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Lv, S-J; Yang, Y; Dwyer, CM; Li, F-K

Published in:
Animal

DOI:
10.1017/S175173111500052X

Print publication: 01/01/2015

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

Shen-Jin Lv[1], Yan Yang[2], Cathy M Dwyer[3], Fu-Kuan Li[1]

[1]*College of Life Sciences, Linyi University, Shuangling Road, Linyi City, 276005, Shandong, China;*

[2]*Linyi Agriculture Science Institute, North Street of Su River, Linyi City, 276009, Shandong, China;*

[3]*Animal & Veterinary Sciences, SRUC, King's Buildings, West Mains Road, Edinburgh EH9 3JG, Scotland, UK;*

Corresponding author. Shen-jin Lv. E-mail: lvshenjin@lyu.edu.cn; lvshenjin_yang@sina.com

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

Abstract

The aim of the experiment was to study the effects of pen size and parity on maternal behaviour of twin-bearing Small Tail Han ewes. Twenty-four ewes were allocated to a 2×2 design (6 per pen) with parity (primiparous or multiparous) and pen size (large: 6.0m×3.0m; small: 6.0m×1.5m) as main effects at Linyi University, Shandong Province, China. Behaviour was observed from after parturition until weaning. All ewes were observed for 6 h every 5 days from 07:00 to10:00 h and 14:00 to 17:00 h. Continuous focal animal sampling was used to quantify the duration of maternal behaviours: sucking, grooming, and following; and the frequency of udder accepting, udder refusing and low-pitched bleating. Oestradiol and cortisol concentration in faeces (collected in the morning every 5 days) were detected with EIA kits. All lambs were weighed 24 hours after parturition and again at weaning at 35 days of age. The small pen size significantly reduced following (P<0.005), grooming (P<0.001) and suckling durations (P<0.05), and the frequency of udder refusals (P<0.001). However, there was a significant interaction with ewe parity, with the decreased grooming and suckling in the small pen largely seen in the multiparous ewes (P<0.001). Independent of pen size multiparous ewes accepted more sucking attempts by their lambs (P<0.05), and made more low-pitched bleats, than primiparous ewes (P<0.001). Multiparous ewes had higher faecal oestradiol concentration than primiparous ewes (P<0.001), and ewes in small pens had higher faecal cortisol than those in larger pens (P<0.001). As lambs increased in age, the duration of maternal grooming, following, and suckling and frequency of udder acceptance and low-pitched bleating all declined, and the frequency of udder refusing increased (P<0.001 for all). Ewe parity, but not pen size, affected lamb weight gain during the observation period (P<0.001). This is the first study to show that pen size, interacting with parity, can affect the expression of maternal behaviour in sheep during lactation. The study is also the first to report on the maternal behaviour of Chinese native sheep breeds (Small Tail Han sheep), with implications for the production of sheep in China.

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

Implications

In many countries there is increasing pressure on land use, such that grazing animals, which would normally be managed extensively, may start to be housed more frequently. However, for sheep, there is little information on appropriate housing and the impact that this may have on stress, growth and behaviour. This study demonstrated that small pen sizes reduced ewe-lamb suckling behaviour and increased udder refusals compared to larger pens, although grooming behaviour was also increased. This was accompanied by increased ewe faecal cortisol and suggests that suckling ewes may be stressed by housing in close confinement, which influences their maternal behaviour.

Introduction

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

In China, economic developments are placing greater pressure on land use, and pasture availability for sheep production is declining with increased use for other agricultural and non-agricultural practices (e.g. Bosing et al., 2014). As a solution to this sheep production is gradually shifting from extensive to intensive management, with increased housing of ewes during pregnancy and lactation, and housing at increasing stocking density. However, there is still little information available about the impact of sheep housing and stocking density on sheep production and welfare, although recent studies suggest that high stocking density results in behavioural disturbances, displacements and altered social interactions (Averòs et al., 2014a, b). It is, therefore, relevant to consider how this might also affect the behaviour of Chinese sheep breeds.

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

The studies mentioned above have focused on the impact of high stocking density on the onset of maternal behaviour. However, the effect of pen size on established maternal behaviour and physiology, when animals are confined in new environments, has not yet been investigated. The neuroendocrine mechanisms of maternal care in ewes, have received considerable research attention in recent years. In particular, oestradiol plays an important role in the onset of maternal behaviour, and cortisol may also influence the expression of maternal behaviour (Dwyer *et al.,* 2004; Meurisse *et al.,* 2005; Bøe *et al.,* 2006; Dwyer, 2014). In general, primiparous ewes are more likely to show inadequate maternal behaviour towards their neonates than experienced ewes (Meurisse *et al.,* 2005; Dwyer and Smith; 2008). This may be because hormonal induction of maternal behaviour in primiparous females is less efficient (Le Neindre *et al.,* 1979). Parity effects on maternal behaviour are not related to circulating oestradiol (Dwyer and Smith, 2008), but the responsiveness to oestradiol induction of maternal behaviour increases with parity (Poindron *et al.,* 1984), which seems to be related to the impact of maternal experience on central oestradiol receptor-α expression (Meurisse *et al.,* 2005). However, most of these studies have focused on hormonal patterns leading to the onset of maternal behaviour, and much less is known about the role of hormonal changes in the maintenance of maternal behaviour.

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

The aim of this project was a) to provide new information on the maternal behaviour of the highly prolific Small Tail Han sheep from parturition to weaning, considering the impact of maternal parity, and b) to investigate whether pen size affected the expression of established maternal behaviour to provide guidance to farmers on husbandry conditions for these sheep. We hypothesise that restrictive housing conditions will increase ewe stress, reduce the expression of maternal behaviour, and affect faecal concentrations of stress and reproductive hormones. Finally, we predict that differences in maternal behaviour will affect the growth of the lamb.

Materials and Methods

Animals, housing and feeding

This study was carried out at Zhong-He farm, Lan-Shan District, Linyi city, Shandong province, China. At the farm, 212 ewes are housed in two big stalls (40.0m×15.0m, about 6.0m^2 per animal). Before the experiment, eighty ewes, which were in good health and condition, were chosen and synchonised in oestrus using progesterone vaginal sponges (Huayu Ltd, Shanghai, China), and were artificially inseminated with semen from 6 rams over two days. Twenty-four pregnant twin-bearing ewes (12 primiparous, 12 multiparous) were selected from the two stalls on the basis of trans-abdominal ultrasound examination. At approximately mid-gestation (75 days), all selected ewes were given ad libitum access to hay. Two hundred g/ewe/day of locally milled ewe nuts (about 200 crude protein per kg dry mass, Huhui Ltd, Linyi, Shandong Province) were provided. From d100 concentrates were fed at a ration of 320 g/ewe/day. Rations were doubled every 15 days until d130 of gestation and then maintained at this level until parturition. At d145 of gestation, ewes were allocated to treatment at random (n=6 per treatment) in a 2×2 design with parity (primiparous (P) or multiparous (M)) and pen size (large (L): 6.0m×3.0m (3.0m^2 per ewe); small (S): 6.0m×1.5m (1.5 m^2 per ewe)) as the factors. Feeders were attached to the outside of the pen walls and one feeder position per ewe was available in all pens. The individual pen walls were made of 1.5 m high solid material with cement to avoid visual and direct physical interactions between groups. The primiparous ewes were aged 1 or 2 years old and the multiparous ewes were 3 years old before pregnancy. The parity of multiparous ewes was 3 or 4.

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

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

Behavioural data collection

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

Faecal collection and analysis

In order to reduce the disturbance of ewes and lambs, a fresh faecal sample of every ewe was collected gently by the stockperson every 5 days in the morning before feeding. About one gram of faecal pellets then were placed into micro-centrifuge tubes and frozen at -80 °C until extraction. The EIA kit (Yuaping Ltd, Shanghai, China) method was used in the experiment to extract the cortisol and oestradiol as described by Palme and Möstl (1997). In brief, 0.5 g faeces were suspended in 4.0 ml methanol and 1.0 ml double distilled water (=80 % methanol) and shaken for 30 minutes at 2000 g. After centrifugation, all the samples were sealed and kept at -20 °C until extraction. As Palme and Möstl (1997) method, 50.0 μl samples were gathered and detected with the EIA kit as instructed by the manufacturers (Pulang Ltd, Beijing, China). The oestradiol EIA had a sensitivity of 20.0pmol/L, an intra coefficient of variation was 0.12 and inter-assay was 0.14. The cortisol EIA had a sensitivity of 10.0μg/L an intra and inter-assay coefficient variation was 0.13 and 0.16, respectively. All the feces samples were assayed for oestradiol concentration in a single assay, and similarly for cortisol concentration.

Data Analysis

For each observation day the total duration of each behaviour for each ewe was calculated for the 6 hour observation period. For behavioural frequencies the mean frequency per 10 minutes was calculated for each observation day. A repeated measures mixed model of variance (REML) was used to analyze the maternal behaviours and hormone concentrations with the following fixed factors in the model: parity (multiparous vs primiparous), pen size (large vs small) and the interaction between parity and pen size. The ewe identity was set as random effect, ewe and lamb age were used as residual term with covariance structure in the model. Before the model was run, all the maternal behaviours were checked to fit a normal distribution. This test generates a Wald statistic (which approximates to a χ^2 statistic), instead of an F statistic, this value is given in the text. A mixed model of variance was used to analyse the effect of the pen size and parity on lamb weight. All the data are shown as a mean, significance was set at $P=0.05$ and all analyses were performed in GenStat (8th Edition) software. It should be noted, however, that only one pen per condition (parity x size) was used and therefore ewe was treated as the experimental unit in the study. Although maternal behaviour is often considered at the ewe level, there may have been pen level factors that could have affected the data, but which could not be disentangled by this design, which is a limitation of this study.

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

Results

Maternal Behaviour

A restricted pen size reduced the amount of following behaviour between ewe and lamb (Table 2, $P<0.005$), reduced ewe grooming ($P<0.001$) and suckling duration ($P<0.05$) and increased udder refusals by the ewe ($P<0.001$). Multiparous ewes also spent more time grooming their lambs (Table 2, $P<0.001$), had a higher frequency of low-pitched bleating ($P<0.001$) and udder acceptance ($P<0.05$), and a lower frequency of udder refusals than primiparous ewes ($P<0.001$). There was, however, a significant interaction between ewe parity and pen size for some maternal behaviours. Multiparous ewes suckled their lambs for longer at a higher stocking density than at a low stocking density but these effects were not seen in primiparous ewes (mean suckling duration (mins): PL=22.29, PS=18.70; ML=14.82, MS=25.17, s.e.d=1.92, W=26.38, d.f.=1, P<0.001). Multiparous ewes also spent more time grooming their lambs in the large pen compared to the small pen, although this effect of pen size was also not seen in the primiparous ewes (mean duration of grooming (min): PL=7.22, PS=7.18; ML=13.04, MS=7.04, s.e.d=0.93, Wald=20.71, d.f.=1, P<0.001).

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

Faecal hormone levels

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

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

Lamb weight

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

Discussion

The result of this study indicated that both parity and pen size had effects on Small Tail Han sheep maternal behaviour during lactation. To our knowledge this is the first paper to describe maternal behaviour in Chinese native sheep breeds. The most significant finding of this study was that housing in pens providing 1.5 m^2 per ewe resulted in changed maternal behaviour (specifically: reduced following, grooming and suckling associated with increased sucking refusals) in comparison to ewes housed at 3 m^2 per ewe, and multiparous ewes appeared to be more influenced by the pen size than primiparous ewes. This was accompanied by increased faecal glucocorticoid metabolites and reduced faecal oestradiol in the small pens.

Few studies have considered the space requirements for housed sheep (other than during transport or at lairage) and very few studies address the impact of housing on the expression of maternal behaviour during lactation. Studies considering what might be an appropriate pen size for ewes have almost invariably considered the space required by the ewe alone (either whilst dry, during pregnancy or lactation only in dairy ewes). Chiumenti (1987) suggests 0.9-1.2 m^2 /head on straw litter and 0.8–1.0 m^2

/head on slatted floor are fit for sheep. However, studies of sheep behaviour suggest that housing at 1-1.5 m^2 (as in the small pens in the present study) results in higher social interactions and reduced activity compared to lower stocking density (Caroprese *et al.,* 2009; Averòs *et al.,* 2014). Conversely, very low space availability increases activity as animals are prevented from lying down when they wish (Bøe *et al.,* 2006). Sevi *et al.* (2009), for lactating dairy ewes, suggests assigning a 2.0 m^2 area per sheep to avoid these behavioural impacts. In the present study, although activity per se was not measured, the increased following time in the larger pens suggests that animals may have been more active than in the smaller pens. In a study of mother-offspring recognition Val-Laillet and Nowak (2006) suggest that lambs kept in small pens take longer to learn to recognize their mothers than lambs in larger pens. This may also have led to the observed increase in following time with more space, if the lambs were better able to recognize and follow their own mother than in small pens, and the reduced sucking refusals (as lambs may be less likely to attempt to suck from a ewe that was not their mother). The increase in grooming behaviour at a lower stocking density may also be associated with improved recognition between ewe and lamb, and a closer bond developing when animals have more space to express maternal behaviour.

In this study there was a significant interaction between ewe parity and pen size for grooming and suckling behaviours. Multiparous ewes expressed a higher amount of grooming behaviour in the larger pen, whereas primiparous ewes were not affected by pen size. Multiparous ewes are known to express a higher quantity and quality of maternal care compared to primiparous ewes (reviewed by Dwyer, 2014). It may be that the smaller pen impaired expression of maternal care in both inexperienced and experienced ewes, but only multiparous ewes were able to show increased maternal care when the environmental conditions permitted this to occur. The higher suckling responses of multiparous ewes in the small pen compared to primiparous ewes might be a greater responsiveness of these experienced ewes to the behaviour of their lambs. However, as this study focussed mainly on ewe behaviour it is not known if lamb behaviour was also altered by the housing conditions.

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

Cortisol is widely measured as an indicator of physiological and psychological stress, and the higher values recorded here in ewes maintained in small pens may indicate greater stress in these animals. In our study, we seldom saw that ewes in the small pens expressed abnormal or stereotypic behaviour, the main effects of low space allowance were reduced time spent grooming and greater udder-refusing frequency (which could be considered as poorer maternal care) than ewes housed in a large pen during lactation. Few studies in sheep have considered the impact of maternal stress on maternal behaviour, and those that have report conflicting results. In studies examining the effects of maternal stress before birth on the onset of maternal behaviour, Hild et al. (2011) found a positive correlation between maternal experience of stress during pregnancy and maternal grooming in sheep. Dwyer *et al.* (2004) reports no relationship between circulating cortisol before birth and maternal behaviour (in a naturally occurring model of differences in circulating cortisol which may not be related to stress) but a negative correlation between postnatal circulating cortisol and maternal behaviour expression in sheep. In rodents, maternal stress induced by unfamiliar male odours produced similar results to those reported here: increased maternal faecal glucocorticoid metabolites and reduced the expression of maternal care (Heiming et al., 2011). Similarly in primates, maternal postpartum cortisol was also correlated with reduced maternal care (Bahr et al., 1998), suggesting that stress during lactation is disruptive for maternal behaviour and may be a consistent response across species.

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

Multiparous ewes are frequently reported to show greater maternal care than primiparous ewes (Dwyer *et al.,* 1998; Dwyer and Smith, 2008). This has been discussed extensively elsewhere (e.g. Dwyer, 2008; 2014), and seems to be related to the increased physiological sensitivity of experienced ewes. The results of the present study indicate that the maternal behaviour of Small Tail Han sheep, like many other breeds of ewe, is also significantly influenced by parity. Although there was no overall effect of parity on suckling behaviour in this study, compared to primiparous ewes, multiparous ewes had a higher frequency of udder accepting and consequently were less likely to refuse sucking than primiparous ewes. These data support those seen in other breeds (e.g. Dwyer and Smith, 2008). Multiparous ewes also had a higher frequency of low-pitched bleating to their lambs compared to primiparous, which may be indicative of greater maternal responsiveness (Dwyer *et al.,* 1998). Low-pitched bleating is also affected by the environment (in outdoor raised sheep), by breed and by nutritional treatment in pregnancy (Shillito-Walser *et al.,* 1984). Our data differ from that of Dwyer *et al.* (1998), however, in different breeds of sheep, who report that there is no significant difference between multiparous and primiparous ewes in low-pitched bleat frequency. This may be related to different sheep breeds, different environments or the timing of observations, which extend for a longer period in the present study but omit the period immediately after parturition which was the focus for the Dwyer *et al.* (1998) study.

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

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

Conclusion

This study is the first to demonstrate that stocking density can affect the expression of maternal behaviour of lactating ewes towards their lambs until weaning. A high stocking density increased udder refusals and reduced following behaviour compared to a low stocking density, and reduced suckling duration and grooming behaviour in multiparous ewes but not primiparous ewes. Stocking density also significantly increased fecal glucocorticoid concentration, and decreased oestradiol concentrations. Other behaviours and lamb growth were affected only by parity. In addition, to our knowledge, no previous research has examined effects on the expression of maternal behaviour in Small Tail Han sheep, a highly prolific and productive Chinese native sheep breed. This is a relatively small study, and whether the effects of pen size on maternal behaviour is repeated in other studies, and in different farm environments remains to be seen. Nevertheless, the study does raise interesting questions about the impact of environment on the maintenance of maternal behaviour, and the role of hormonal factors in maternal behaviour after the initial onset of maternal care.

Acknowledgements

The authors wish to thank Jiang Zi-ming and Yin Zuo-guo for animal husbandry and technical assistance at gestation and parturition time, and Xu Song-shan, Wan Wen-wen and Li Jing for the observation data collection and hormone assays. This study was supported by the National Natural Science Foundation of China (Grant No: 31001027; 31272480); Promotive research fund for young and middle-aged scientists of Shandong Province (Grant No: BS2011NY004), Domesticated Animal Germ plasm Resources Platform of China, Shandong Modern Agriculture Industry Technology System-Sheep Industry Innovation Team (Grant No: SDAIT-09-011-15). SRUC receives grant-in-aid from the Scottish Government.

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Table 1: Definition of behaviours expressed by ewes and lambs

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

Table 2: Effects of maternal parity and pen size on maternal behaviour and lamb weight for primiparous and multiparous ewes. All values are means, with standard errors in parentheses. Significance was determined by linear mixed models (REML), statistic is Wald (w, approximates to χ2; d.f.=1 throughout).

	Pen Size*			Parity		
Behaviour	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

*Large pens provided 3 m^2 per ewe, small pens provided 1.5 m^2 per ewe

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

	Age of lamb (days)								*Sed*	*P* 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

Figure captions

Fig 1. The effect of lamb age (in days) on ewe faecal cortisol concentration (µg/L) for multiparous ewes in large pens (solid square and lines), multiparous ewes in small pens (open square, broken lines), primiparous ewes in large pens (solid circle and broken lines), and primiparous ewes in small pens (open circle and lines).

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