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2 **Pen size and parity effects on maternal behaviour of Small Tail Han sheep**

3
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15
16 Short title: Pen size and parity effects on ewe maternal behaviour

17
18 **Abstract**

19 The aim of the experiment was to study the effects of pen size and parity on maternal
20 behaviour of twin-bearing Small Tail Han ewes. Twenty-four ewes were allocated to a
21 2x2 design (6 per pen) with parity (primiparous or multiparous) and pen size (large:
22 6.0m×3.0m; small: 6.0m×1.5m) as main effects at Linyi University, Shandong
23 Province, China. Behaviour was observed from after parturition until weaning. All
24 ewes were observed for 6 h every 5 days from 07:00 to 10:00 h and 14:00 to 17:00 h.
25 Continuous focal animal sampling was used to quantify the duration of maternal
26 behaviours: sucking, grooming, and following; and the frequency of udder accepting,

27 udder refusing and low-pitched bleating. Oestradiol and cortisol concentration in
28 faeces (collected in the morning every 5 days) were detected with EIA kits. All lambs
29 were weighed 24 hours after parturition and again at weaning at 35 days of age. The
30 small pen size significantly reduced following ($P<0.005$), grooming ($P<0.001$) and
31 suckling durations ($P<0.05$), and the frequency of udder refusals ($P<0.001$). However,
32 there was a significant interaction with ewe parity, with the decreased grooming and
33 suckling in the small pen largely seen in the multiparous ewes ($P<0.001$).
34 Independent of pen size multiparous ewes accepted more sucking attempts by their
35 lambs ($P<0.05$), and made more low-pitched bleats, than primiparous ewes
36 ($P<0.001$). Multiparous ewes had higher faecal oestradiol concentration than
37 primiparous ewes ($P<0.001$), and ewes in small pens had higher faecal cortisol than
38 those in larger pens ($P<0.001$). As lambs increased in age, the duration of maternal
39 grooming, following, and suckling and frequency of udder acceptance and low-pitched
40 bleating all declined, and the frequency of udder refusing increased ($P<0.001$ for all).
41 Ewe parity, but not pen size, affected lamb weight gain during the observation period
42 ($P<0.001$). This is the first study to show that pen size, interacting with parity, can
43 affect the expression of maternal behaviour in sheep during lactation. The study is
44 also the first to report on the maternal behaviour of Chinese native sheep breeds
45 (Small Tail Han sheep), with implications for the production of sheep in China.

46

47 **Key words:** Pen size, Parity, Small Tail Han sheep, Maternal behaviour, Cortisol

48

49 **Implications**

50 In many countries there is increasing pressure on land use, such that grazing animals,
51 which would normally be managed extensively, may start to be housed more
52 frequently. However, for sheep, there is little information on appropriate housing and

53 the impact that this may have on stress, growth and behaviour. This study
54 demonstrated that small pen sizes reduced ewe-lamb suckling behaviour and
55 increased udder refusals compared to larger pens, although grooming behaviour was
56 also increased. This was accompanied by increased ewe faecal cortisol and suggests
57 that suckling ewes may be stressed by housing in close confinement, which
58 influences their maternal behaviour.

59

60 **Introduction**

61 China is the largest sheep producing nation in the world (183 million head in 2012:
62 www.faostat.org) yet comparatively little is known about the management and
63 behaviour of these sheep. Many indigenous sheep breeds exist, of both fat-tailed and
64 thin-tailed breeds, including the Small Tail Han sheep originating from Mongolia. This
65 breed is highly prolific, producing 2.61 to 2.65 lambs per litter (Chang et al., 1998)
66 with non-seasonal oestrus. Mature ewes can have 3 parturitions every two years, with
67 some animals producing 2 litters every year. Anecdotally, the level of lamb mortality
68 ranges from 5% to 20% in different Small Tail Han sheep enterprises in China, which
69 is comparable to studies in other breeds, but not acceptable from both welfare and
70 production perspectives. The behaviour of the ewe and lamb are known to be
71 important for the survival of the offspring (e.g. reviewed by Dwyer, 2014). However, to
72 our knowledge there are no published studies describing the maternal behaviour of
73 Chinese indigenous sheep breeds.

74

75 In China, economic developments are placing greater pressure on land use, and
76 pasture availability for sheep production is declining with increased use for other
77 agricultural and non-agricultural practices (e.g. Bosing et al., 2014). As a solution to

78 this sheep production is gradually shifting from extensive to intensive management,
79 with increased housing of ewes during pregnancy and lactation, and housing at
80 increasing stocking density. However, there is still little information available about the
81 impact of sheep housing and stocking density on sheep production and welfare,
82 although recent studies suggest that high stocking density results in behavioural
83 disturbances, displacements and altered social interactions (Averòs et al., 2014a, b).
84 It is, therefore, relevant to consider how this might also affect the behaviour of
85 Chinese sheep breeds.

86

87 High stocking densities affect sheep behaviour, physiology, and welfare (Sevi et al.,
88 1999; Caroprese et al., 2009; Averòs et al., 2014a, b) and may also affect
89 mother-young interactions (Averòs et al., 2014; Dwyer, 2014). Crowded conditions
90 may exacerbate lamb desertion or separation behaviour in paddocks (Winfield, 1970),
91 and are likely to be even greater in indoor housing conditions. Ewes giving birth in
92 crowded conditions are often unable to isolate themselves from the flock and may be
93 interfered with by other ewes, or their lambs may become separated and be
94 abandoned (Alexander *et al.*, 1983). Gonyou and Stookey (1985) concluded that the
95 use of cubicles reduced the incidence of poor maternal behaviour in housed ewes by
96 giving them an opportunity to isolate themselves from other ewes.

97

98 The studies mentioned above have focused on the impact of high stocking density on
99 the onset of maternal behaviour. However, the effect of pen size on established
100 maternal behaviour and physiology, when animals are confined in new environments,
101 has not yet been investigated. The neuroendocrine mechanisms of maternal care in
102 ewes, have received considerable research attention in recent years. In particular,

103 oestradiol plays an important role in the onset of maternal behaviour, and cortisol may
104 also influence the expression of maternal behaviour (Dwyer *et al.*, 2004; Meurisse *et*
105 *al.*, 2005; Bøe *et al.*, 2006; Dwyer, 2014). In general, primiparous ewes are more likely
106 to show inadequate maternal behaviour towards their neonates than experienced
107 ewes (Meurisse *et al.*, 2005; Dwyer and Smith, 2008). This may be because hormonal
108 induction of maternal behaviour in primiparous females is less efficient (Le Neindre *et*
109 *al.*, 1979). Parity effects on maternal behaviour are not related to circulating oestradiol
110 (Dwyer and Smith, 2008), but the responsiveness to oestradiol induction of maternal
111 behaviour increases with parity (Poindron *et al.*, 1984), which seems to be related to
112 the impact of maternal experience on central oestradiol receptor- α expression
113 (Meurisse *et al.*, 2005). However, most of these studies have focused on hormonal
114 patterns leading to the onset of maternal behaviour, and much less is known about
115 the role of hormonal changes in the maintenance of maternal behaviour.

116

117 In many studies, the concentration of cortisol, as an indicator of the activation of the
118 hypothalamic pituitary adrenal axis in response to stress, is measured in blood as an
119 indicator of animal physiological status (Palme and Möstl; 1997), but may only capture
120 acute responses. Non-invasive techniques, such as faecal sampling, can offer an
121 effective method to reduce the stress of sampling, and allow data to be integrated
122 over a longer period of time (Möstl and Palme; 2002). This study used faecal sampling
123 as a method to assess the impact of housing density on the physiological responses
124 of the ewes.

125

126 The aim of this project was a) to provide new information on the maternal behaviour of
127 the highly prolific Small Tail Han sheep from parturition to weaning, considering the

128 impact of maternal parity, and b) to investigate whether pen size affected the
129 expression of established maternal behaviour to provide guidance to farmers on
130 husbandry conditions for these sheep. We hypothesise that restrictive housing
131 conditions will increase ewe stress, reduce the expression of maternal behaviour, and
132 affect faecal concentrations of stress and reproductive hormones. Finally, we predict
133 that differences in maternal behaviour will affect the growth of the lamb.

134

135 **Materials and Methods**

136 *Animals, housing and feeding*

137 This study was carried out at Zhong-He farm, Lan-Shan District, Linyi city, Shandong
138 province, China. At the farm, 212 ewes are housed in two big stalls (40.0m×15.0m,
139 about 6.0m² per animal). Before the experiment, eighty ewes, which were in good
140 health and condition, were chosen and synchronised in oestrus using progesterone
141 vaginal sponges (Huayu Ltd, Shanghai, China), and were artificially inseminated with
142 semen from 6 rams over two days. Twenty-four pregnant twin-bearing ewes (12
143 primiparous, 12 multiparous) were selected from the two stalls on the basis of
144 trans-abdominal ultrasound examination. At approximately mid-gestation (75 days),
145 all selected ewes were given ad libitum access to hay. Two hundred g/ewe/day of
146 locally milled ewe nuts (about 200 crude protein per kg dry mass, Huhui Ltd, Linyi,
147 Shandong Province) were provided. From d100 concentrates were fed at a ration of
148 320 g/ewe/day. Rations were doubled every 15 days until d130 of gestation and then
149 maintained at this level until parturition. At d145 of gestation, ewes were allocated to
150 treatment at random (n=6 per treatment) in a 2×2 design with parity (primiparous (P)
151 or multiparous (M)) and pen size (large (L): 6.0m×3.0m (3.0m² per ewe); small (S):
152 6.0m×1.5m (1.5 m² per ewe)) as the factors. Feeders were attached to the outside of

153 the pen walls and one feeder position per ewe was available in all pens. The individual
154 pen walls were made of 1.5 m high solid material with cement to avoid visual and
155 direct physical interactions between groups. The primiparous ewes were aged 1 or 2
156 years old and the multiparous ewes were 3 years old before pregnancy. The parity of
157 multiparous ewes was 3 or 4.

158

159 During the parturition period, the pens were checked every morning by the
160 stockperson to find neonates born during the previous 12 h. Each newborn was
161 inspected and weighed. If parturition was observed during the day, to avoid
162 interrupting the process of mother-neonate bonding, neonates were not checked
163 immediately. However, assistance at lambing was provided if required and 3
164 primiparous ewes were assisted for dystocia.

165

166 All ewes and neonates were marked by plastic ear-tags and with different colour
167 numbers using stockmarker (an odourless product designed for animal use) for easy
168 identification. The pens were cleaned before morning feeding and the ewes and
169 lambs condition were checked gently by the stockperson. In addition to natural light,
170 artificial lighting was kept on about 8.0 h every day. Forty-four of the 48 twin lambs
171 born to the ewes survived throughout the experimental period. One of the multiparous
172 ewes in the large pen lost one of her lambs. Two primiparous ewes in the large pen
173 and one primiparous ewes in the small pen each lost one lamb. The lambs were
174 weighed again at the age of 35 days (weaning). Of the surviving lambs 23 were male
175 and 21 female, and the distribution of lamb sexes were approximately balanced
176 across treatment groups (PL: 5 male, 5 female; PS: 6 males, 5 females; ML: 5 males,
177 6 females; MS: 7 males, 5 females).

178

179 *Behavioural data collection*

180 Before the parturition period, ewes were habituated to the workers presence by an
181 additional 3-day period before the formal experiment started. In each pen, a 4 channel
182 video camera monitoring system (Huiya Ltd, Shenzhen, China) was installed on the
183 wall. The data were collected for 35 days with continuous focal observations of each
184 pen made on the day of birth and every 5 days thereafter (8 observations in total) until
185 weaning at d35. On each observation day animals were observed for 6 hours: 3 hours
186 in the morning (from 07:00 to 10:00 h) and 3 in the afternoon (from 14:00 to 17:00 h),
187 resulting in 48 hours of observation per animal. Table 1 gives the definition of ewe and
188 lamb behaviours observed as either events or states according to an ethogram
189 devised from existing work in this area (Dwyer and Lawrence, 1998; Hild et al., 2011).
190 Behaviours directed towards one or both lambs were recorded, and lamb behaviours
191 were summed as litter responses. The durations of sucking, grooming and following of
192 individual ewes and their lambs were recorded as described by Pickup and Dwyer
193 (2011), and considered as the duration occurring in 6 hours. The behaviours of
194 udder-accepting, udder-refusing and low-pitched bleating of every ewe were recorded
195 as events, each hour of observation was divided into 6 x 10 minute bins to facilitate
196 data analysis and the data were considered as the frequencies expressed within 10
197 minute periods. Prior to observation, the three observers who collected all the
198 behavioural data were trained for 10 days with pre-existing video records to reduce
199 inter-observer variability.

200

201 *Faecal collection and analysis*

202 In order to reduce the disturbance of ewes and lambs, a fresh faecal sample of every
203 ewe was collected gently by the stockperson every 5 days in the morning before

204 feeding. About one gram of faecal pellets then were placed into micro-centrifuge
205 tubes and frozen at -80°C until extraction (Pulang Ltd, Shanghai, China)
206 method was used in the experiment to extract the cortisol and oestradiol as described
207 by Palme and Möstl (1997). In brief, 0.5 g faeces were suspended in 4.0 ml methanol
208 and 1.0 ml double distilled water (=80 % methanol) and shaken for 30 minutes at 2000
209 g. After centrifugation, all the samples were sealed and kept at -20°C until extraction.
210 As Palme and Möstl (1997) method, 50.0 μl samples were gathered and detected with
211 the EIA kit as instructed by the manufacturers (Pulang Ltd, Beijing, China). The
212 oestradiol EIA had a sensitivity of 20.0pmol/L, an intra coefficient of variation was 0.12
213 and inter-assay was 0.14. The cortisol EIA had a sensitivity of 10.0 $\mu\text{g/L}$ an intra and
214 inter-assay coefficient variation was 0.13 and 0.16, respectively. All the feces samples
215 were assayed for oestradiol concentration in a single assay, and similarly for cortisol
216 concentration.

217

218 *Data Analysis*

219 For each observation day the total duration of each behaviour for each ewe was
220 calculated for the 6 hour observation period. For behavioural frequencies the mean
221 frequency per 10 minutes was calculated for each observation day. A repeated
222 measures mixed model of variance (REML) was used to analyze the maternal
223 behaviours and hormone concentrations with the following fixed factors in the model:
224 parity (multiparous vs primiparous), pen size (large vs small) and the interaction
225 between parity and pen size. The ewe identity was set as random effect, ewe and
226 lamb age were used as residual term with covariance structure in the model. Before
227 the model was run, all the maternal behaviours were checked to fit a normal
228 distribution. This test generates a Wald statistic (which approximates to a χ^2 statistic),

229 instead of an F statistic, this value is given in the text. A mixed model of variance was
230 used to analyse the effect of the pen size and parity on lamb weight. All the data are
231 shown as a mean, significance was set at $P=0.05$ and all analyses were performed in
232 GenStat (8th Edition) software. It should be noted, however, that only one pen per
233 condition (parity x size) was used and therefore ewe was treated as the experimental
234 unit in the study. Although maternal behaviour is often considered at the ewe level,
235 there may have been pen level factors that could have affected the data, but which
236 could not be disentangled by this design, which is a limitation of this study.

237

238 All experimental procedures were performed according to authorization granted by
239 the Chinese Ministry of Agriculture. All procedures involving animals were approved
240 by the animal care and use committee at the institution where the experiment was
241 conducted.

242

243 **Results**

244 *Maternal Behaviour*

245 A restricted pen size reduced the amount of following behaviour between ewe and
246 lamb (Table 2, $P<0.005$), reduced ewe grooming ($P<0.001$) and suckling duration
247 ($P<0.05$) and increased udder refusals by the ewe ($P<0.001$). Multiparous ewes also
248 spent more time grooming their lambs (Table 2, $P<0.001$), had a higher frequency of
249 low-pitched bleating ($P<0.001$) and udder acceptance ($P<0.05$), and a lower
250 frequency of udder refusals than primiparous ewes ($P<0.001$). There was, however, a
251 significant interaction between ewe parity and pen size for some maternal behaviours.
252 Multiparous ewes suckled their lambs for longer at a higher stocking density than at a
253 low stocking density but these effects were not seen in primiparous ewes (mean

254 suckling duration (mins): PL=22.29, PS=18.70; ML=14.82, MS=25.17, s.e.d=1.92,
255 W=26.38, d.f.=1, $P<0.001$). Multiparous ewes also spent more time grooming their
256 lambs in the large pen compared to the small pen, although this effect of pen size was
257 also not seen in the primiparous ewes (mean duration of grooming (min): PL=7.22,
258 PS=7.18; ML=13.04, MS=7.04, s.e.d=0.93, Wald=20.71, d.f.=1, $P<0.001$).

259

260 The duration of suckling, grooming and following behaviour declined with increasing
261 lamb age (Table 3, $P<0.001$ for all behaviours), as did the frequency of low pitched
262 bleating ($P<0.001$) and udder acceptance ($P<0.001$). In contrast the frequency of
263 udder refusals increased with lamb age (Table 3, $P<0.001$).

264

265 *Faecal hormone levels*

266 Pen size had a significant effect on cortisol concentration, with higher values for ewes
267 kept in the smaller pens (Figure 1, Wald statistic=32.75, d.f.=1, $P<0.001$). Cortisol
268 concentrations varied significantly with time (Wald =29.01, d.f.=7, $P<0.001$) but no
269 overall or consistent pattern of change was detected. There were no significant effects
270 of parity or interactions between parity and pen size in fecal cortisol concentrations.

271

272 Multiparous ewes had significantly higher faecal oestradiol concentrations than
273 primiparous ewes (Figure 2, Wald =28.37, d.f.=1, $P<0.001$), and ewes in larger pens
274 also had significantly higher oestradiol (Wald =4.56, d.f.=1, $P<0.05$). As with cortisol
275 there were significant effects of time on faecal oestradiol (Wald =31.55, d.f.=7,
276 $P<0.001$) but no consistent pattern of change was seen. There were no significant
277 interactions between parity and pen size on oestradiol concentration (Wald=2.04,
278 d.f.=1, $P=0.169$).

279

280 *Lamb weight*

281 The weight of lambs of multiparous ewes tended to be heavier than those of
282 primiparous ewes at birth (Table 2, Wald=3.05, d.f.=1, $P=0.096$). By weaning at 35
283 days lamb weight was significantly greater in lambs of multiparous ewes compared to
284 primiparous (Wald=11.12, d.f.=1, $P<0.005$). There were no significant effects of pen
285 size or the interactions between pen size and parity for lamb weaning weight.

286

287 **Discussion**

288 The result of this study indicated that both parity and pen size had effects on Small
289 Tail Han sheep maternal behaviour during lactation. To our knowledge this is the first
290 paper to describe maternal behaviour in Chinese native sheep breeds. The most
291 significant finding of this study was that housing in pens providing 1.5 m² per ewe
292 resulted in changed maternal behaviour (specifically: reduced following, grooming and
293 suckling associated with increased sucking refusals) in comparison to ewes housed at
294 3 m² per ewe, and multiparous ewes appeared to be more influenced by the pen size
295 than primiparous ewes. This was accompanied by increased faecal glucocorticoid
296 metabolites and reduced faecal oestradiol in the small pens.

297

298 Few studies have considered the space requirements for housed sheep (other than
299 during transport or at lairage) and very few studies address the impact of housing on
300 the expression of maternal behaviour during lactation. Studies considering what might
301 be an appropriate pen size for ewes have almost invariably considered the space
302 required by the ewe alone (either whilst dry, during pregnancy or lactation only in dairy
303 ewes). Chiumenti (1987) suggests 0.9-1.2 m² /head on straw litter and 0.8–1.0 m²

304 /head on slatted floor are fit for sheep. However, studies of sheep behaviour suggest
305 that housing at 1-1.5 m² (as in the small pens in the present study) results in higher
306 social interactions and reduced activity compared to lower stocking density
307 (Caroprese *et al.*, 2009; Averòs *et al.*, 2014). Conversely, very low space availability
308 increases activity as animals are prevented from lying down when they wish (Bøe *et*
309 *al.*, 2006). Sevi *et al.* (2009), for lactating dairy ewes, suggests assigning a 2.0 m²
310 area per sheep to avoid these behavioural impacts. In the present study, although
311 activity per se was not measured, the increased following time in the larger pens
312 suggests that animals may have been more active than in the smaller pens. In a study
313 of mother-offspring recognition Val-Laillet and Nowak (2006) suggest that lambs kept
314 in small pens take longer to learn to recognize their mothers than lambs in larger
315 pens. This may also have led to the observed increase in following time with more
316 space, if the lambs were better able to recognize and follow their own mother than in
317 small pens, and the reduced sucking refusals (as lambs may be less likely to attempt
318 to suck from a ewe that was not their mother). The increase in grooming behaviour at
319 a lower stocking density may also be associated with improved recognition between
320 ewe and lamb, and a closer bond developing when animals have more space to
321 express maternal behaviour.

322

323 In this study there was a significant interaction between ewe parity and pen size for
324 grooming and suckling behaviours. Multiparous ewes expressed a higher amount of
325 grooming behaviour in the larger pen, whereas primiparous ewes were not affected by
326 pen size. Multiparous ewes are known to express a higher quantity and quality of
327 maternal care compared to primiparous ewes (reviewed by Dwyer, 2014). It may be
328 that the smaller pen impaired expression of maternal care in both inexperienced and

329 experienced ewes, but only multiparous ewes were able to show increased maternal
330 care when the environmental conditions permitted this to occur. The higher suckling
331 responses of multiparous ewes in the small pen compared to primiparous ewes might
332 be a greater responsiveness of these experienced ewes to the behaviour of their
333 lambs. However, as this study focussed mainly on ewe behaviour it is not known if
334 lamb behaviour was also altered by the housing conditions.

335

336 In this study, pen size significantly affected the concentration of both oestradiol and
337 cortisol in ewe faeces during lactation, with ewes in the smaller pen having higher
338 cortisol and lower oestradiol than ewes in larger pens. Oestradiol concentration plays
339 an important role in inducing expression of maternal behaviour at parturition
340 (Meurisse *et al.*, 2005; Dwyer, 2014), although its role in the maintenance of maternal
341 behaviour is not well described. Whether the higher oestradiol in ewes housed at
342 greater space allowance can be related to their apparently greater expression of
343 maternal behaviour requires further investigation.

344

345 Cortisol is widely measured as an indicator of physiological and psychological stress,
346 and the higher values recorded here in ewes maintained in small pens may indicate
347 greater stress in these animals. In our study, we seldom saw that ewes in the small
348 pens expressed abnormal or stereotypic behaviour, the main effects of low space
349 allowance were reduced time spent grooming and greater udder-refusing frequency
350 (which could be considered as poorer maternal care) than ewes housed in a large pen
351 during lactation. Few studies in sheep have considered the impact of maternal stress
352 on maternal behaviour, and those that have report conflicting results. In studies
353 examining the effects of maternal stress before birth on the onset of maternal

354 behaviour, Hild et al. (2011) found a positive correlation between maternal experience
355 of stress during pregnancy and maternal grooming in sheep. Dwyer *et al.* (2004)
356 reports no relationship between circulating cortisol before birth and maternal
357 behaviour (in a naturally occurring model of differences in circulating cortisol which
358 may not be related to stress) but a negative correlation between postnatal circulating
359 cortisol and maternal behaviour expression in sheep. In rodents, maternal stress
360 induced by unfamiliar male odours produced similar results to those reported here:
361 increased maternal faecal glucocorticoid metabolites and reduced the expression of
362 maternal care (Heiming et al., 2011). Similarly in primates, maternal postpartum
363 cortisol was also correlated with reduced maternal care (Bahr et al., 1998), suggesting
364 that stress during lactation is disruptive for maternal behaviour and may be a
365 consistent response across species.

366

367 McNatty *et al.* (1972) have shown that an average of 28 days is needed for cortisol to
368 return to "normal" levels when sheep are brought from pasture into an animal house.
369 Although in the present study there is a significant effect of time on faecal cortisol and
370 oestradiol there is no consistent pattern to suggest animals are habituating or
371 adjusting to the changed housing environment.

372

373 Multiparous ewes are frequently reported to show greater maternal care than
374 primiparous ewes (Dwyer *et al.*, 1998; Dwyer and Smith, 2008). This has been
375 discussed extensively elsewhere (e.g. Dwyer, 2008; 2014), and seems to be related
376 to the increased physiological sensitivity of experienced ewes. The results of the
377 present study indicate that the maternal behaviour of Small Tail Han sheep, like many
378 other breeds of ewe, is also significantly influenced by parity. Although there was no

379 overall effect of parity on suckling behaviour in this study, compared to primiparous
380 ewes, multiparous ewes had a higher frequency of udder accepting and consequently
381 were less likely to refuse sucking than primiparous ewes. These data support those
382 seen in other breeds (e.g. Dwyer and Smith, 2008). Multiparous ewes also had a
383 higher frequency of low-pitched bleating to their lambs compared to primiparous,
384 which may be indicative of greater maternal responsiveness (Dwyer *et al.*, 1998).
385 Low-pitched bleating is also affected by the environment (in outdoor raised sheep), by
386 breed and by nutritional treatment in pregnancy (Shillito-Walser *et al.*, 1984). Our data
387 differ from that of Dwyer *et al.* (1998), however, in different breeds of sheep, who
388 report that there is no significant difference between multiparous and primiparous
389 ewes in low-pitched bleat frequency. This may be related to different sheep breeds,
390 different environments or the timing of observations, which extend for a longer period
391 in the present study but omit the period immediately after parturition which was the
392 focus for the Dwyer *et al.* (1998) study.

393

394 The Small Tail Han sheep is noted for its fecundity, thus good maternal behaviour
395 would be important to increase lamb survival. The neonatal lamb will not survive
396 (without human intervention) if females do not nurse and care for their young (Nowak
397 *et al.*, 2000; Dwyer, 2008). In this study, the results suggest that maternal experience
398 plays an important role in the maternal behaviour expression of Chinese ewes, and
399 that multiparous ewes had a higher faecal concentration of oestradiol than
400 primiparous ewes during lactation. Previous studies have suggested that there is a
401 significant positive correlation between circulating oestradiol (before birth) and the
402 subsequent expression of maternal behaviour (low pitched bleating and grooming) at
403 birth (Dwyer *et al.*, 2004). This study extends those observations by suggesting that

404 higher oestradiol throughout lactation may also be related to greater expression of
405 maternal grooming and low-pitched bleating.

406

407 A small space allowance per animal has been shown to reduce feed intake and
408 weight gain in 11-week old growing lambs (Horton et al., 1991). However, in this
409 study, we did not see similar effects on lamb weight. This may be because, at the
410 lamb ages used in the present study, the lambs were almost entirely dependent on
411 the ewe for their nutrition and so were relatively protected from the effects stocking
412 density on feeding and social disturbance that may influence growth. This is
413 supported by the significant effect of ewe parity on lamb growth, which suggests that it
414 is the ability of the ewe to provide milk to her lamb which was the main determinant of
415 lamb weight gain. Younger ewes are known to produce milk with significantly less
416 protein, casein and fat compared to older ewes (Sevi *et al.*, 2000). Although stocking
417 density did appear to affect suckling duration, at least in the multiparous ewes, this
418 was not associated with reduced milk supply and so poorer lamb growth in the smaller
419 pens.

420

421 **Conclusion**

422 This study is the first to demonstrate that stocking density can affect the expression of
423 maternal behaviour of lactating ewes towards their lambs until weaning. A high
424 stocking density increased udder refusals and reduced following behaviour compared
425 to a low stocking density, and reduced suckling duration and grooming behaviour in
426 multiparous ewes but not primiparous ewes. Stocking density also significantly
427 increased fecal glucocorticoid concentration, and decreased oestradiol
428 concentrations. Other behaviours and lamb growth were affected only by parity. In
429 addition, to our knowledge, no previous research has examined effects on the

430 expression of maternal behaviour in Small Tail Han sheep, a highly prolific and
431 productive Chinese native sheep breed. This is a relatively small study, and whether
432 the effects of pen size on maternal behaviour is repeated in other studies, and in
433 different farm environments remains to be seen. Nevertheless, the study does raise
434 interesting questions about the impact of environment on the maintenance of maternal
435 behaviour, and the role of hormonal factors in maternal behaviour after the initial
436 onset of maternal care.

437

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448

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534

535

536 **Table 1:** Definition of behaviours expressed by ewes and lambs

537

Behaviour	Description
Sucking(min)	Lamb holds the teat in its mouth, appears to be sucking for at least 5 s.
Grooming(min)	Ewe licking and nibbling movements directed towards the lamb
Following(min)	Lamb staying with ewe or walking with the ewe at less than its body length
Udder accepting(freq)	Ewe keeps still to let the lamb suck
Udder refusing(freq)	Ewe moving, backing or circling, preventing the lamb from attempting to suck or reach the udder
Low-pitched bleating(freq)	Ewe bleating with a low pitched “rumble” or “mmm” call

538 **Table 2:** Effects of maternal parity and pen size on maternal behaviour and lamb weight for primiparous and multiparous ewes. All
 539 values are means, with standard errors in parentheses. Significance was determined by linear mixed models (REML), statistic is
 540 Wald (w, approximates to χ^2 ; d.f.=1 throughout).

Behaviour	Pen Size*			Parity		
	Large	Small	Statistics	Multiparous	Primiparous	Statistics
Grooming (min)	10.13 (0.71)	7.11 (0.38)	W=21.24, P<0.001	10.04 (0.74)	7.20 (0.32)	W=18.81, P<0.001
Following (min)	78.88 (5.37)	60.23 (4.82)	W=11.07, P=0.003	73.37 (5.58)	65.74 (4.73)	W=1.85, P=0.189
Suckling (min)	21.93 (1.60)	18.55 (1.38)	W=6.21, P=0.022	19.99 (1.53)	20.49 (1.47)	W=0.14, P=0.717
Udder acceptance (freq)	4.55 (0.32)	4.57 (0.31)	W=0.00, P=0.978	5.57 (0.22)	3.55 (0.36)	W=7.03, P<0.05
Udder refusal (freq)	7.00 (0.36)	9.54 (0.52)	W=15.18, P<0.001	5.45 (0.25)	11.09 (0.46)	W=74.90, P<0.001
Low-pitched bleats (freq)	6.79 (0.40)	6.57 (0.41)	W=0.07, P=0.798	8.04 (0.45)	5.41 (0.30)	W=25.37, P<0.001

Lamb weight at 24 h (kg)	1.42 (0.05)	1.43 (0.06)	W=0.00, P=0.947	1.50 (0.05)	1.35 (0.05)	W=3.05, P=0.096
Weight at weaning at 35d (kg)	7.38 (0.32)	7.14 (0.25)	W=0.29, P=0.598	7.99 (0.29)	6.53 (0.12)	W=11.12, P<0.005

541 *Large pens provided 3 m² per ewe, small pens provided 1.5 m² per ewe

542 **Table 3:** The effect of increasing lamb age on the expression of maternal behaviour. Values are means with pooled standard error of
 543 difference (sed).

	Age of lamb (days)								<i>Sed</i>	<i>P</i> value
	1	5	10	15	20	25	30	35		
Grooming (min)	15.87 ^a	12.24 ^b	10.55 ^b	9.18 ^c	6.81 ^d	6.72 ^d	4.37 ^e	3.21 ^e	0.95	<0.001
Following (min)	149.97 ^a	119.14 ^b	90.55 ^c	70.43 ^d	55.65 ^e	38.03 ^f	21.28 ^g	11.38 ^g	5.05	<0.001
Sucking (min)	43.40 ^a	36.06 ^b	26.10 ^c	20.11 ^d	13.83 ^e	10.92 ^f	6.88 ^g	4.65 ^g	1.38	<0.001
Udder-accepting (freq)	7.25 ^a	6.13 ^b	5.67 ^{bc}	5.41 ^c	4.29 ^d	3.41 ^d	2.13 ^e	2.21 ^e	0.50	<0.001
Udder-refusing (freq)	4.67 ^a	6.54 ^b	7.42 ^b	7.04 ^b	8.96 ^c	9.79 ^{cd}	10.46 ^d	11.29 ^d	0.67	<0.001
Low-pitched bleating (freq)	11.75 ^a	9.67 ^b	8.17 ^c	6.48 ^d	6.29 ^d	4.46 ^e	4.04 ^e	2.96 ^f	0.67	<0.001

544 **Figure captions**

545

546 **Fig 1.** The effect of lamb age (in days) on ewe faecal cortisol concentration ($\mu\text{g/L}$) for
547 multiparous ewes in large pens (solid square and lines), multiparous ewes in small
548 pens (open square, broken lines), primiparous ewes in large pens (solid circle and
549 broken lines), and primiparous ewes in small pens (open circle and lines).

550

551 **Fig 2.** The effect of lamb age (in days) on ewe faecal oestradiol concentration
552 (pmol/L) for multiparous ewes in large pens (solid square and lines), multiparous
553 ewes in small pens (open square, broken lines), primiparous ewes in large pens (solid
554 circle and broken lines), and primiparous ewes in small pens (open circle and lines).

555