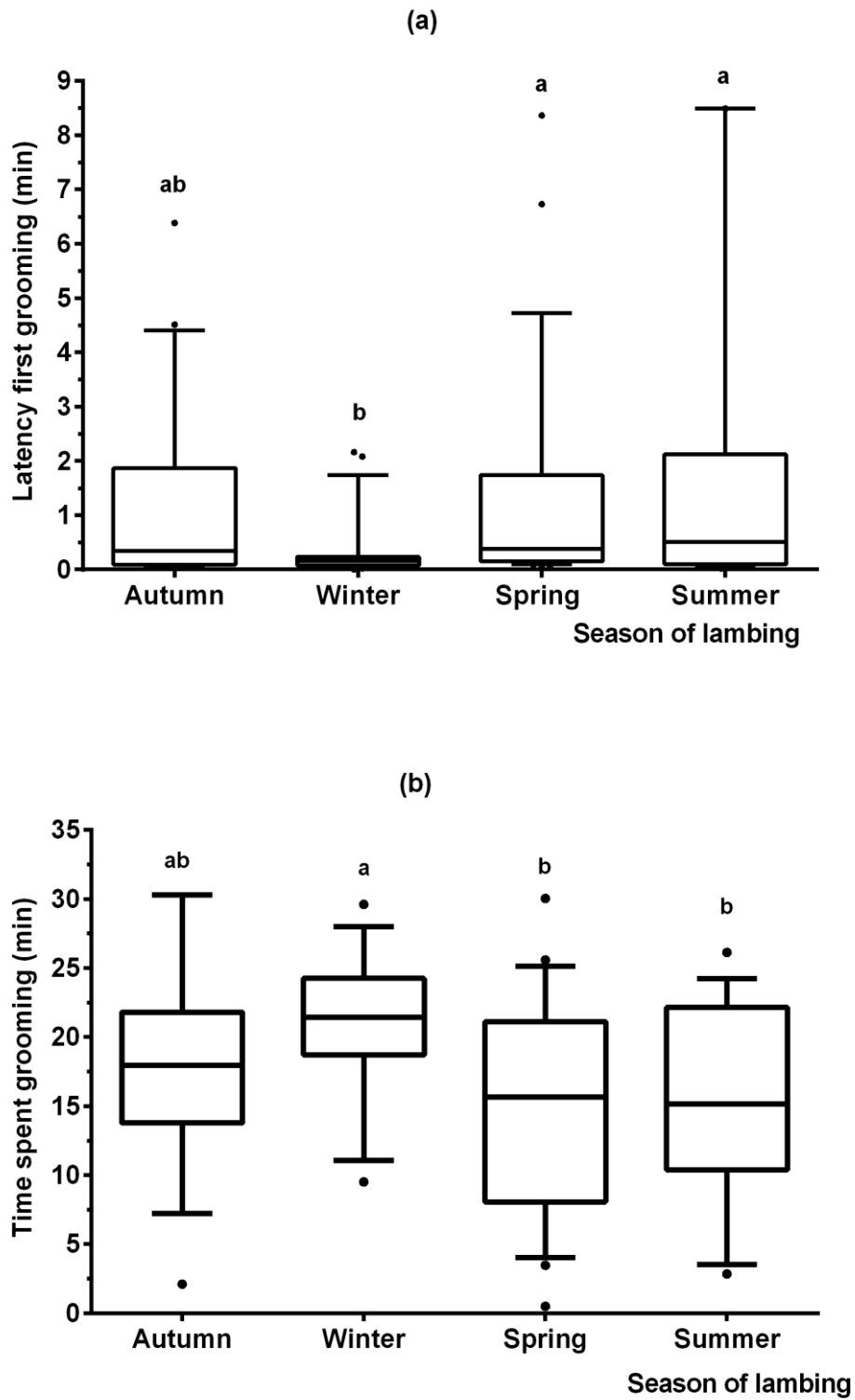
578 **Figure 1** Postnatal grooming activity of D'man ewes according to lambing season: (a) time
579 interval between full expulsion of each lamb and first grooming given each of them;(b) and total
580 duration of grooming during the first 30 min after giving birth to the first lamb. Values are
581 medians (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 within1 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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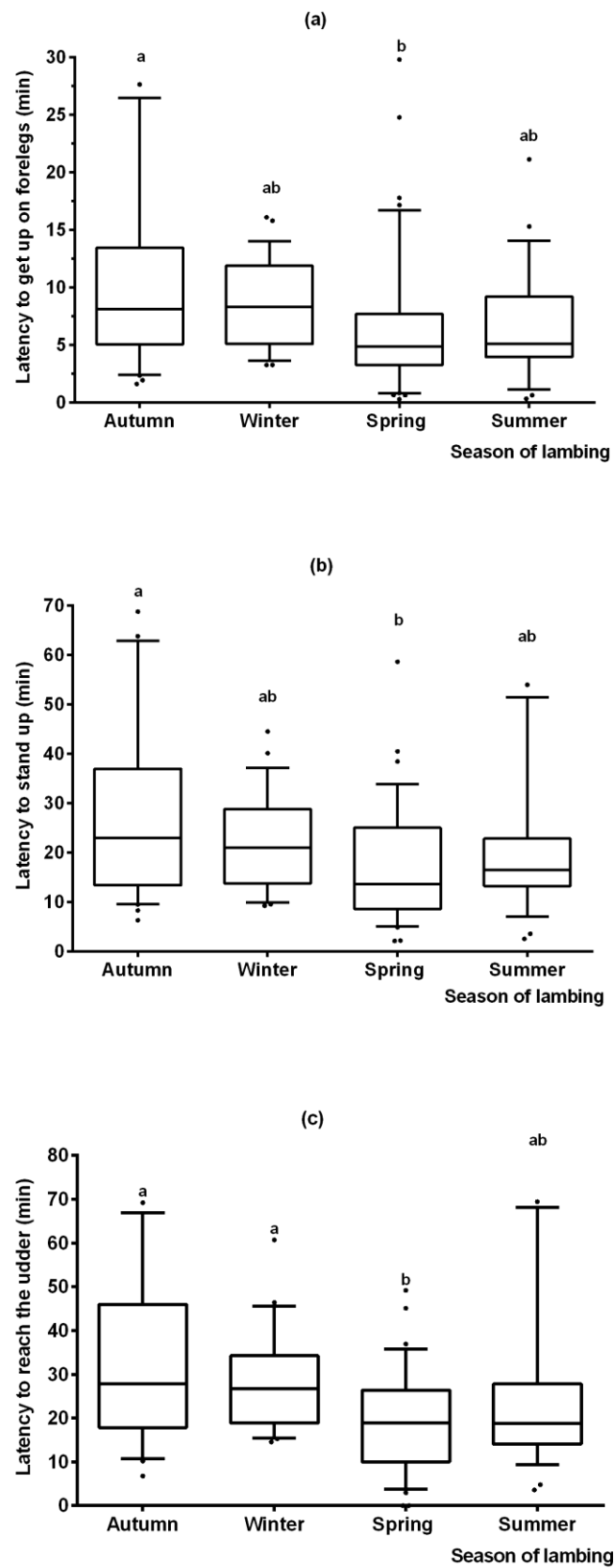


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Figure 1

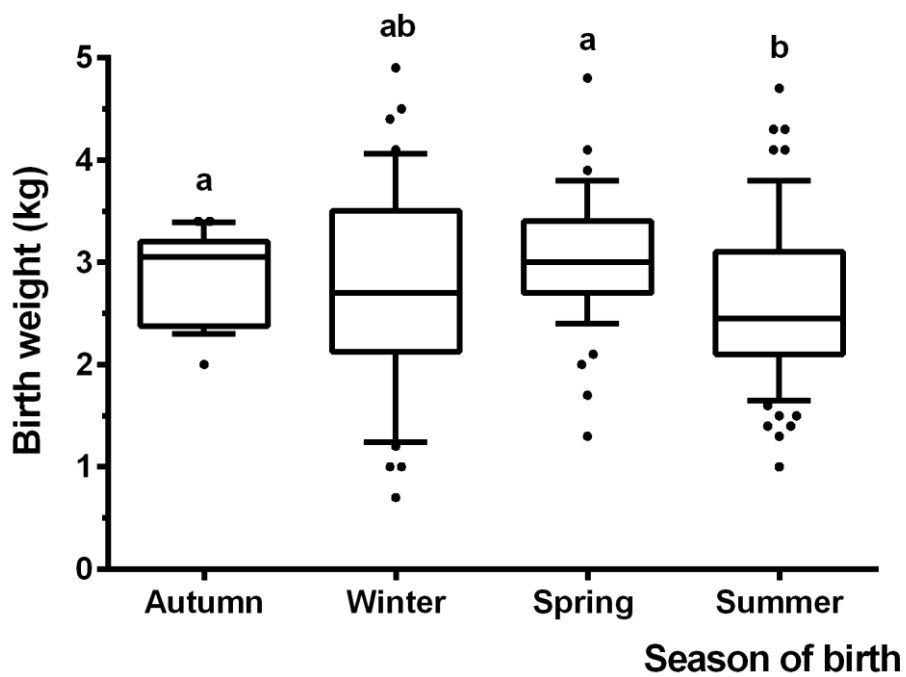
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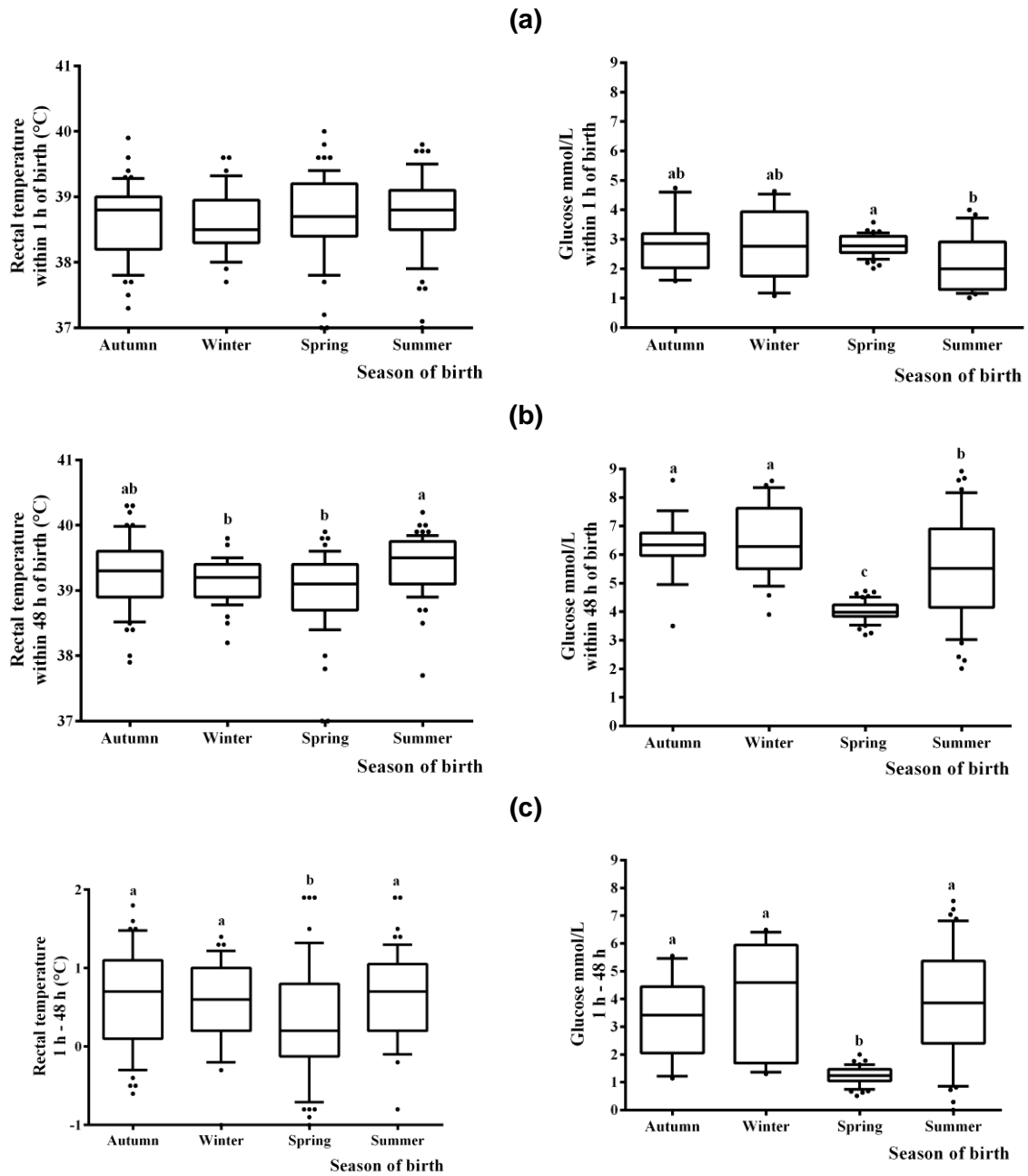
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Figure 2



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602 **Figure 3**

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Figure 4