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Postnatal maternal behaviour expression depends on lambing difficulty in Merino ewes



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ABSTRACT

Dystocia, a prolonged or non-progressive birth event, is the main contributor to lamb mortality in Australia and across the world. Dystocia can cause neonatal hypoxia, central nervous system (CNS) damage leading to increased risk of starvation, exposure and mismothering, and death. These prolonged birth events can also cause fatigue, injury and death in the ewe. Dystocia may interrupt the expression of maternal behaviour and the strength of the ewe-lamb bond, and consequently lamb survival. This study focused on the effect of dystocia on ewe behaviour in the 2 h post-lambing. A total of 18 ewes were chosen for continuous behaviour annotation and analysis (dystocic (n = 9) and eutocic (n = 9)) based on the quality of video recordings, length of stage 2 parturition and classification by a single experienced observer. Dystocic ewes showed significantly lower expression of maternal behaviours and a significantly greater expression of avoidance behaviours compared to eutocic ewes. Additionally, dystocic ewes performed fewer behaviours in total compared to eutocic ewes.

Dystocia can significantly affect the quality and quantity of ewe maternal behaviour expression, leading to increased avoidance of the lamb, increased risk of maternal disinterest, and increased risk of death for the lamb. If dystocic events can be identified quickly and accurately, measures can be taken to ensure the ewe and lamb recover successfully.

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1. Introduction

Appropriate and sufficient maternal care, and a strong, exclusive dam-young bond are crucial for the survival of the neonate [4,27,30], especially in extensive production systems. In sheep, the lamb relies on the ewe for nutrition [26], primary immunological support, and physical protection from flock members and predators [14,31]. Dysfunction of maternal behaviour without human intervention leads to a weakened dam-young bond and increases the risk of mortality for the neonate [9]. Additionally, lamb behaviour such as vocalising and teat-seeking reinforces maternal behaviour [28], and similarly, a dysfunction in lamb behaviour can lead to poor ewe-lamb bond formation, and increased risk of lamb mortality [7,29].

A common cause for behaviour dysfunction in periparturient ewes and neonatal lambs is dystocia. Dystocia is a prolonged or non-progressive labour, and can manifest as maternal exhaustion,

disinterest, injury or death [4,7,16], and in the neonate as hypoxic brain injury, CNS damage, low vigour and stillbirth [12,20,32]. Dystocia is a major contributor to neonatal lamb mortality across the world, most notably in extensive outdoor lambing practices. It is also an animal welfare concern of increasing importance, as studies have shown dystocia to affect not only lamb, but also ewe health and survival [21,32].

In this trial we made use of video recordings of ewe behaviour at lambing which were collected to provide annotations for a project developing a birth-detection algorithm in ewes [34]. In this original trial, the main goal of the study design had been to collect a diverse range of lambing behaviours and a range of parturition duration. The study cohort included therefore ewes which had been mated to different sire types and which included single- and twin-bearing ewes. In the present study, we selected suitable video recordings from this existing study cohort, in keeping with the goal to reduce the numbers of animals used in animal research by reuse of data. We analysed ewe behaviour for 2 h immediately post-birth to determine the differences in ewe behaviour transitions and performance of specific behaviours associated with maternal care and bonding between eutocic and dystocic lambing events. We hypothesise that birth difficulty will affect the

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expression of maternal behaviour.

2. Materials and methods

2.1. Animal ethics approval

Animal experimentation was undertaken at the CSIRO FD McMaster Laboratory, Armidale, NSW, Australia between March and October 2018 and conducted according to the Australian Code for the Use and Care of Animals in Research and Teaching. Experimental protocols were approved by the CSIRO Armidale Animal Ethics Committee under Animal Research Approval number 18/11.

2.2. Animals and management

Pregnant Merino ewes were selected from two larger flocks joined separately to either Merino or Border Leicester rams. A total of 100 ewes were selected based on due date with the aim to allow lambing over a three-week period and a secondary aim to include a balance for parity (maiden ($n = 54$) and mature ewes ($n = 46$)) and for sire breed (Merino ($n = 48$) and Border Leicester ($n = 52$)). All twin-bearing ewes were included in the experiment ($n = 12$) and the rest were single-bearing ($n = 88$). The main aim of the experiment was to collect complete lambing records of lambing events, including high-quality video records.

To facilitate identification of ewes on video records, ewes were side branded with stock paint (Siromark, Heiningen, Bibra Lake, WA) with a unique number. The 20 ewes expected to lamb first were moved into the supervised observation paddock (approx. 35 m \times 75 m) and the rest of the ewes were moved to adjacent holding paddocks. Ewes had *ad libitum* access to water and pasture grass and were supplemented with a mix of fava beans and pellets once daily (200 g/day; sheep pellets based on wheat, millrun and lucerne; 17.5% protein, 2.5% fat, 17% fibre, 20% ADF, 34% NDF) and lucerne hay (200 g/day per ewe) twice daily.

Health checks were performed twice daily, and any abnormalities were recorded on paper and reported to the principal investigator and the site veterinarian.

Once a ewe had lambed, if the ewe and her lamb appeared fit, healthy and well bonded, they were moved to a larger paddock adjacent to the lambing enclosure. Pregnant ewes from the holding paddocks were brought into the lambing enclosure to replace the ewes that had lambed, maintaining a group of 20 ewes at any given time in the lambing enclosure. This rotation continued until all ewes had lambed. For the last three ewes, ewes and lambs remained in the enclosure until all lambs were born to avoid isolation of an individual or very small number of ewes in the enclosure.

2.3. Lambing data collection

Ewes were first checked at 6:30 a.m. followed by multiple times, at least hourly, during the day, with the last check at 5:30 p.m. If a ewe was observed to be in active labour, experienced animal carers would check on her every 30 min until birth. According to the approved animal research protocol, ewes were provided assistance if no progression was made after 2 h from observations of protruding fetal membranes or fetal body parts; however, in the present study, none of the recorded ewes required assistance. For the first 2 h after a lamb was born, ewe and offspring were left undisturbed to allow bonding to take place. At 2 h after lambing for lambs born during the day, or on the following morning for lambs born during the night, lambs were weighed using hand-held scales (Tru Test, QLD, Australia). The sex was determined by visual inspection and the lamb was tagged by visual and RFID tags in both ears (Shearwell, VIC, Australia). Of each lamb born in the supervised lambing enclosure, the time of birth was identified from the stored video data and recorded. Of the collected lambing data, lambing date and time of day were recorded for the use in the presented study. Lamb breed and sex were recorded and reported but not statistically analysed as the study design did not support this. Other lambing data was collected as part of regular husbandry practices and not included in further analyses.

2.4. Behaviour data

The supervised lambing paddock was set up with 10 day and night video surveillance cameras (Hikvision Digital Technology Co, Hangzhou, China) positioned around the paddock to cover the entire area. The cameras were connected to digital video recorders and footage captured using IVMS4200 software (Hikvision Digital Technology Co). Intensity of labour was classified subjectively *post hoc* using stored video records by a single observer familiar with lambing ewes, taking into account the duration of the birth. Birth events were classified as normal/eutocic or difficult/dystocic based on the duration from the first observation of abdominal contractions to expulsion of the lamb. A birth that exceeded 120 min from the first abdominal contractions was classed as a dystocic event. Quality and completeness of the video record was noted for the time period of 2 h following birth. Ewes with video records with poor visibility, for example due to poor lighting or angling away from the cameras, were excluded from the study. Of the videos collected during this study, nine difficult/dystocic events were selected for detailed analysis which were characterised by extended duration of the overall parturition process from first observation of abdominal contraction to the completed expulsion of a live, viable lamb. Of the recorded normal labour events, nine events were chosen for detailed annotation which were comparable to the date and time of day to the difficult events. Behavioural Observation Research Interactive Software (BORIS [18]) was used to continuously annotate a period of 2 h after full expulsion of the lamb. An ethogram was developed by observing normal and post-lambing-specific behaviours, then grouping like behaviours and eliminating rare or singular behaviours. The ethogram was sorted into categories including care, locomotion, feeding and grooming (Table 1).

2.5. Data processing and analysis

Behaviour transitions (BT), recorded as the number of behaviour changes observed in the 2 h after lambing, were recorded for each ewe. Behaviour transitions include all transitions between every recorded behaviour, divided by the total observed time. The metric BT was a calculation output provided by the behaviour annotation software package BORIS [18].

Data were entered into MS Excel (Microsoft, Redmont, WA, USA), where data were organised and descriptive statistics were performed (e.g. mean, range, standard deviation).

The range of behaviours performed by each ewe were recorded and tabled as total number of occurrences and total duration for the observation period and expressed as percentage of the time budget within the observation period. Comparisons between the two birth types were made for each individual behaviour and for each behaviour category.

Statistical analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria; Team, 2013).

When analysing count data (eg. BT), type III Wald chi square tests were used. When comparing values between eutocic and dystocic labours (eg. time spent grooming) Student's t-tests were used. Where individual behaviours were of insufficient frequency to support statistical analysis, observations for the behavioural category were combined and reanalysed. For all tests, significance was considered achieved when $P \leq 0.05$.

3. Results

3.1. Overall frequency of dystocic events

Of the 100 ewes enrolled in this study, 69 lambing events were captured on video; 52 were classified as eutocic events and 17 were classified as dystocic events. Additionally, 14 of these dystocic events had durations of stage 2 parturition exceeding 120 min. The average duration of stage 2 parturition for eutocic lambing events for this study was 56.78 ± 23.03 min, and 220.67 ± 113.91 min ($P = 0.001$) for dystocic lambing events.

The following results are reported for the 18 ewes selected for detailed continuous annotation.

Table 1

Ethogram used to identify and annotate post-lambing behaviour in BORIS annotation program. Behaviours were grouped in categories and assigned a type (point for single instance behaviours, or state for continuous behaviours) for ease of use in BORIS.

| Behaviour | Category | Type | Description |
|----------------------------|------------|-------------|---|
| Sucking | Care | State event | Ewe lets the lamb suckle |
| Nudging | Care | State event | Ewe uses head or front foot to move the lamb |
| Watching the lamb | Care | State event | Ewe looking in the direction of the lamb; close to it or not; no physical contact; standing or lying |
| Communication-vocalisation | Care | Point event | Ewe nearby the lamb or not; mouth opens; abdominal muscles contract |
| Graze | Feeding | State event | Grazing; lamb nearby or not; standing or moving 2-3 steps |
| Drinking | Feeding | State event | Ewe drinking at the trough |
| Eating placenta | Feeding | State event | Ewe eating placenta or foetal membranes on the ground or off the lamb |
| Sniffing | Grooming | State event | Ewe's nose is close to or touching the lamb; mouth closed and not moving |
| Licking | Grooming | State event | Ewe's nose is touching the lamb; licking the lamb or its membranes; mouth is moving |
| Bonding | Grooming | State event | Ewe close to the lamb; performing behaviours like grooming or nudging; when the lamb is suckling |
| Walking | Locomotion | State event | Moving forward with a steady 4-beat gait |
| Circling | Locomotion | Point event | Ewe turns around the lamb or spins around itself; head in the direction of the lamb or not; turning 360° or more |
| Stepping | Locomotion | Point event | Ewe moves 2 or 3 steps and stops; head up or down; forwards or backwards |
| Scratching | Locomotion | State event | Ewe scratching the ground with one hoof; head up or down |
| Dodging | Locomotion | Point event | Avoidance movements when the lamb approaches to suckle |
| Get up | Locomotion | Point event | Ewe gets up from the ground and stands on its four legs |
| Standing | Locomotion | State event | Not moving for at least 4 s; four feet on the ground: head up or not; lamb nearby or not; not looking at the lamb |
| Lying | Locomotion | State event | Ewe is on the ground; legs tucked or stretched; head on the ground or up |
| Looking elsewhere | Locomotion | State event | Ewe does not look in the direction of its lamb; standing or lying; head up or down; up to 3-4 s |
| Other | | Point event | Unclassified behaviour |
| Not visible | | State event | Ewe out of frame or unable to be seen clearly |

3.2. Distribution of lamb breed and litter size

The distribution of lamb breed and litter size was uneven between eutocic and dystocic labour events which were deemed of sufficient quality and selected for annotation (Table 2). Preference in selection was given to single-bearing ewes to minimise the effect of litter size on perinatal ewe behaviour. The nine ewes in the eutocic group only had single lambs; five of which were pure Merino, and four Border Leicester crosses. Eight ewes in the dystocic group had single lambs and one had a set of twins. All ewes in the dystocic group had Border Leicester cross-bred lambs, except for one ewe bearing a single Merino lamb. The structure of the available data set did not support analysis of lamb breed and litter size effects on maternal ewe behaviour, and the data analysis was therefore limited to the effect of lambing difficulty on observed ewe behaviour.

3.3. Difficult lambing events lead to lower maternal behaviour expression

3.3.1. Grooming

To analyse grooming behaviour, the behaviours in the grooming category (sniffing, licking and bonding) were combined. All eutocic ewes performed grooming behaviours, whereas only 78% of dystocic ewes performed grooming behaviours. On average, eutocic ewes spent significantly more time performing grooming behaviours compared to dystocic ewes (eutocic = 38.16 ± 4.57%, dystocic = 14.20 ± 5.47%; $P = 0.006$). Additionally, the average number of grooming events performed was significantly higher for eutocic ewes than dystocic ewes (eutocic = 247 ± 27, dystocic = 85 ± 39; $P = 0.005$, Fig. 1).

3.3.2. Licking

When licking was analysed as a separate behaviour, all eutocic ewes were observed to lick their lambs, whereas only 83% of dystocic ewes

Table 2

Distribution of birth difficulty, sire breed and litter size of the 18 ewes selected for detailed annotation.

| Frequencies of Labour type in the selected study cohort | | | |
|---|------------|------------|------|
| Labour type | Lamb breed | Birth type | |
| | | Single | Twin |
| Normal | Merino | 5 | – |
| | cross-bred | 4 | – |
| Difficult | Merino | 1 | – |
| | cross-bred | 7 | 1 |

performed licking behaviours. On average, eutocic ewes spent significantly more time performing licking behaviours compared to dystocic ewes (eutocic = 29.40 ± 3.65%, dystocic = 11.32 ± 4.18%; $P = 0.04$). Additionally, the average number of licking events performed was significantly higher for eutocic ewes than dystocic ewes (eutocic = 166.56 ± 14.45, dystocic = 51.11 ± 24.14; $P = 0.02$).

3.3.3. Bonding

Of the eutocic ewes, 78% performed bonding behaviours, whereas only 33% of dystocic ewes performed bonding behaviours. On average, eutocic ewes spent more of their time performing bonding behaviours compared to dystocic ewes (eutocic = 5.45 ± 5.34%, dystocic = 0.25 ± 0.56%). Additionally, the average number of bonding events was higher for eutocic ewes than dystocic ewes (eutocic = 24.00 ± 5.45, dystocic = 4.00 ± 2.12). These results were not statistically analysed as there were too few values in the dystocic group.

3.3.4. Circling

All eutocic ewes performed circling behaviours, whereas 78% of dystocic ewes performed circling behaviours. On average, eutocic ewes performed significantly more circling events than dystocic ewes (eutocic = 85.19 ± 68.87, dystocic = 29.56 ± 31.62; $P = 0.02$).

3.3.5. Looking elsewhere

All eutocic ewes performed looking elsewhere behaviours, whereas only 78% of dystocic ewes performed looking elsewhere behaviours. On average, eutocic ewes performed more looking elsewhere events compared to dystocic ewes (eutocic = 93.18 ± 57.58, dystocic = 28.00 ± 38.45; $P = 0.004$). Additionally, eutocic ewes spent more time looking elsewhere than dystocic ewes (eutocic = 276.46 ± 172.62 s, dystocic = 82.86 ± 111.59 s; $P = 0.003$).

3.4. Dystocic events lead to greater expression of avoidance-type behaviours

3.4.1. Walking

All ewes were seen to perform walking behaviours during the 2 h post-lambing. On average, eutocic ewes spent 110.17 ± 64.78 s performing walking behaviours, nearly half the time dystocic ewes spent performing walking behaviours (209.61 ± 202.45 s). Due to the high standard deviation, this result is statistically non-significant ($P = 0.09$).

3.4.2. Stepping

All ewes were seen to perform stepping behaviours during the 2 h

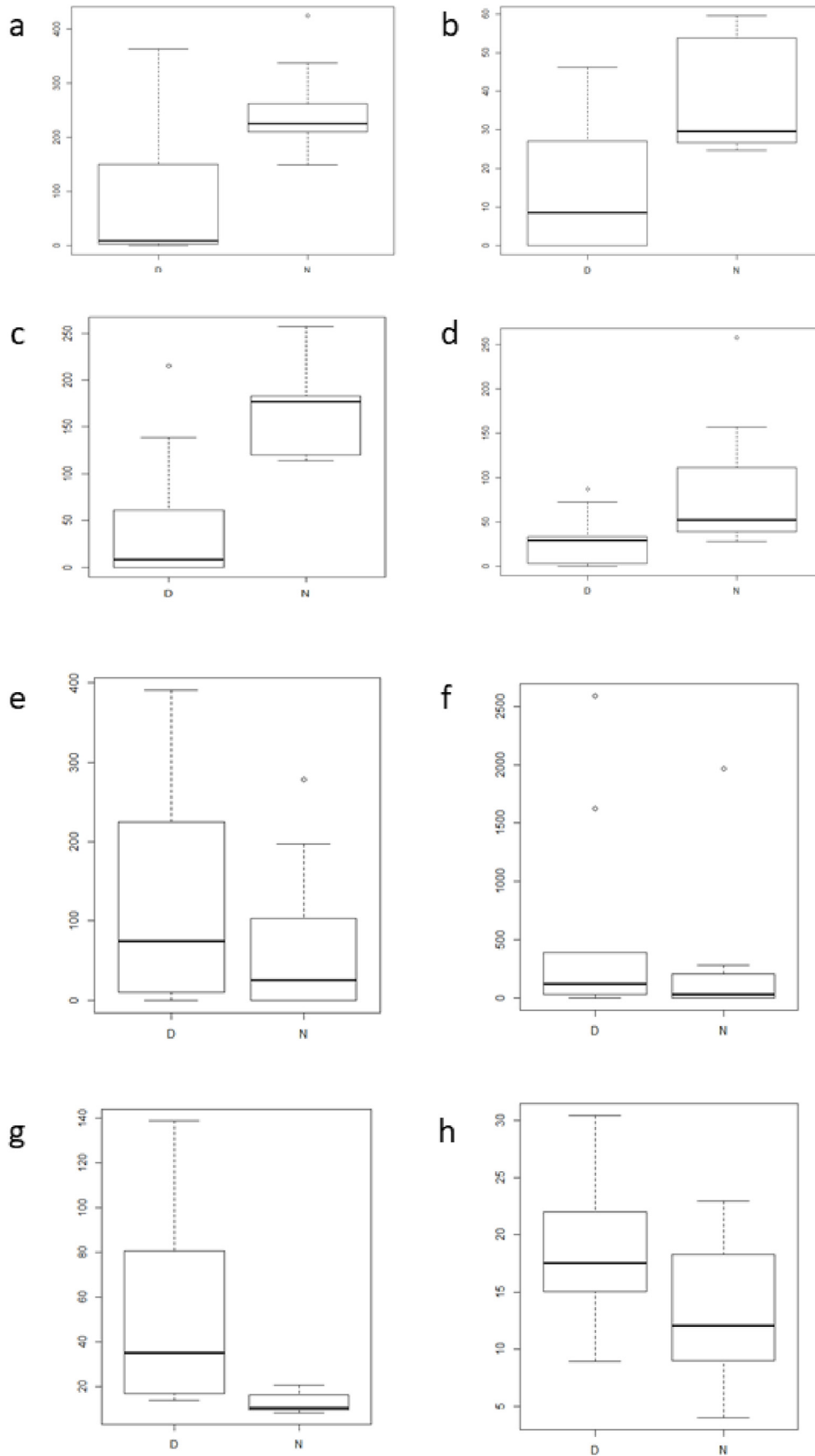


Fig. 1. Behavioural observations for Grooming (a, b), Licking (c), Circling (d), Lying (e, f) and Standing (g, h) in the 2 h period post-partum for dystocic (D) and eutocic/normal (N) ewes. Y axes represent total numbers of occurrences (a, c, d); proportion of total time budget in percent (b, h), duration mean in minutes (e, g) or total duration in minutes (f). Boxes represent interquartile ranges, solid lines within the boxes indicate the median value. Whiskers represent the data range.

post-lambing. On average, eutocic ewes performed significantly fewer stepping events than dystocic ewes (eutocic = 28.56 ± 8.81 , dystocic = 71.56 ± 10.72 ; $P = 0.001$).

3.4.3. Lying

There were no statistical differences between birth types for number of lying occurrences, total or mean duration of lying events.

3.5. Standing

All ewes were seen to perform standing behaviours during the 2 h post-lambing. On average, eutocic ewes spent less time standing compared to dystocic ewes (eutocic = 1167.25 ± 172.92 s, dystocic = 2768.47 ± 631.34 s; $P = 0.03$).

3.6. Dystocic events lead to lower behavioural transitions compared to eutocic events

In the 2 h immediately following birth, eutocic ewes had higher total number of BT compared to dystocic ewes (eutocic = 451.99 ± 61.83 , dystocic = 222.73 ± 50.66 ; $P = 0.016$).

4. Discussion

The aim of this trial was to investigate the differences in behaviour immediately post-lambing between ewes that have experienced a eutocic labour and a dystocic labour.

4.1. Dystocic events lead to lower maternal behaviour expression

Merinos are highly gregarious, and in an attempt to return to the flock quickly, may move from the birth site before the lamb is able to follow [2] or abandon mute or low-vigour lambs [24]. Dystocia can affect the expression of correct postnatal behaviour in the ewe and the lamb, leading to poorer outcomes compared to eutocia. In this study, we examined the effect of duration of lambing on the postpartum behaviour of Merino ewes. Sire breeds used in the study included both Merino and Border Leicester rams, but it was outside the scope of this study to investigate the effect of the lamb genotype on the behavioural expression of the ewe, as the available video data for dystocic ewes included mainly ewes carrying cross-bred lambs. From the evidence presented in previous studies, it appears that lamb breed has no effect on neonatal survival in the first 24 h of birth [19,22] although Kleeman et al. observe higher survival from 24 h to 3 months of age in Border Leicester cross-bred lambs compared to pure Merino lambs. In a genetic analysis of historic records of over 14,000 lambs, Hatcher et al. (2010) found very low heritabilities for lamb viability and the performance of the dam or rearing abilities. In summary, genetic studies of lamb survival provide no direct evidence of an effect of lamb genotype on neonatal lamb survival. Earlier studies of the development of the ewe-lamb bond had found that lamb breed influenced the lambs' ability to recognise their mother in arena tests [25] and effects of lamb breed on lamb behaviour may lead to differences in maternal behaviour of same-breed ewes, as reviewed by Lindsay et al. [23]. It must be emphasised that in the present study, no conclusions can be drawn on the effect of lamb breed on maternal behaviour due to the limitations of the study. Similarly, no conclusion can be drawn from our data set on the validity of our observations for twin-bearing ewes as only one twin-bearing ewe was included in the study cohort.

It has been well described that maternal care immediately post birth is essential for the health, wellbeing and survival of the neonate [4,20,27] including physical protection and ingestion of colostrum [29]. A link between delayed maternal care and lamb mortality was established early on by Arnold and Morgan [3] who observed greater risk of lamb mortality when the start of maternal behaviours was delayed after parturition. The dysfunction of maternal behaviour expression increases the risk of insufficient bonding, leaving the lamb more vulnerable to starvation, exposure and abandonment [13,29]. Maternal care behaviours include grooming and licking, which are integral behaviours to facilitate an

exclusive and strong ewe-lamb bond [11,26]. [7] reported a longer latency to interact with the lamb for dystocic ewes compared with eutocic ewes, and [8] described poorer lamb survival outcomes for lambs that had experienced birth asphyxia, a common outcome for dystocic lambing events. Lamb vigour and vitality contribute to successful bonding and increased chance of survival [17], as active lambs are quicker to stand and quicker to suck. Dystocic events can cause hypoxic brain injury and physical injury to the lamb [32], decreasing the lamb's ability to stand, successfully seek the udder and follow the ewe appropriately.

In our study, a comprehensive ethogram was developed for the study of ewe post-birth behaviour, and this ethogram was used to investigate the differences of dystocic and eutocic ewe behaviour through continuous behavioural annotations and the analysis of behavioural time budgets. In the present study, dystocic ewes spent less time on maternal care behaviours overall and also displayed fewer bouts of maternal care. Dystocic ewes also spent less time circling and looking elsewhere, measures of ewe vigilance and protection of the lamb [31].

4.2. Dystocic events lead to greater expression of avoidance-type behaviours

In addition to the poorer quality of maternal care given by dystocic ewes, these ewes performed more avoidance type-behaviours. For example, a greater proportion of dystocic ewes performed lying behaviours compared to eutocic ewes, and dystocic ewes spent more time walking, stepping and standing. Bonding is facilitated by 'approach' behaviours, rather than 'avoid' behaviours, so it is reasonable to assume that an increase in 'avoid' behaviours results in poorer bond formation [30]. These avoidance-type behaviours are performed instead of maternal care behaviours, reducing the quality of the ewe-lamb bond and increasing the risk of lamb mortality [10]. It has been described that dystocic ewes are more likely to perform rejection-type behaviours, including longer latency to groom [1], rejection of suckling attempts [26], and rejecting/abandoning their lamb [7,15].

4.3. Dystocic events lead to lower total behavioural transitions compared to eutocic events

Dystocic ewes performed fewer behaviour transitions on average in the 2 h immediately following birth compared to eutocic ewes. This finding agrees with previous studies that describe dystocic ewes to be less active in the period after lambing [7]. Birth is a significant event requiring large amounts of energy to fuel myometrial contraction and increased activity of the dam [33,35]. It has been shown that dams experience pronounced hyperglycaemia around birth [6], likely to facilitate the final stages of parturition and provision of adequate aftercare [5]. As dystocia is a prolonged or non-progressive labour, the depletion of these energy reserves lead to maternal exhaustion and a reduced ability to provide satisfactory aftercare [16].

In our study, we found that overall ewe behaviour differed between eutocic and dystocic ewes. As behaviour research is laborious, remote sensing techniques are becoming more appealing. Lambing events as such can be detected from on-animal devices [34]. Behavioural metrics and more detailed classification of sheep behaviour from movement data may help in the future the development of algorithms to identify difficult labour events and associated impaired maternal behaviour.

5. Conclusion

Ewes that had experienced a difficult labour performed fewer behaviour transitions in the 2 h immediately following birth compared with ewes that had experienced a normal birth. More specifically, ewes that had experienced a difficult lambing event performed less maternal care behaviours compared with ewes that had experienced a normal labour. Ewes spent less time grooming, licking and bonding with their lambs, and also performed fewer circling and looking elsewhere events. Finally, ewes that had experienced a difficult lambing event spent more time performing avoidance behaviours such as walking, stepping and standing, and were more likely to perform lying behaviours compared to ewes that had experienced a normal lambing event.

These findings suggest that dystocia has a marked effect on the ability of the ewe to provide the same amount of care to her lamb(s) compared with eutocic ewes. Reduced maternal care in the period following birth is likely to affect the development of the bond between ewe and lamb and in consequence the chance of survival of the neonatal lamb. Our work has demonstrated that dystocia affects the amount of maternal care provided by the ewe following birth and has defined overall characteristics of ewe behaviour following birth. Further work is required to address the effects of litter size and other factors on maternal ewe behaviour.

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CRediT authorship contribution statement

Amellia Redfearn: Investigation, Methodology, Writing – original draft. **Estelle Janodet:** Data curation, Methodology. **Jody McNally:** Investigation, Project administration. **Heather Brewer:** Investigation, Project administration. **Emma Doyle:** Supervision. **Rebecca Doyle:** Supervision. **Sabine Schmoelzl:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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