Physiological and behavioural patterns associated with prolonged lambing events in sheep

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Certification

I certify that this work does not contain material which has been or will be submitted for the award of any other degree or diploma in my name at any other university or tertiary institution. To the best of my knowledge, this work does not contain material previously written or published by another person, except where due reference has been made in the text.

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[redacted]

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Love Amellia

P.S. To those that tried to steal my light, silence my voice, make me doubt my ability and question my worth: Look at me now. I did it. I won. I persevered, I trusted myself, and I BLOODY DID IT!

P.P.S. Sheep rule. That is all.

Preface

This thesis is a collection of manuscripts intended to be published as separate articles. At least one manuscript has been accepted for publication, and therefore qualifies this thesis to be completed as a 'Thesis by publication'.

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Abstract

Dystocia is one of the most important issues faced by sheep producers nationally and internationally. In Australia specifically, dystocia directly and indirectly accounts for 50-75% of all neonatal lamb mortalities. Similar figures have been reported in flocks in New Zealand and the UK. Dystocia has been the subject of research for decades; however, the outcome studied is almost always 'number of lambs dead', therefore excluding dystocic events that did not result in death.

This thesis is comprised of a series of experimental manuscripts investigating dystocia and its effect on maternal behaviour pre- and post-birth; assessing on-animal accelerometers to identify differences in eutocic and dystocic ewe activity pre-birth; and using pen-side blood analysis techniques to identify maternal blood markers that may be indicative of a dystocic event before and after birth.

Dystocia has a marked effect on the behaviour of the ewe in the hours before birth. Dystocic ewes are significantly more active overall – identified by accelerometer data alongside traditional observation and annotation methods – but reach the peak in their activity 3 h before birth, whereas eutocic ewes reach the peak in activity at birth. There is also a significant difference in ewe behaviour after birth, with dystocic ewes performing fewer maternal care behaviours and more avoidance behaviours. Finally, there are multiple blood markers seen to be different in eutocic and dystocic ewes before and immediately after birth; however they are cohort-dependent.

Dystocia is a complex and multifaceted problem, and requires a multifaceted approach in order to appropriately address the underlying issues and develop practical detection and prevention strategies.

Background

Historically, the sheep industry has been highly valuable to Australia's economy, with the first international trade of wool occurring in 1807. By 1830, the value of exported Merino wool reached $2 million (equivalent to $243 million in 2022), quickly becoming the country's main export. Currently, Australia is the largest sheepmeat producer and exporter in the world, valued at approximately $7.2 billion in 2019 (Meat & Livestock Australia, 2020), and the value of high quality Merino wool exports was approximately $4.4 billion in 2017-18 (ABARES, 2017).

In a report prepared by Lane et al. (2015) listing endemic diseases for the red meat industries, the greatest national cost to the sheep industry was perinatal mortality, valued at approximately AU$540 million per year. This figure is around $100 million more than internal parasites, and over double the cost of flystrike, both of which are well-known issues and are readily accepted and addressed. This indicates more focus should be put on perinatal lamb mortality, its causes and preventions.

This review will explore the drivers of neonatal lamb mortality with a focus on dystocia, and factors that may put some ewes and lambs at higher risk. It will also begin to discuss various methods of animal monitoring with the aim of improving ewe and lamb outcomes.

Lamb mortality

Lamb mortality is highest in the first three days of life, and is an issue faced in every sheep production system (Alexander & Peterson, 1961). Despite improvements in flock productivity, with an increased focus on selection for Number of Lambs Weaned (NLW), mortality rates have remained troublingly high at a national average of 20% (Hinch & Brien, 2014; Kelly, 1992; Refshauge et al., 2016). The increased twinning rates may offset the gains made in lamb survival, as twins have lower survival rates than singles (Purser & Young, 1964). Decades of research into lamb mortality have successfully described management strategies that can reduce lamb mortality to some degree; however mortality is still too high. Despite the knowledge that there is a role of genetics in lamb survival, so far there has not been a phenotype described that could be implemented in breeding programs to improve lambing outcomes.

Static mortality rates may also be explained by a lack of understanding of the underlying causes. To be able to address lamb mortality, the cause of death must first be identified accurately. Studies from as early as the 1950s have investigated the causes of neonatal mortality in sheep and they classed predation or starvation/mismothering to be the main cause of mortality (McHugh & Edwards, 1958; Moule, 1954). In the decade following, similar results were published; starvation/mismothering and predation as primary causes of lamb losses (McDonald, 1966; Moore et al., 1966; Smith, 1964). One study reported that almost 67% of lamb deaths were as a result of starvation/mismothering. There was however one study that reflected results that are more commonly seen in newer research; dystocia was the leading cause of perinatal death at 53.6%, and starvation/mismothering and predation accounted for 6.8% and 7.4% respectively (Hughes et al., 1964). The research that followed on from these studies showed similar trends. While the percentages of lambs lost to starvation/mismothering remained high, fewer lambs were lost to predation, and more due to dystocia. The changes in reported data are most likely due to more thorough *post mortem* examinations, including observation of the central nervous system (CNS) for oedema, haemorrhage or lesions indicative of dystocia (Holst, 2004) leading to a more accurate diagnosis of cause of death. In an extensive study collecting data from more than 3000 lamb *post mortems* across various locations, breeds and years, it was reported that dystocia was the leading cause of lamb mortality (Refshauge et al., 2016). Dystocia, stillbirth with CNS lesions (dystocia 2) and birth injury (dystocia 3) accounted for 9%, 21% and 18% of all lamb deaths respectively (Figure 1).

From the advances in *post mortem* techniques and more accurate determination of the cause of death, it is now evident that the greatest single contributor to neonatal lamb mortality is dystocia. A meta-analysis performed by Bruce et al. (2021) came to very similar conclusions.

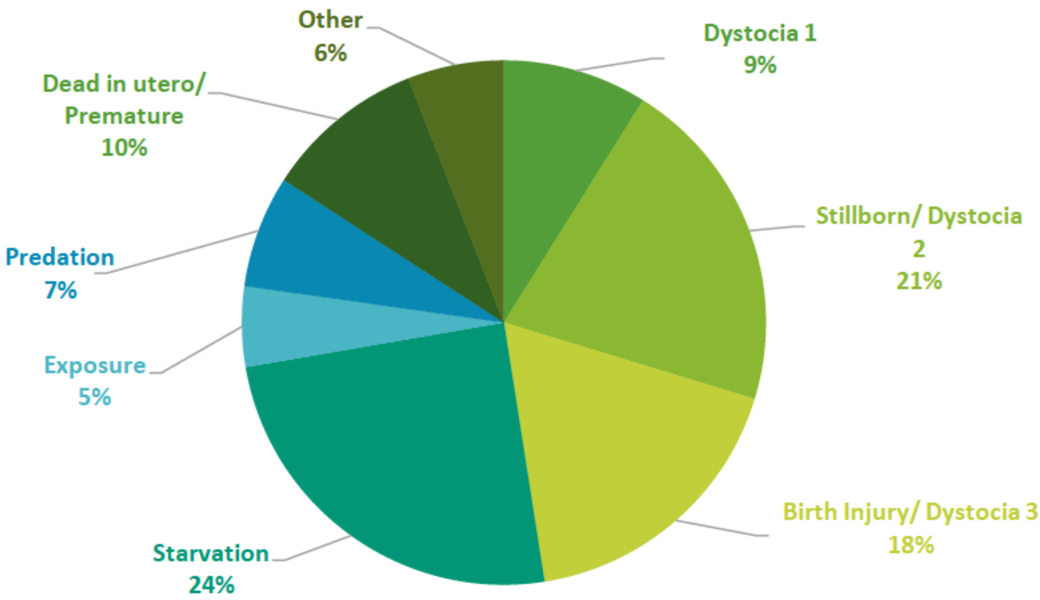

Figure 1 Causes of neonatal lamb mortality adapted from Refshauge et al., (2016). Dystocia accounts for 48% of lamb deaths, starvation accounts for 24% and exposure, predation, premature and other account for 5%, 7%, 10% and 6% respectively.

Dystocia

Labour consists of three phases: phase 1 – cervical dilation and movement of the foetus into the birth canal; phase 2 – active labour, pronounced abdominal straining and expulsion of the foetus, and; 3 – expulsion of the placenta. Generally speaking, dystocia is characterised as a prolonged or non-progressive phase 2 of labour. As it is the leading cause of lamb mortality and it increases the risk of ewe mortality, dystocia poses significant welfare and economic issues (Bickell et al., 2010; Bruce et al., 2021; Lane et al., 2015; McHugh et al., 2016).

Dystocia is a multifactorial issue influenced by environmental, lamb and ewe factors including – but not limited to – maternal nutrition (Dwyer et al., 2003; Mulvaney et al., 2008), uterine inertia (Jacobson et al., 2020), ewe breed (Everett-Hincks et al., 2014; Holst & Allan, 1992; Holst et al., 2002), ewe parity (Kleemann & Walker, 2005; McDonald, 1966; Purser & Young, 1964; Robertson et al., 2017), foetopelvic disproportion (Jacobson et al., 2020), malpresentation (Jackson, 2004); litter size (Everett-Hincks et al., 2005; Holst et al., 2002; McHugh et al., 2016; Schreurs et al., 2010), sire breed (Dwyer & Bünger, 2012) and birth weight (Holst & Allan, 1992; Holst et al., 2002; McHugh et al., 2016).

Dystocia can cause injury, hypoxia, CNS damage and death of the lamb (Holst et al., 2002; Jacobson et al., 2020; Refshauge et al., 2016). If the lamb survives birth, the CNS damage can reduce the lamb's vigour, or diminish its ability to stand, suckle and follow its dam, increasing the risk of death by starvation, exposure and predation (Dwyer et al., 1996). It can be argued that the functional impairment that leaves the lamb vulnerable to starvation, exposure and predation is directly caused by dystocia, and therefore the cause of death is primary dystocia and secondary starvation/exposure/predation.

Dystocia can cause pain, exhaustion, injury, maternal behaviour dysfunction and even death of the dam (Bickell et al., 2010; Darwish & Ashmawy, 2011; El-Hamamy & Arulkumaran, 2005; Jacobson et al., 2020). Maternal behaviour dysfunction, including expression of rejection behaviours, can occur after a prolonged or difficult birth event and will in turn leave the lamb vulnerable to other causes of death (Dwyer et al., 2003; Jacobson et al., 2020).

Maternal behaviour

In mammalian species, especially in livestock, the survival of the neonate is almost completely dependent on the dam for survival. Appropriate and sufficient maternal care is crucial for establishing a strong and exclusive dam-young bond (Bickell et al., 2010; Nowak et al., 2011; Nowak & Boivin, 2015). In sheep, the lamb relies on the ewe for nutrition (Nowak, 2000), primary immunological support (Nowak & Poindron, 2006) and physical protection from flock members and predators (Dwyer, 2014; Pickup & Dwyer, 2011). Successful dam-young bonding is a co-ordinated and sustained effort by both the dam and the neonate (Dwyer, 2014; Nowak, 2000). In sheep, ewes tend to pick an isolated area for the birth site where she will stay for the immediate postparturient period (Nowak & Poindron, 2006). This is less common in highly gregarious breeds such as Merinos (Alexander et al., 1983) where ewes return to the flock within a few hours. Once the lamb is born, the ewe almost immediately begins licking the lamb, starting at the head and working her way down the lamb's body. Licking serves to dry and stimulate the lamb (Alexander, 1988; Nowak & Poindron, 2006), remove birth fluids and allows the ewe to learn the lamb's unique scent (Poindron et al., 2007). While the ewe is licking the lamb, she is also emitting low-pitched 'maternal' bleats, thought to be involved with bond formation (Dwyer, 2014; Nowak, 1996) and recognition after the immediate post-parturient period (Pickup & Dwyer, 2011). Once the lamb successfully stands and begins teat-seeking, the ewe will stand still and assume a 'sucking' posture with her hind legs extended and belly lifted to enable easy access to the udder (Alexander, 1988; Nowak, 2000). These interactions are reinforced by the lamb's efforts to vocalise, stand and locate the udder (Dwyer et al., 2003). Behaviour dysfunction is when a breakdown occurs with one behaviour or multiple behaviours. For example, if a ewe does not lick her lamb, the bond between her and her lamb may be weak or easily broken (Nowak & Poindron, 2006). Additionally, if a lamb has low vigour or it is not vocalising, ewes may become disinterested and abandon the lamb (Bickell et al., 2010; Dwyer et al., 2003; Nowak & Poindron, 2006). Dysfunction of bond-forming behaviour expression is often caused or exacerbated by dystocia (Alexander, 1960; Alexander et al., 1993), as the ewe may be exhausted and disinterested in the lamb, and the lamb may have cognitive impairments due to hypoxic brain injury (Bickell et al., 2010; Darwish & Ashmawy, 2011). It has been reported that ewes are able to distinguish between the bleat of a healthy lamb and a hypoxic lamb, and will engage more readily with the healthy lamb (Morton et al., 2018). It is therefore imperative that the underlying causes of dystocia are recognised, animals are managed properly to reduce the risk of dystocia, and incidences of dystocia are correctly identified and addressed appropriately.

Management and prevention strategies

As technology improves and becomes more accessible, a greater number of producers are embracing the use of technology to make informed decisions in their animal breeding systems. One such example is real-time ultrasound scanning. A skilled operator can provide fast, cost-effective and accurate information on pregnancy status (pregnant or dry), litter size (singleton, twin, higher multiple) and even foetal age, generally accurate within 4 days (Fowler & Wilkins, 1984; Jones & Reed, 2017). This information allows the ewes to be separated for targeted management not only on pregnancy status, but on litter size, which is strongly recommended by the literature to improve performance and decrease mortality (Behrendt et al., 2011; Jacobson et al., 2020; Oldham et al., 2011). Additionally, knowing how many lambs to expect, and when to expect them ensures the producer is prepared for lambing, and collects accurate information on survival rates for their flock (Jones & Reed, 2017).

Proper nutrition is essential for optimum animal performance and is especially important for pregnant and lactating ewes. In the peri-conception period, a restricted feed intake and an *ad libitum* intake have shown to be detrimental to conception rate (Parr, 1992), especially in ewe lambs (Mulvaney et al., 2008). In a study following the effect of peri-conceptional nutrition on gestation length, growth of the foetus and neonatal behaviour, Kleemann et al. (2015) reported that ewes on the low nutrition treatment produced lambs that were smaller, had less peri-renal fat and greater liver mass than those fed maintenance and above. Similarly, Vonnahme et al. (2003) reported greater liver mass, smaller foetus size, lower maternal weight and lower maternal and foetal glucose concentrations in ewes that were feed-restricted in early- to mid-gestation. Holst & Allan (1992) also investigated the effect of mid-pregnancy feed-restriction, and they reported that feed-restricted ewes had a longer gestation period, therefore producing heavier lambs than non-feed-restricted ewes. Kenyon et al. (2011) investigated various nutritional treatments on the performance of ewes and their lambs. Despite changes in ewe liveweight, no differences were seen in any production parameters measured. They also concluded that there was no benefit in feeding twin-bearing ewes above pregnancy maintenance requirements in mid- to late-pregnancy. Another study by McGovern et al. (2015) was performed to investigate late-gestation nutritional treatments on foetal development and growth to weaning. They reported lambs born to high-nutrition ewes were larger and quicker to stand and suck, and were heavier at weaning. There appears to be conflicting information surrounding ewe nutrition, as results may be dependent on timing, ewe factors and environmental factors among others. It is clear that nutrition influences ewe and lamb performance, and has an impact on the risk of dystocia and lamb survival. It is therefore crucial that ewes are monitored and fed according to their requirements during the different stages of pregnancy to achieve optimum performance.

Animal monitoring is one of the most important practices in any animal production scenario. Observing an animal, their environment, their feeding patterns and movements can provide a wealth of information about the health and welfare of that animal, and provide guidance on management decisions. This is particularly important in pregnant and lactating animals, as their needs are vastly different from non-pregnant animals. As nutrition is an important influence on labour outcome, it may be possible to use blood biochemistry to monitor dam health around parturition and detect possible disturbances that may increase the likelihood of adverse outcomes. For example, disturbances in maternal ion concentrations (calcium, potassium, sodium) may lead to dystocia, as they play essential roles in myocyte excitability and uterine contractility (Bernstein et al., 2014; Brainard et al., 2007; Dunford et al., 2020; Jones et al., 2004). Additionally, as glucose appears to be the main energy source for myometrial cells (Rizzo et al., 2011; Steingrímsdóttir et al., 1993), an energy deficit in parturient ewes may lead to weak contractions, poor labour progression and exhaustion. There are several other physiological markers of a prolonged labour, including elevated creatinine (Ali et al., 2016; Ghoneim et al., 2016; Salazar, 2014), elevated lactic acid and disturbed acid-base markers (Musaba et al., 2019; Quenby et al., 2004). These markers may be used to detect or confirm a dystocic event; however, more research needs to be done to evaluate their suitability for predicting dystocia.

Behaviour monitoring has become more popular in recent years as a tool to assess stock health and welfare and as a way to inform management decisions (Nawroth et al., 2019). In late-gestation and during labour, the behaviour of the dam may change, including increased isolation, nesting behaviours and decreased feed intake (Alexander, 1988; Dwyer & Lawrence, 2005; Miedema et al., 2011; Mukasa-Mugerwa & Mattoni, 1990; Waters et al., 2021). Observation of these behavioural changes can enable stock people to be vigilant and present when and if assistance is needed. There are reports of behavioural differences between eutocic and dystocic birth events (Barrier et al., 2012; Proudfoot et al., 2009; Wehrend et al., 2006); however these are much more difficult to observe in a commercial setting due to large flock size (Chang et al., 2020).

Remote monitoring of animal behaviour is an attractive alternative to traditional observation methods (Brown et al., 2013; Riaboff et al., 2022), and may be especially useful in monitoring parturition progress. Some studies have investigated the use of accelerometers to detect lambing in ewes (Gurule et al., 2021; Smith et al., 2020). While the activity of lambing ewes was able to be detected and differentiated from a non-lambing day, there have not been any studies comparing the behaviour/activity of eutocic ewes in comparison to dystocic ewes. This may be an important area to continue research, as the detection and prevention of dystocia is the most promising method of improving neonatal lamb survival.

A better understanding of dystocia, in particular the effects of dystocia on ewe behaviour, will help to inform future research into better management of dystocia and through this prevention of lamb mortality. Researching dystocia is, however, made difficult by the labour-intensive nature of direct observation. Understanding of differences in observable behaviour will help develop research tools to differentiate between normal and dystocic lambing events, and such tools can then be used to investigate the underlying causes of dystocia at scale. Remote sensing of livestock behaviour has in recent years developed into a promising approach to detect various behaviours (Greenwood et al. 2017). In the main, sensor-based detection algorithms focus on frequent behaviours such as grazing, walking or lying, to name a few (Hu et al. 2020). Rare events such as lambing require specialised approaches (Dobos et al. 2014) (Smith et al. 2020). Understanding effects of dystocia on ewe behaviour can help not only better understand the effects of dystocia on reproductive outcomes but also help refine birth detection algorithms to differentiate between normal and dystocic lambing events.

Research questions

From the review of the literature, a list of research questions to investigate in this thesis were developed:

1. Are there observable differences in ewe behaviour between eutocic and dystocic lambing events? [Chapter 2]
2. How does dystocia affect the behaviour expression of ewes after birth? [Chapter 3]
3. Can accelerometers be used to capture the differences in lambing activity between eutocic and dystocic ewes? [Chapter 2]
4. Can penside blood analysis be used to identify and/or predict dystocic events in the periparturient period? [Chapter 4]

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Chapter 2: Pre-partum behavioural transitions differ depending on labour difficulty in Merino ewes

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Pre-partum behavioural transitions increase with difficult birth events in Merino ewes

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Abstract

Lambing is a period of increased risk for lambs and ewes. Despite decades of research, lamb survival remains an issue of high importance to the Australian sheep industry. Across different breeds, locations and seasons, birth injury due to dystocia (prolonged and/or difficult labour) has been conclusively identified as the leading cause of neonatal lamb mortality. In this trial, we focused on ewe behaviour in the 5.5 h leading up to birth and asked whether behaviour metrics and/or remote-sensing technology could be used to identify any differences between normal and difficult births. Lambing ewes were monitored by surveillance cameras for the entire lambing period. A group of ewes were selected for detailed annotation (eutocic n = 9; dystocic n = 6; classification by a single experienced observer). Videos were continuously annotated for the period of 5.5 h leading up to birth using Behavioural Observation Research Interactive Software (BORIS).

Dystocic ewes performed significantly more behaviour transitions than eutocic ewes ($P < 0.001$) and a significant interaction of time and birth difficulty was seen on behaviour transitions over the 5.5 h before birth ($P < 0.001$). Accelerometer data reflected behavioural data; dystocic ewes spent more time active ($P = <0.001$) and less time resting ($P = <0.001$) than eutocic ewes.

Dystocia has a significant effect on ewe behaviour transitions in the 5.5 h before lambing. This difference may help detect and predict dystocic lambing events.

Key words: dystocia, behaviour transitions, remote sensing, ethogram, sheep

Implications

Dystocic ewes performed more behaviour transitions in the 5.5 h before birth, but less behaviour transitions in the final 30 mins before birth.

During eutocia, behaviour transitions increase as birth approaches, with a peak in behaviour transitions at birth. Dystocic events reach this peak too early, and behaviour transitions decline until birth.

Ewe behaviour transitions may be used to identify eutocic and dystocic lambing events.

Behavioural studies could be paired with emerging technologies to collect more efficient observations of lambing events for future research.

Introduction

Dystocia, or a difficult and prolonged lambing event, is the largest contributing factor to neonatal lamb mortality in Australia (Refshauge et al., 2016). These difficult lambing events can increase the risk of injury and mortality in both ewe and lamb (Bickell et al., 2010; McHugh et al., 2016). There have been numerous studies undertaken to understand the causative factors of dystocia with little success in reducing lamb mortality (Hinch & Brien, 2014). It is true that lambing percentages have increased over the last 50 years as twinning rates have increased, and overall farm profitability has improved; however, the percentage of lambs lost has remained constant (Jacobson et al., 2020). Dystocia, and therefore neonatal lamb mortality, is a complex issue, influenced by nutrition (Dwyer et al., 2003; Mulvaney et al., 2008), ewe breed (Everett-Hincks et al., 2014; Holst & Allan, 1992; Holst et al., 2002), ewe parity (Alexander et al., 1993; McHugh et al., 2016), litter size (Everett-Hincks et al., 2005; Holst et al., 2002; McHugh et al., 2016; Schreurs et al., 2010) and birth weight (Holst and Allan, 1992; Holst et al., 2002; McHugh et al., 2016) to name a few. As no one variable is to blame, novel approaches to managing dystocia must therefore be explored.

Behaviour is becoming more commonly used for assessing stock health and welfare, and increasingly to make informed management decisions (Nawroth et al., 2019). As in-person observation is difficult with large flock sizes typical of commercial operations (Chang et al., 2020), remote-sensing technology proves an attractive option (Brown et al., 2013; Riaboff et al., 2022).

Previous studies in dairy cattle have showed that there are behavioural indicators of the commencement of parturition, identified as increasing frequency of tail lifts (Miedema et al., 2011), while Barrier et al. (2012) reported some behavioural differences between normal and dystocic calving events during parturition, including increased restlessness and lying behaviours. Neck-, ear- and tail-mounted accelerometers and inclinometers have been used to detect activity changes associated with parturition in livestock; however, it remains largely novel (Chang et al., 2020; Saint-Dizier & Chastant-Maillard, 2015).

This trial was performed in order to record the lambing behaviours of ewes in the hours leading up to the birth of the lamb with cameras and accelerometers, and analyse the differences in behaviour and activity. We hypothesised that difficult/dystocic birth events would be characterised by increased activity before birth. Additionally, we hypothesised that the accelerometers used would be able to record and report these different activity levels.

Materials and methods

Animals and management

Two hundred Merino ewes of mixed ages were joined either with Merino or Border Leicester rams (two rams for 100 ewes) for five weeks in March/April 2018. A large number of ewes was enrolled for twofold reasons; i) to increase the chance of observing a number of dystocic lambing events; and b) to meet the requirements of a concomitant study (Smith et al., 2020).

At 80 days after joining, ewes were scanned by a commercial ultrasound scanning provider for pregnancy status and parity/litter size. From this group, 100 ewes were selected for inclusion in the experiment based on predicted date of birth within a three-week period. Numbers were approximately balanced for parity (primiparous (n = 54) and multiparous ewes (n = 46)) and sire breed (Merino (n = 48) and Border Leicester (n = 52)). All twin-bearing ewes were enrolled in the trial (n = 12), and the rest was made up by single-bearing ewes (n = 88). Below average twinning rates prevented complete balancing of single- and twin-bearing ewes in this trial.

Two weeks before predicted lambing dates, ewes were assessed for Body Condition Score (BCS) by palpation of the lumbar region by a single assessor, paint branded with a unique number (Siromark, Heininger, Bibra Lake, WA) and the 20 ewes expected to lamb first were moved to the supervised lambing enclosure (approximately 75 m x 35 m). The rest of the ewes were moved to nearby holding paddocks.

Ewes had *ad libitum* access to pasture grass and water. Supplementary feed was provided in the form of lucerne hay twice daily (200 g/day per ewe) and a mix of sheep pellets and fava beans once daily (200 g/day per ewe; sheep pellets based on wheat, millrun and lucerne; 17.5% protein, 2.5% fat, 17% fibre, 20% ADF, 34% NDF). Throughout the experiment, rations were adjusted to maintain a BCS of ewes between 2.5 and 3.5. Health checks were performed multiple times a day, and any abnormalities – including illness or injury – were recorded and reported to the principal investigator and if necessary, the veterinarian on site.

Accelerometer deployment and collection

In the week before lambing was expected to commence, the first group of ewes was brought into the yards adjacent to the lambing enclosure. Each ewe was fitted with an ActiGraph tri-axial accelerometer (ActiGraph Corp, Pensacola, FL, USA) sampled at 10 Hz. The accelerometer was positioned on the left side of the neck, approximately halfway along the length of the neck. The accelerometers were mounted on an adjustable elastic strap with a plastic snap buckle, making them quick to fit and minimising handling time. Date and time of deployment, accelerometer number, ewe number, along with any comments were recorded for each accelerometer deployment. All accelerometers were removed in the middle of the lambing period for one day to charge. Each accelerometer was refitted to the same ewe it was removed from.

At least 48 h after a ewe had lambed, the ActiGraph was removed and date and time of removal was recorded. As pregnant ewes were brought into the enclosure, they were fitted with an accelerometer.

Lambing data

Ewes were first checked at 6:30am, and multiple times during the day, with the last check at 5:30pm. At least 2 h after a lamb was born, it was caught, weighed, sexed and tagged. The ewe was observed for any abnormalities and the ewe and lamb were observed to ensure the ewe called for the lamb and the lamb followed the ewe and sucked – indicating sufficient bonding. If there was an issue with bonding, the lamb and ewe were moved into a single pen indoors until sufficiently bonded. Of each lamb born in the supervised lambing enclosure, the time of birth was identified from the videos and recorded.

Ewes were monitored during parturition to ensure the wellbeing of the ewe and lamb(s). Once a ewe was identified as being in labour, progress was checked every 30 min until birth. If progress was not made within 1 h of membranes at the vulva, 1 h of feet visible, or 1 h of nose visible, the ewe was caught, manually assisted and excluded from further analysis. Assisted ewes were penned, monitored and treated (if necessary) before being released.

Behaviour data

The supervised lambing paddock was set up with 10 day- and night- vision video surveillance cameras (Hikvision Digital Technology Co, Hangzhou, China) positioned around the paddock to cover the entire area. Once a ewe had lambed, the video records were checked for a number of key events and descriptors. These included the first signs of lambing which ranged from isolation from the group, pawing at the ground all the way to contractions and seeing parts of the lamb; the first occurrence of pushing indicating active labour; first appearance of birth fluids, lamb parts, lamb head; and full expulsion of the lamb; as well as the visual quality of the videos, whether the ewe was able to be seen clearly the whole time; whether the recording was complete, and the birth type- whether the birth was normal (eutocic) or difficult (dystocic). All key events and descriptors were identified by a single observer. Mean length of parturition (from first signs to birth) was calculated for the selected ewes, and an annotation period of 330 min (5.5 h) was chosen to include as much of the dystocic events as possible without excessive annotation of eutocic events.

Dystocic events generally exceeded 120 min of active labour; however, the observer used observed behaviour to confirm difficulty. From the complete, clear and high-quality videos, nine eutocic and six dystocic labour events were selected for more detailed analysis. The eutocic events were selected to approximately match the date and time of each dystocic event. Three additional eutocic events were chosen to increase sample size. Dystocic events that occurred at different times of day were selected to ensure time of day was not a confounding factor.

Behavioural Observation Research Interactive Software (BORIS; Friard and Gamba, 2016) was used to continuously annotate a period of 5.5 h before full expulsion of the lamb. An ethogram was developed by observing normal and lambing-specific behaviours. After using the ethogram to annotate several lambing events, like behaviours were grouped together and rare or singular behaviours were removed. The refined ethogram (Table 1) was used to annotate the chosen lambing events in BORIS. Each behaviour was listed and grouped into categories (agitation, feeding, foetal events and locomotion).

Table 1 Ethogram for annotating lambing ewe 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
Aggression	Agitation	Point	Sheep strikes another with their head or leg.
Agitated	Agitation	Point	Kicking or biting at midsection; stomping feet- picking one foot up and putting it down with force greater than a regular step; shifting weight between feet while standing.
Lying intention	Agitation	Point	Going onto knees and getting back up.
Pawing	Agitation	Point	Sheep strikes the ground with hoof of a foreleg more than twice. Sheep may move dirt in the process. Head may be up or down.
Eating	Feeding	State	Grazing or consuming feed from the trough.
Ruminating	Feeding	State	Sheep is chewing without having eaten immediately beforehand. May be standing or lying.
Amnion burst	Foetal events	Point	Amniotic sac burst; “waters breaking”; can see more membranes protruding.

Foetus expelled	Foetal events	Point	All parts of the lamb are outside of the ewe.
Foetal parts	Foetal events	Point	Lamb parts visible- usually toes first, but can be nose.
Head out	Foetal events	Point	Head of the lamb is completely outside the ewe.
Mucus	Foetal events	Point	The first time mucus is visible at the vulva of the ewe.
Pushing	Foetal events	State	During labour, abdominal muscles visibly contract, ewe may vocalise, lift head or stretch out legs. May be standing or lying.
Circling	Locomotion	State	Turning 360˚ or more. Sheep may turn on the spot or walk in a circle smaller than 2m diameter. Head may be up or down.
Lying	Locomotion	State	Sheep is on the ground, with legs tucked under, or stretched out. Head may be up or down.
Pacing	Locomotion	State	Walking the same track more than twice in succession.
Shuffling	Locomotion	State	Sheep moves no more than 4 steps in succession before stopping. Head may be up or down. May be repeated.
Standing	Locomotion	State	Not moving; all four feet on the ground. Head may be up or down, ears relaxed.
Walking	Locomotion	State	Forwards movement with a balanced, even four-beat gait.
Not visible		State	Ewe is not able to be seen clearly or at all.
Other		Point	Behaviour does not fit into any other category.

Data processing

A summary of the annotation was provided as an output by BORIS and was split into 30 min blocks. The metric of interest, behavioural transitions (BT), was calculated as the number of behaviour changes for each 30 min block. BT was recorded for each ewe observed for 5.5h before the lamb was born. Additionally, total BT (sum of all behaviour transitions over 5.5h) was calculated for each ewe.

The range of behaviours performed by each ewe were recorded and tabled. Comparisons between the two birth types were made for each individual behaviour and for each behaviour category.

For comparison of BT relative to start of parturition, time 0 was set to -210 minutes for eutocic events and –330 minutes for dystocic events.

Accelerometer data were analysed for the same period of time before birth for the same ewes.

Statistics

Statistical analyses were performed using R (R Foundation for Statistical Comptuting, Vienna, Austria; Team, 2013). A GLM with binomial distribution (logit link function) was used to analyse the outcome of lambing, presented as 0 = normal labour, 1 = difficult labour.

GLMs with Poisson distribution (log link function) were used for count data (eg, behavioural transitions).

A repeated measures GLM (GLMER) with Poisson distribution (log link function) was used to analyse count data over time (eg, behavioural transitions over time). Individual ewe ID was fitted as a random factor to account for repeated measures from each animal.

For GLMs, the interactions between lamb breed, litter size and labour difficulty were included. For GLMER, the interaction of difficulty and time was included.

When analysing binary outcomes (normal or difficult labour), chi square tests were used. When analysing count data (behavioural transitions), type III Wald chi square tests were used.

Accelerometer data were loaded onto a computer and analysed for a period of 5.5 h before birth, using ActiLife v6.13.3 (ActiGraph Corp, Pensacola, FL, USA). The metric used for this analysis was "activity" and had two levels; resting and active. The 'resting' category in this context included both quietly standing and laying down. The 'active' category included light and moderate activity levels. For both birth types, the average percentage of time spent in both activity levels was calculated. Student's t-tests were used in Genstat (VSN International, 2019) to compare the percentage of time spent in each activity level (± SD) between the two birth types; and between activity levels for both birth types.

For all tests, significance was considered achieved when $P \leq 0.05$.

Results

Labour length for eutocic events was 56.78 ± 23.03 min of stage 2 parturition, whereas dystocic events were on average 220.67 ± 113.91 min ($P = 0.001$; Figure 1).

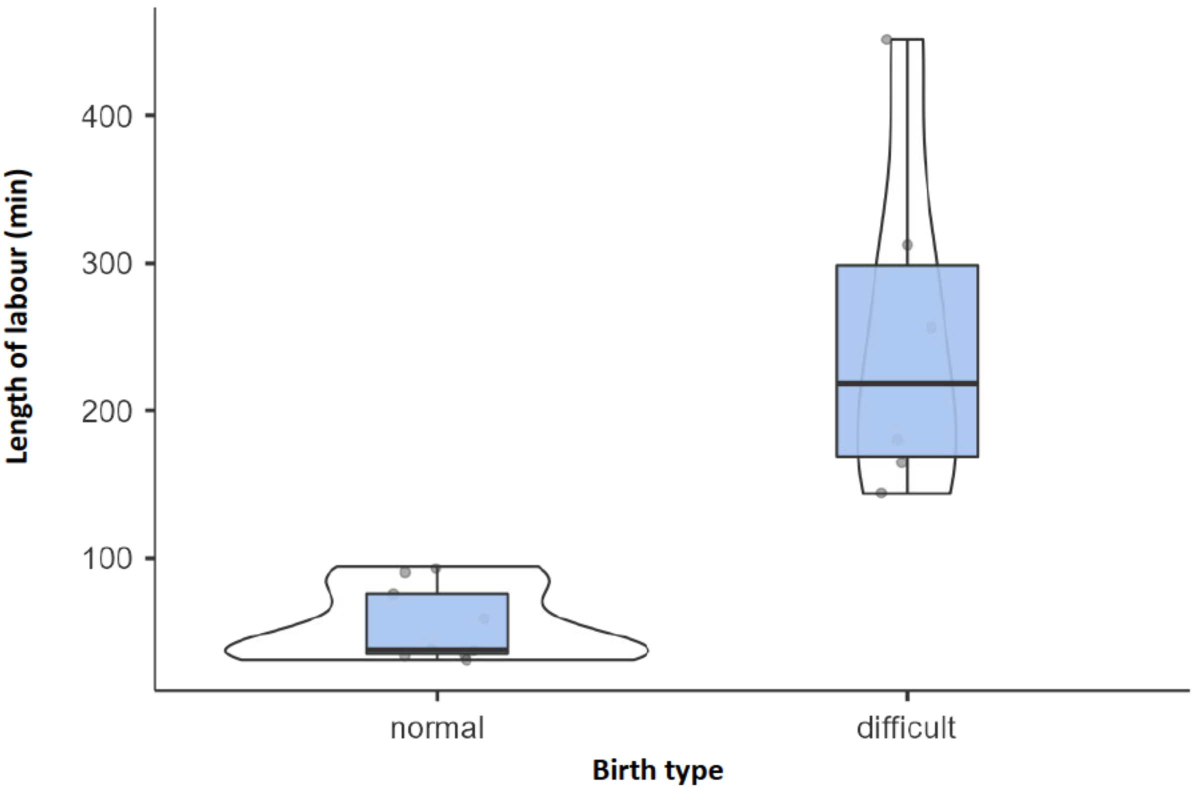

Figure 1 Length (min) of stage 2 parturition (active labour) for lambing events classed as "normal" (eutocic) or "difficult" (dystocic).

Lamb breed may influence lambing difficulty, but litter size may not

Of the 15 ewes selected for detailed annotation, nine ewes experienced a normal labour, and six ewes experienced a difficult labour (Table 2). Of the normal labours, there were seven singles and two sets of twins; four pure Merinos and five Border Leicester crosses. Of the difficult labours, there were five singles and one set of twins; zero Merinos and six Border Leicester crosses. In this experiment, litter size did not influence labour difficulty; however, lamb breed did ($P = 0.02$).

Table 2 Distribution of ewes according to difficulty, sire breed and litter size selected for detailed annotation.

Birth difficulty	Sire Breed	Litter Size	Number of ewes
Eutocic	Merino	Single	4
		Twin	-
	Border Leicester	Single	3
		Twin	2
Dystocic	Merino	Single	-
		Twin	-
	Border Leicester	Single	5
		Twin	1

Birth difficulty influences total BT

Litter size and lamb breed had no effect on total BT. Dystocic ewes changed behaviours significantly more frequently than eutocic ewes in the 5.5 hours before birth (dystocic = 754 ± 141.57, eutocic = 398 ± 57.71; P < 0.0001).

In contrast to results for the 5.5 h period before birth, in the final 30 min before birth, eutocic ewes moved between behaviours significantly more than dystocic ewes (eutocic = 106 ± 49.39, dystocic = 70 ± 39.00; P < 0.0001). Litter size and lamb breed had no effect on BT in the final 30 minutes of labour.

The interaction of time and difficulty influences BT over 5.5 h of labour

Significant time x difficulty interactions occurred for BT over the 5.5 h of observation (P < 0.001; Figure 2) when analysing the data in 30 min time brackets. From 330 to 60 minutes before lambing, BT was higher for dystocic lambing events compared to eutocic lambing events. The difference increased from 210 minutes before lambing and the highest BT along with the strongest difference between birth types was observed at 150 minutes before lambing. After this peak, dystocic ewes performed decreasing numbers of behaviour transitions until the lamb was born. BT was higher for eutocic than for dystocic birth events in the final 30 minutes before lambing.

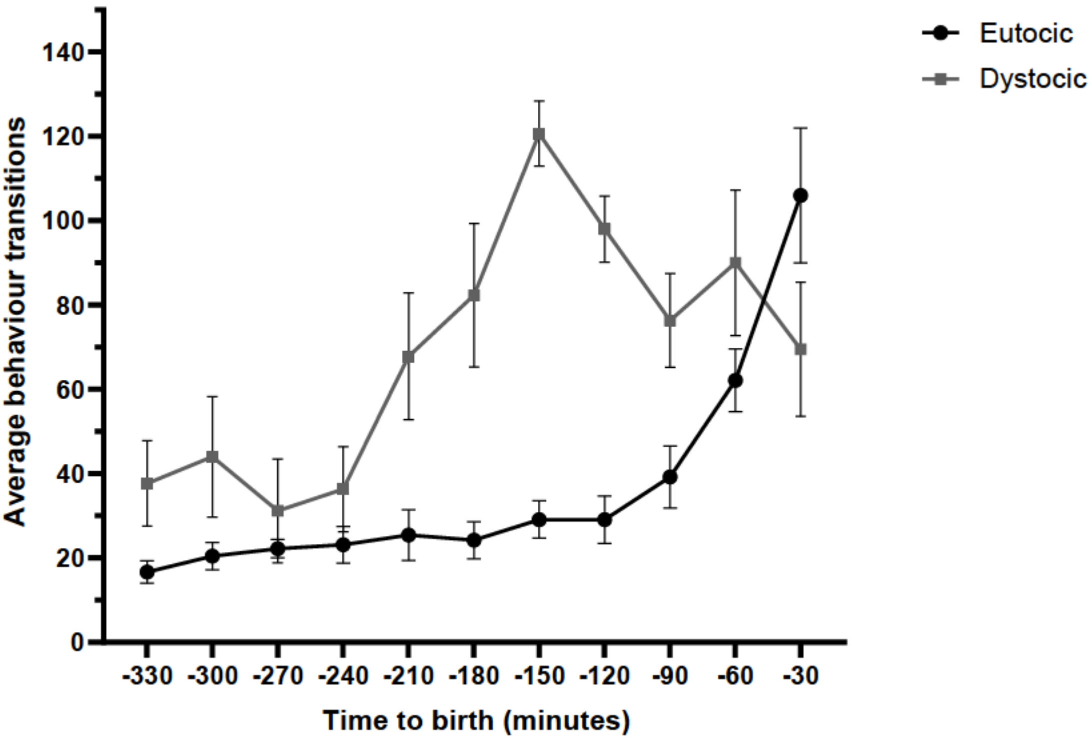

Figure 2 Average BT (mean values ± SEM error bars) in 30 min blocks over 330 min leading up to the birth of the lamb(s) for eutocic and dystocic birth events.

When comparing the curves in Figure 3, there were similar slopes apparent in eutocic ewes from -210 to 0 and in dystocic ewes from -330 to -150. To investigate the development of BT relative to the start of parturition rather than relative to birth, time points were adjusted by resetting time 0 to the timepoints at -210 minutes for eutocic events and -330 minutes for dystocic events (Figure 3). There were no significant differences of BT between eutocic and dystocic ewes at any time point for the period of 180 minutes from start of parturition.

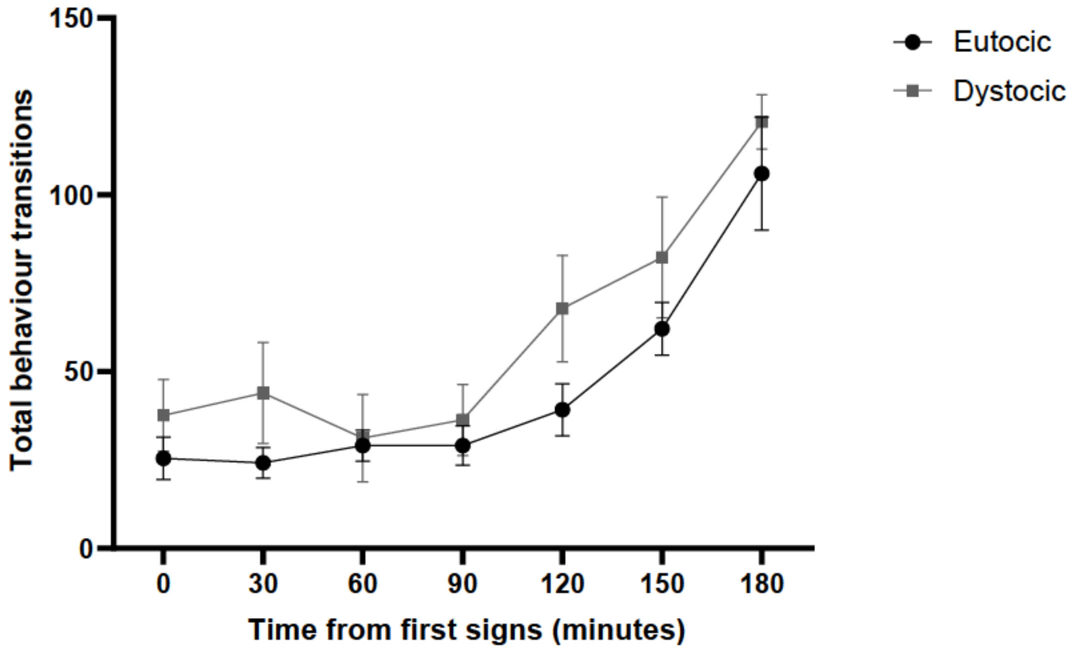

Figure 3 Behaviour transitions from time of first signs of parturition until the peak of behaviour transitions for eutocic and dystocic lambing events. Peak behaviour transitions occured at birth for eutocia, but not for dystocia

When considering individual behaviours, there were significant differences between eutocic and dystocic birth events in the frequency of which behaviours were performed (Table 3), but there were no behaviours that were performed only in eutocia or only in dystocia.

Table 3 Total frequency (mean ± SEM) of behaviours performed by ewes experiencing eutocia or dystocia in the 5.5 h preceding birth.

	Total # Occurrences		
Behaviour	**Eutocia**	**Dystocia**	***P* value**
Walking	131.33 ± 8.34	202.33 ± 34.08	0.09
Standing	132.22 ± 8.37	214.50 ± 32.08	0.05
Lying	29.11 ± 5.02	60.00 ± 11.07	0.04
Eating	8.56 ± 2.72	10.67 ± 8.38	0.82
Ruminating	2.11 ± 0.63	5.00 ± 2.66	0.33
Circling	9.00 ± 1.30	16.83 ± 11.98	0.54
Agitated	1.89 ± 1.07	9.50 ± 4.19	0.13
Lying Intention	3.00 ± 1.03	1.00 ± 0.52	0.11
Pawing	21.22 ± 8.32	102.17 ± 27.31	0.03
Aggression	3.44 ± 1.62	3.50 ± 2.92	0.99
Pushing	14.33 ± 2.80	74.17 ± 4.68	<0.0001
Shaking	2.89 ± 0.84	3.33 ± 1.58	0.81

Accelerometers to identify dystocia

Eutocic ewes spent significantly more time resting than dystocic ewes (eutocic = 0.70 ± 0.059, dystocic = 0.53 ± 0.084; $P < 0.001$; Figure 4).

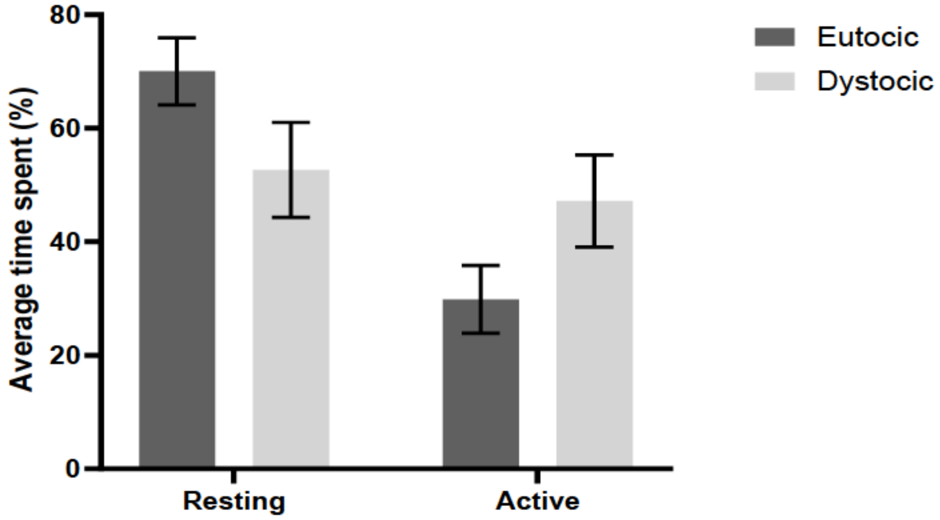

Figure 4 Percentage of time (mean ± SD error bars) spent resting and active in the 5.5 h before birth for ewes that have experienced a eutocic or dystocic labour.

Eutocic ewes spent significantly more time resting than active in the 5.5 h prior to birth (resting = 0.70 ± 0.059, active = 0.30 ± 0.059; $P < 0.001$). Additionally, dystocic ewes spent equivalent time resting and active in the same period (resting = 0.53 ± 0.084, active = 0.47 ± 0.084; $P = 0.15$).

Discussion

This trial was designed to identify behavioural differences between eutocic and dystocic lambing events in a group of 15 detailed annotations. There were differences between birth types in the frequency at which specific behaviours were performed; however, there were no behaviours exclusive to eutocia or dystocia. Rather than individual behaviour metrics, BT was more indicative of birth difficulty in the 5.5 h before birth.

The present study was designed to draw conclusions about the effects of birth difficulty on BT; however, lamb breed or litter size may have had an effect on the results. Sample sizes were not sufficient to be able to make generalised conclusions on the effect of lamb breed or litter size, although in this particular trial, we saw that lamb breed influenced labour difficulty, but litter size did not. Further investigation into the whole mob will provide clarity to the extent of the effects of lamb breed and litter size. Some breeds are predisposed to labour difficulty (McHugh et al., 2016) and this risk may be increased by crossing larger framed rams and smaller framed ewes, such as Merino ewes with Border Leicester rams, as were used in this trial, and were found to be more likely to experience a difficult labour than ewes carrying pure Merino lambs.

The observation of litter size not showing an effect on birth difficulty agrees with previous research by Alexander et al. (1993) stating that litter size had no effect on labour difficulty. Twin lambs have lower survival rates than single lambs; however, this can be attributed to lower birth weights rather than complications with birth (Purser & Young, 1964). In contrast, McHugh et al. (2016) found that twinning ewes had lower difficulty scores than single-bearing ewes, although the ewes in that study were not Merino ewes, and hence results may not be directly comparable.

Birth difficulty influences BT

In our trial, birth difficulty had a significant effect on total BT; however, lamb breed and litter size did not. Dystocic ewes performed significantly more behaviour transitions than eutocic ewes in the 5.5 h preceding birth.

Several studies have reported post-birth behavioural differences between eutocic and dystocic lambing events in ewes (Darwish & Ashmawy, 2011) and lambs (Dwyer, 2003; Dwyer & Bünger, 2012); however, little is known so far about pre-birth behaviour in sheep. In humans, pre- and during-labour predictors of dystocia include anxiety, dehydration, height, age (Nahaee et al., 2020), obesity, gestational diabetes (Catalano et al., 2012) and elevated glucose concentration (Popova et al., 2016). A link between anxiety and increased activity has been explored in adult humans by Biddle et al. (2021). While a correlation between sitting and risk of depression was shown, correlation to anxiety was not found.

There were a number of differences in behaviour frequency for certain behaviours (Table 3), yet there were no behaviours that were exclusive to birth difficulty. Isolating and targeting a single behaviour is unlikely to assist in detecting and identifying dystocia, therefore average BT (of all behaviours) was used as the metric of interest. Similarly, a greater frequency of postural changes in the 24 h before calving was reported by Proudfoot et al. (2009), indicating that dystocia could be identified by behavioural observation. Additionally, Barrier et al. (2012) reported that dystocic cows displayed more contractions than normal cows, were more restless (greater frequency of postural changes) and raised their tails for longer than normal cows. Contrastingly, Miedema et al. (2011) and Barraclough et al. (2020) reported no behavioural differences between normal and difficult calving events in dairy cows. Behavioural observations may be useful in identifying dystocia; however, more research is required to develop consistent and reliable classifications.

Labour difficulty influenced the number of behaviour transitions in the final 30 minutes before the lamb was born. Eutocic ewes performed more behaviours in the final 30 min than dystocic ewes. This fits with the concept that for normal birth events, in readiness for giving birth, energy is mobilised in the form of glycogen, and used to meet the energetic demands of uterine contractions, delivery of the neonate and provision of adequate aftercare (Chew & Rinard, 1979; Comline & Silver, 1972). Dystocic ewes, using their stored energy during prolonged labour, may deplete energy stores by the time the lamb is born, leaving them unable to provide sufficient aftercare (Bickell et al., 2010; Nahaee et al., 2020; Rietema et al., 2015). There is also evidence for severe dystocia causing metabolic disruptions in the neonate, reducing the ability of the neonate to actively bond (Nowak et al., 2000; Nowak and Poindron, 2006) and survive without sufficient aftercare (Bellows & Lammoglia, 2000).

Prolonged labour in humans and cattle is associated with a stronger experience of pain (Barraclough et al., 2020; Nystedt et al., 2005) and it is reasonable to postulate that dystocia in sheep is experienced as a painful event by the ewe. It is possible that behaviour before birth is affected by pain experienced in the birth process. Pain is often associated with specific behaviours such as standing in stature position or recumbency after castration, mulesing or tail docking (Small et al., 2018). In our analysis, we found no singularly informative difference between eutocic and dystocic events in individual behaviours. Similarly, no behavioural difference of any individual behaviour was found in dairy cows (Barraclough et al., 2020); rather, behavioural transition frequency varied between the birth types.

Interestingly, we found that peak activity in dystocic vs eutocic birth differed by around 3 h. This corresponds to one of the criteria for dystocia in humans according to the World Health Organisation as summarised by Nystedt et al. (2005).

The interaction of time and difficulty influences BTF over 5.5 h of labour

We observed a significant interaction of time and for BT over the 5.5 h annotation period. In eutocic ewes the number of behaviour transitions increased over time, starting around 2.5-3 h before birth, and peaking when the lamb was born (Figure 2). Mukasa-Mugerwa and Mattoni (1990) reported an increase in restlessness around 3 h before parturition in normal birth events in *Bos indicus* cows; however, the behavioural observations were not continuous or detailed. In the same time period, dystocic ewes reached the peak of their behaviour transitions at -150 mins. This suggests a strong similarity in behaviour patterns for lambing in stage 1 of parturition. If then parturition does not progress through the birth canal in a dystocic labour event, birth does not occur at peak BT. When time points from start of increased activity and BT to peak BT were compared between dystocic and eutocic ewes (Figure 3), we did not find any significant differences between BT at any time point. The peak BT for eutocic ewes was at birth, whereas the peak for dystocic ewes was 2.5 h before birth. This confirms that BT characterises the progression of parturition in a meaningful way. Application of BT as a metric may be utilised to characterise lambing events and support identification of dystocic events. The approach taken here requires labour-intensive observation. Replacement of human operators through emerging technology may assist classification of labour events. This in turn will enable further studies into the underlying causes of dystocia in lambing ewes.

Accelerometers to identify dystocia

Remote-sensing devices are not recent additions to livestock management toolkits, as they have been used in grazing systems for over 20 years to replace human observation (Trotter et al., 2008; Turner et al., 2000). Global Positioning System (GPS) tracking collars have been deployed on cattle to monitor grazing activity and pasture utilisation at regular intervals across several days, to inform management decisions including pasture rotation and feed supplementation (Turner et al., 2000). As technologies have improved, so have data collection capabilities. Specific behaviours are now able to be identified, including walking, resting, grazing and ruminating, and can be monitored in near real-time (Bailey et al., 2018).

Parturition detection in livestock using remote sensing devices is a relatively recent development and is discussed thoroughly in reviews by Chang et al. (2020) and Saint-Dizier & Chastant-Maillard (2015). Neck-, ear- and tail-mounted accelerometers and inclinometers have been used to detect activity changes, belts to monitor uterine contractions, and intravaginal devices to monitor temperature changes, amniotic membrane rupture or birth of the neonate, some of which may be broken upon expulsion. There are some challenges faced when appropriating these devices for use in sheep, as they are much smaller in size, usually have some wool during lambing that may interfere with contraction detection, and in Australia, most sheep are tail-docked, rendering tail-mounted sensors incompatible. A recent study by Gurule et al. (2021) assessed the use of ear-tag accelerometers to detect parturition-related behaviour in ewes. They found it difficult to identify specific behaviours; however, like (Smith et al., 2020) and in the current trial, they were able to use activity and inactivity to identify lambing.

In this trial we used small accelerometers attached around the ewes' necks with an elasticated strap. We found that dystocic ewes simultaneously spent more time active and less time resting than eutocic ewes in the period before birth. Similar results were found in behavioural studies conducted in cattle (Barrier et al., 2012; Proudfoot et al., 2009), with reports of increased restlessness and a higher frequency of postural changes in dystocic calving events. Studies by Barraclough et al. (2020) and Miedema et al. (2011) collected contrasting results, as they did not see any differences in behaviour between eutocic and dystocic calving events.

Additionally, dystocic ewes spent equivalent time active and resting, whereas eutocic ewes spent significantly more time resting than active in the same time period. This can be attributed to the fact that dystocic events are generally longer than eutocic events. In this trial, eutocic ewes spent around 56 min in stage 2 parturition, and dystocic ewes spent around 221 min in stage 2 parturition. Similar lengths of eutocia were described by Schuenemann et al. (2011) but manual assistance was provided to the cows if labour exceeded 80 min without progress. Additionally, Darwish & Ashmawy (2011) used time intervals to determine birth difficulty, the dystocic ewes had longer stage 2 parturition than eutocic ewes; however, they did not disclose actual length of parturition.

Conclusion

In this trial, we aimed to describe the behavioural differences between eutocic ewes and dystocic ewes in the hours preceding birth, using visual behaviour annotation and accelerometer data. We found that dystocic ewes performed more BT over the 5.5 h before birth, but fewer BT in the last 30 min of labour compared to eutocic ewes. When looking at the pattern of BT over the observation period, there were similarities seen between eutocic and dystocic birth events, indicating that BT meaningfully reflects progression of parturition. Dystocic labour extends past when the lamb should be born, causing the over-exertion of the ewe and the observable decrease in behaviour transitions. Behaviour can be a valuable, non-invasive tool for reproduction research; however, current approaches involve time-intensive processes, requiring skilled observers and specific technology. As accelerometer data collected in this study reflected the annotated videos, research into these technologies may result in more efficient observations to improve research addressing dystocia in sheep.

Animal Ethics Approval

All animal experimentation was conducted in accordance with the ARRIVE guidelines during August-September 2018 at CSIRO Chiswick research station, FD McMaster Laboratory, Armidale NSW. Animal ethics approval was granted by the CSIRO Armidale Animal Ethics Committee under Animal Research Authority 18/11.

Data and model availability

None of the data were deposited in an official repository as they are part of a larger project with IP implications. The data will be made publicly accessible in the future once the project has been completed. Before then, data can be made available under material transfer agreements

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Amellia Redfearn: Investigation, Methodology, Writing- Original Draft. **Jody McNally**: Investigation, Project Administration. **Daniel Hutchinson**: Data Curation, Methodology. **Rebecca Doyle**: Formal analysis, Supervision. **Heather Brewer**: Investigation, Project Administration. **Emma Doyle**: Supervision. **Sabine Schmoelzl**: Conceptualisation, Supervision, Funding acquisition, Writing-Reviewing and Editing.

Declaration of interest

None

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Chapter 3: Postnatal maternal behaviour expression depends on lambing difficulty in Merino ewes

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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.

Keywords

dystocia, ethogram, ewe-lamb bond, lamb survival

Introduction

Appropriate and sufficient maternal care, and a strong, exclusive dam-young bond are crucial for the survival of the neonate (Bickell et al., 2010; Nowak et al., 2011; Nowak & Boivin, 2015), especially in extensive production systems. In sheep, the lamb relies on the ewe for nutrition (Nowak et al., 2000), primary immunological support, and physical protection from flock members and predators (Dwyer & Lawrence, 2005; Pickup & Dwyer, 2011). Dysfunction of maternal behaviour without human intervention leads to a weakened dam-young bond and increases the risk of mortality for the neonate (Dwyer, 2003). Additionally, lamb behaviour such as vocalising and teat-seeking reinforces maternal behaviour (Nowak et al., 2007), and similarly, a dysfunction in lamb behaviour can lead to poor ewe-lamb bond formation, and increased risk of lamb mortality (Darwish & Ashmawy, 2011; Nowak & Poindron, 2006).

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 (Bickell et al., 2010; Darwish & Ashmawy, 2011; El-Hamamy & Arulkumaran, 2005), and in the neonate as hypoxic brain injury, central nervous system (CNS) damage, low vigour and stillbirth (Dwyer et al., 1996; Hinch & Brien, 2014; Refshauge et al., 2016). 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 (Jacobson et al., 2020; Refshauge et al., 2016).

In this trial we made use of video recordings of ewe behaviour which were collected to provide annotations for a project developing a birth-detection algorithm in ewes (Smith et al., 2020). 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 diminish the expression of maternal behaviour.

Materials and methods

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 Ethics Approval number 18/11.

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, Heininger, Bibra Lake, WA) with a unique number. The 20 ewes expected to lamb first were moved into the supervised observation paddock (approx. 35m x 75m) 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 and they appeared fit, healthy and well bonded (teat-seeking behaviour and sucking observed by the lamb, lamb-seeking and vocalising observed by the ewe) 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.

Lambing data

Ewes were first checked at 6:30 am followed by multiple times, at least hourly, during the day, with the last check at 5:30 pm. If a ewe was observed to be in active labour, experienced animal carers would check on her every 30 minutes until birth. According to the approved animal research protocol, ewes were provided assistance if no progression was made after 2 hours 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 recorded 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.

Behaviour data

The supervised lambing paddock was set up with 10 day/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 minutes 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 included in the study, nine normal and nine difficult labour events were selected for detailed analysis. Behavioural Observation Research Interactive Software (BORIS; Friard and Gamba, 2016) 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).

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 seconds; 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 the lamb; standing or lying; head up or down; up to 3-4 seconds
Other		Point event	Unclassified behaviour

Not visible	State event	Ewe out of frame or unable to be seen clearly

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 (Friard and Gamba, 2016).

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 $P \leq 0.05$.

Results

Overall frequency of dystocic events

Of the 100 ewes enrolled in this study, 69 lambing events that were captured on video; 52 were classified as eutocic and 17 were classified as dystocic events. Additionally, 14 of these dystocic events had durations of stage 2 parturition exceeding 120 minutes. 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.

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 selected for annotation (Table 2). The ewes in the eutocic group only had single lambs; five of which were pure Merino, and four Border Leicester crosses. The ewes in the dystocic group had mainly Border Leicester cross lambs; seven of which were single lambs and one set of twins. The final ewe in the dystocic group had a single Merino lamb.

Table 2 Distribution of birth difficulty, sire breed and litter size of the 19 ewes selected for detailed annotation.

Birth difficulty	Sire Breed	Litter Size	Number of lambs
Eutocic	Merino	Single	5
		Twin	-
	Border Leicester	Single	4
		Twin	-
Dystocic	Merino	Single	1
		Twin	-
	Border Leicester	Single	7
		Twin	1

Difficult lambing events lead to lower maternal behaviour expression

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; Figure 1).

Licking

When licking was analysed as a separate behaviour, all eutocic ewes performed licking behaviours, whereas only 83% of dystocic ewes 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).

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 difficult group.

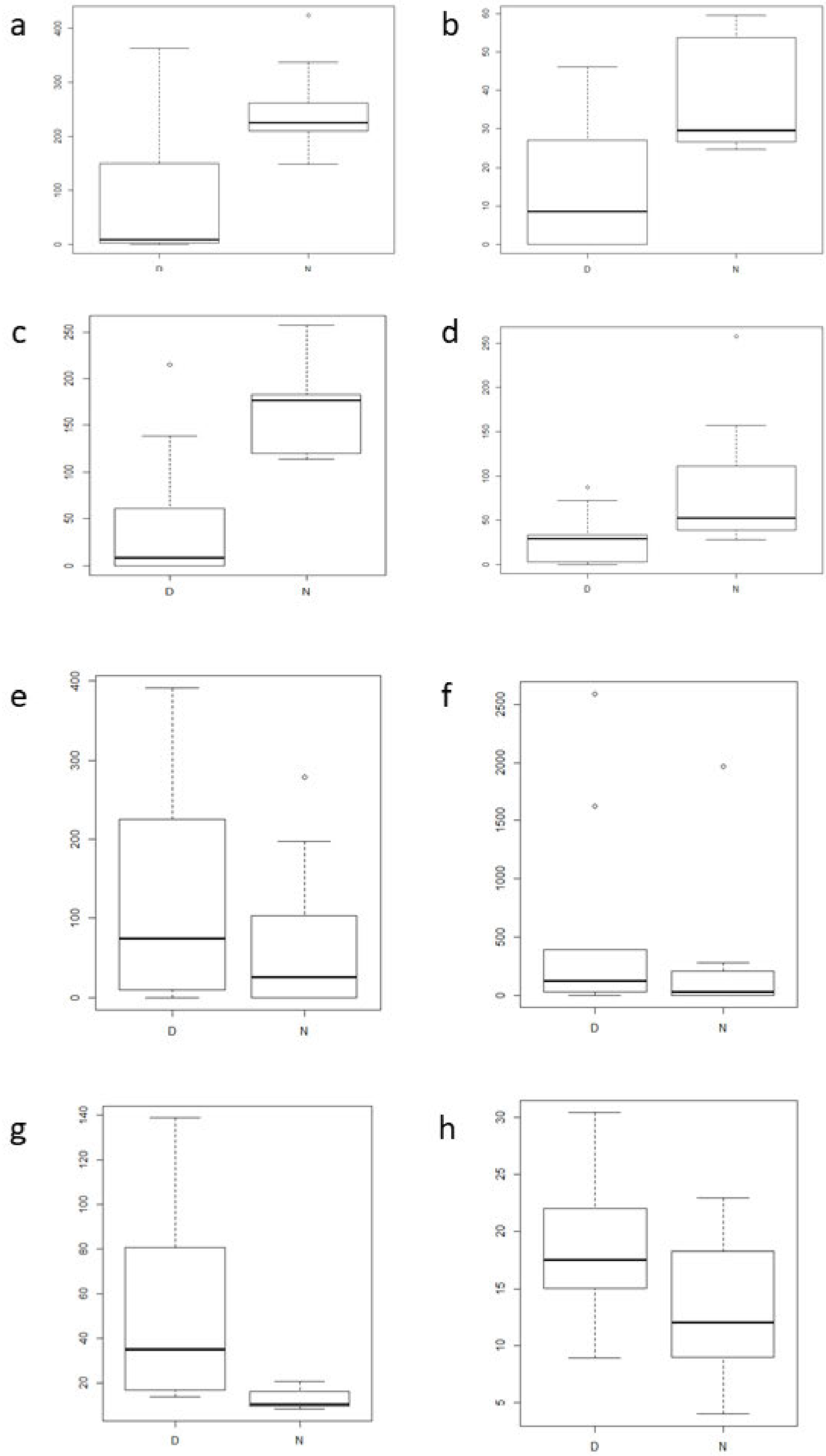

Figure 1 Behavioural observations for Grooming (a, b), Licking (c), Circling (d), Lying (e, f) and Standing (g, h) in the 2 h period post-partum for dystocic (D) and eutocic/normal (N) ewes. Y axes represent total numbers of occurrences (a, c, d); proportion of total time budget in percent (b, h), duration 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.

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).

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 sec, dystocic = 82.86 ± 111.59 sec; P = 0.003).

Dystocic events lead to greater expression of avoidance-type behaviours

Walking

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

Stepping

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

Lying

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

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 sec, dystocic = 2768.47 ± 631.34 sec; P = 0.03).

Dystocic events lead to lower behavioural transitions compared to eutocic events

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

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.

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 (Alexander et al., 1983) or abandon mute or low-vigour lambs (Lynch & Alexander, 1977). 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.

It has been well described that maternal care immediately post birth is essential for the health, wellbeing and survival of the neonate (Bickell et al., 2010; Hinch & Brien, 2014; Nowak et al., 2011) including physical protection and ingestion of colostrum (Nowak & Poindron, 2006). A link between delayed maternal care and lamb mortality was established early on by Arnold & Morgan (1975) 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 (Dwyer & Bünger, 2012; Nowak & Poindron, 2006). Maternal care behaviours include grooming and licking, which are integral behaviours to facilitate an exclusive and strong ewe-lamb bond (Dwyer, 2014; Nowak et al., 2000). Darwish & Ashmawy (2011) reported a longer latency to interact with the lamb for dystocic ewes compared with eutocic ewes, and Dutra & Banchero (2011) 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 (Everett-Hincks et al., 2005), as active lambs are quicker to stand and quicker to suck. Dystocic events can cause hypoxic brain injury and physical injury to the lamb (Refshauge et al., 2016), 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 (Pickup & Dwyer, 2011).

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 (Nowak & Boivin, 2015). 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 (Dwyer, 2008). It has been described that dystocic ewes are more likely to perform rejection-type behaviours, including longer latency to groom (Alexander, 1988), rejection of suckling attempts (Nowak, 2000), and rejecting/abandoning their lamb (Darwish & Ashmawy, 2011; Dwyer et al., 2003).

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 (Darwish & Ashmawy, 2011). Birth is a significant event requiring large amounts of energy to fuel myometrial contraction and increased activity of the dam (Rizzo et al., 2011; Steingrímsdóttir et al., 1993). It has been shown that dams experience pronounced hyperglycaemia around birth (Comline & Silver, 1972), likely to facilitate the final stages of parturition and provision of adequate aftercare (Chew & Rinard, 1979). 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 (El-Hamamy & Arulkumaran, 2005).

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 (Smith et al. 2020). 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.

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.

Animal Ethics Approval

All animal experimentation was conducted in accordance with the ARRIVE guidelines during August-September 2018 at CSIRO Chiswick research station, FD McMaster Laboratory, Armidale NSW. Animal ethics approval was granted by the CSIRO Armidale Animal Ethics Committee under Animal Research Authority 18/11.

Data and model availability

None of the data were deposited in an official repository.

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Declaration of interest

None

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Type of work	Page number/s
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Chapter 4: Using pen-side measurable blood parameters to predict or identify dystocic lambing events

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Using pen-side measurable blood parameters to predict or identify dystocic lambing events

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Simple Summary

Prolonged or non-progressive labour is the greatest risk factor for loss of newborn lambs in Australia and poses significant welfare and economic concerns worldwide. In this study, we set out to investigate whether pen-side technology could be used to predict which ewe would be at risk of prolonged labour. In our pilot trial we found potentially useful markers. We next developed a sampling protocol by looking at changes of candidate markers over time in normal lambing events. Finally, we searched for blood markers which could distinguish between normal and difficult lambing events, sampling pre-birth (estimated one week before birth), at birth (within 3 h) and post-birth (16-26 h). Possible predictors of lambing difficulty were chloride, haematocrit, and haemoglobin, sampled one week before birth; creatinine, sampled at birth; and acid-base related parameters after birth. In conclusion, we found pen-side analysis of blood markers showed promise to identify dystocic lambing events. More information is required to decide whether pen-side diagnostics could be useful to identify and predict dystocic lambing in the future.

Abstract

Dystocia is the greatest contributor to neonatal lamb mortality in Australia and poses significant welfare and economic concerns worldwide. In this study, we set out to investigate whether pen-side analysis technology could be employed to detect blood parameters predictive of dystocic labour events in sheep. In a pilot trial we collected and analysed blood samples in pen-side assays for glucose, lactate, pH, pCO_2, pO_2, base excess, HCO_3, TCO_2, sO_2, lactate sodium, potassium, chloride, calcium, urea nitrogen, creatinine, haematocrit, haemoglobin, and anion gap. From the pilot data, we identified creatinine, TCO_2, chloride and calcium as potentially useful markers. To develop a time course and to establish variability of the selected blood parameters, a time series of samples was collected from 12 ewes, from mid-gestation to 48 h after birth. For the main trial, blood samples were collected at mid- and late gestation for glucose determination, and for the full set of blood parameters at three timepoints before, at and after birth. Possible predictors of lambing difficulty were chloride, haematocrit, and haemoglobin, sampled one week before birth; creatinine, sampled at birth; and blood pH and base excess after birth. In conclusion, we found pen-side analysis of blood markers showed promise to identify dystocic lambing events.

Keywords: sheep; ewe; reproduction; production; physiology; rapid testing; time course

Introduction

Dystocia is an important welfare and economic issue faced in animal production industries in Australia, and across the world. It is the greatest contributor to neonatal lamb mortality (Refshauge et al., 2016), and lamb and ewe morbidity in Australia (Jacobson et al., 2020). Over many years of research, lamb mortality rates have remained troublingly high as described by (Hinch & Brien, 2014; Refshauge et al., 2016) irrespective of increased lambing rates, driven by increased average litter size. Much of the existing body of research investigates the effect of management (Kenyon et al., 2011; Mulvaney et al., 2008) and genetic factors (Brien et al., 2010; Bunter et al., 2020) on lamb mortality. Lamb loss is the end point of a multifactorial complex process with considerable influence of uncontrollable environmental factors such as prevailing weather around the time of lambing, which means research data on lamb survival tend to be highly variable, making research project effortful and progress slow. In our research, we have been focusing on better understanding the underlying causes of dystocia as the main risk factor leading to lamb loss (Refshauge et al., 2016). Blood biochemistry analyses are able to provide insights into individual animals' health and welfare status by identifying disease presence, metabolic disturbances and stress hormone levels (Ahmed et al., 2020; Morris et al., 2002). Traditionally, samples are collected, stored and processed in a laboratory. These tests can be costly, and may take days to process, especially for large sample sizes. It has been reported that the type of collection tube used, temperature at which samples are stored, the length of time samples are stored and the number of times samples are frozen and thawed significantly impacts the results received (Morris et al., 2002). Therefore, the development of point-of-care analysis technology has introduced fast, portable, affordable and easy to use laboratory-equivalent tests to veterinary and research settings (Chong & Reineke, 2016; Fletcher, 2016). These technologies have made multifactorial health and welfare investigations more easily carried out, on an individual and group basis.

In this study, we made use of a series of lambing trials designed to collect behavioural data during lambing (Smith et al., 2020) to collect blood samples and obtain information on maternal blood parameters. In an initial pilot trial, samples were collected immediately after birth, and 24 h after birth. This was followed by a time course trial to refine our sampling protocol. Finally, in the main trial phase, pre- and post-birth samples were collected, endeavouring to identify differences in the blood profiles between eutocic and dystocic lambing events.

Materials and Methods

Animal experimentation was undertaken at the CSIRO FD McMaster Laboratory, Armidale, NSW, Australia between March 2018 and November 2019 and conducted in accordance with 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 Ethics Approval numbers 18/11 and 19/10.

Animal management

All animals were treated in the same manner and followed the same lambing protocols unless stated otherwise. All trials were made up of pure Merino ewes of varying ages (2-7 years old), mated to either Merino or Border Leicester rams, and carrying either singles or twins. Each trial was balanced as far as possible for even numbers of these combinations.

Approximately one week before lambing was due to commence, ewes were brought into the yards. The 20 ewes that were due to lamb first were drafted and were assigned to group 1, and the rest of the ewes were divided evenly into groups based on due date. All ewes were side branded with a unique number with stock safe paint (Siromark, Heininger, Bibra Lake, WA) to facilitate identification from video records. Group 1 was moved into the supervised lambing enclosure equipped with 10 day- and night-vision cameras (Hikvision Digital Technology Co, Hangzhou, China) recording continuously for the entire trial period, and the remaining groups were held in nearby 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 twice daily. Ewes in the main trial were supplemented with thiamine powder mixed in with their daily feed when thiamine deficiency symptoms appeared in this cohort (see results section "*Main trial*").

Ewes were left undisturbed while lambing, unless there were obvious signs of distress, foetal malpresentation (one leg back, two legs back, breech), or there was no progress achieved in 1 h after foetal membranes or foetal parts appeared. If assistance was given, the ewe was restrained in a lateral lying position by one handler, and progression of the lamb through the birth canal was assisted by another handler. The ewe and lamb were monitored closely until the ewe was observed to be grooming the lamb and allowing it to suckle.

Once a ewe had lambed in the lambing enclosure, time of birth and birth intensity (eutocic/normal or dystocic/difficult) were documented from the video recordings. Birth intensity was determined by a single observer familiar with lambing ewes. Ewe and lamb pairs that appeared fit, healthy and well bonded were moved into an adjacent paddock, or alternatively to hospital pens to allow treatment of ewes or to facilitate bonding. The ewe was replaced by a pregnant ewe from the holding paddock, keeping the number of ewes in the lambing enclosure at 20. This was continued until all ewes had lambed.

Blood sampling

Blood samples were collected by jugular venepuncture into 10mL Lithium Heparin vaccutainer tubes. Samples were analysed with the iStat-1 handheld analyser (Abbot Point of Care, Princeton NJ, USA) using a combination of cartridges (Table 1). Cartridge types were changed for time course and main trials due to changes in availability.

Peripheral blood glucose was measured in the Time Course and Main Trial phases, using an AccuCheck handheld glucometer (AccuChek, Roche Diabetes Care, North Ryde, NSW, Australia) via capillary puncture in the ear.

Table 1 Blood analysis parameters by trial

Cartridge type	Blood parameters analysed	Trial used
CG4+	Na, K, Cl, Ca (as ionised calcium), TCO_2, Glucose, Urea, Creatinine, Haematocrit (PCV), Haemoglobin, Anion Gap	Pilot
CHEM8+	pH, pCO_2, pO_2, Base Excess, HCO_3, TCO_2, sO_2, Lactate	Pilot
EC8+	Na, K, Cl, TCO_2, Urea, Glucose, Haematocrit (PCV), pH, pCO_2, HCO_3, Base Excess, Anion Gap, Haemoglobin	Time Course Main Trial
Crea	Creatinine	Time Course Main Trial

Pilot trial

The pilot trial was conducted over three lambing cohorts in 2018. A total of 37 ewes were sampled for the pilot trial from a total of 420 lambing ewes. The first cohort lambed in April-May (blood samples collected from 15 out of 221 ewes; n = 15; 13 single/2 twin births), the second in August-September (n = 100; blood samples collected from 10 out of 100 ewes; n = 10; 9 single/ 1 twin births), and the third in October-November (blood samples collected from 12 out of 99 ewes; n = 12; 7 single / 5 twin births). For the first cohort, if a ewe was found less than 2 h post-lambing, she was eligible for a blood sample. The ewe and her lamb(s) were brought into the lambing shed, a blood sample was collected, and the ewe and her lamb(s) were then moved to the adjacent paddock. For the subsequent two cohorts, timing of blood sampling was adjusted to allow sufficient time to bond for ewe and lamb. Hence, blood samples were collected 2-4 h post-birth and a second sample was added at 24 h post-birth. Analysis of the samples was as described above.

Time course

Twelve ewes of a lambing cohort of 100 ewes were selected for a blood sampling time course. All ewes were carrying singleton pure Merino lambs. At two time points during gestation – mid gestation approximately 80 d gestation, and late gestation approximately 140 d gestation – all ewes of the cohort (n = 100) were brought into the yards for blood glucose determination. Additionally, the ewes selected for indoor lambing had a blood sample collected for analysis with the iStat analyzer (for cartridge details see Table 1).

The six ewes first due to lamb were brought into an indoor group lambing pen (4 m x 3 m) with straw bedding and two day/night surveillance cameras. Ewes were provided with free access to water, *ad libitum* lucerne hay and a daily ration of sheep pellets and fava beans as above.

The ewes were checked every 2-3 h for signs of lambing, and when first signs appeared, the ewe was monitored every 30 minutes until birth. Once a ewe had lambed, she was moved with her lamb into a single pen (1.5 m x 1.5 m) adjacent to the lambing pen. A blood sample was collected within 2 h of lambing and blood glucose was recorded. This protocol was repeated at 4 h, 8 h 24 h and 48 h post-lambing. For ewes which lambed outside the observation period no further blood samples were collected. After all bloods were collected, the ewe and lamb were moved into a paddock with other ewes and lambs, and one of the other selected pregnant ewes was brought into the indoor lambing pen.

Only complete sample sets for all time points were used for the time course analysis. A complete sample set was obtained from a total of six ewes for the time course of iStat analysis.

Main trial

At two time points during gestation, at 80 days post-joining for mid- gestation, and at 140 days post-joining for late-gestation, ewes (n = 100) were brought into the yards for blood glucose determination. One week before lambing was due to commence, the 20 ewes that were expected to lamb first based on foetal size at pregnancy scanning were brought into the lambing shed, had their blood glucose measured and recorded and a blood sample was collected. The rest of the ewes were brought into the lambing shed in groups of 20 (based on due date) in two- or five-day intervals for the same blood sampling protocol. This method was employed to reduce stress on the ewes, and to allow sampling of all ewes at around one week before lambing.

Some ewes in this trial showed symptoms of thiamine deficiency in the first week of observation. The likely cause was nutritional management of the ewes before entering the trial which was affected by severe drought conditions in 2018/19. Under these conditions, diets were low in fibre and high in pulses and grains. Over prolonged periods of time, this can change the rumen biome affecting thiamine production. No symptoms had been diagnosed up until end of pregnancy and it is hypothesised that the additional thiamine demands of the growing foetus may have triggered the symptoms in previously borderline deficient ewes. Once diagnosed by a veterinarian, all ewes were supplemented with vitamin thiamine powder with hard feed every day. All ewes were given a thiamine intramuscular injection, and ewes that were acutely symptomatic were given thiamine intravenously. Treated ewes became ineligible for further sample collection. Overall, 11 out of 100 ewes were treated for acute thiamine deficiency. Of the 11 treated ewes, 6 ewes recovered and 5 ewes were euthanised by captive bolt stun and exsanguination as per approved protocol. Any ewe that was found within 3 h of lambing was eligible for a birth blood sample. She was brought into the lambing shed with her lamb, a blood sample was collected and blood glucose was recorded. She was then either penned outside or returned to the lambing enclosure, depending on evident secure ewe-lamb bonding and appropriate maternal behaviour. On the following day, between 16 h and 26 h post-lambing, a second sample was collected from the ewe. The ewe and lamb were then moved to an adjacent paddock.

If a ewe with newborn lamb had not been found until more than 3 h after lambing, most commonly on the following morning, a single post-birth blood sample was collected at this time, at 16 h to 26 h after lambing and then ewe and lamb were released into the adjacent paddock.

Blood samples were successfully collected at birth, within 3 h of birth, from 27 ewes (n = 27; 17 single / 10 twin births) and on the day following birth within 16-26 h from 30 ewes (n = 30; 18 single / 12 twin births).

Statistical analysis

All data were recorded on paper and then entered into MS Excel at the end of the trial (Microsoft, Redmont, WA, USA). Excel was used to clean the data, organise it and perform basic descriptive statistics (mean, range, standard deviation).

Statistical tests were performed using Genstat (VSN International, 2021). Unpaired Student's t-Tests were used to compare blood marker values between eutocic and dystocic births, and for comparing mid-gestation values and subsequent values in the time course. The significance level was $p < 0.05$.

Results

All ewes in this study, including dystocic ewes, successfully delivered their lambs unassisted within the time limits allowed by the study protocol.

Pilot trial

In the first lambing cohort, 15 ewes were sampled 0-2 h after birth from eutocic (n = 11; 9 single/ 2 twin births) and dystocic (n = 4; 4 single/ 0 twin births) lambing events. This cohort was analysed separately to cohorts 2 and 3 due to changes made to the sampling protocol. Potassium (eutocic = 4.07 ± 0.33 mmol/L, dystocic = 3.85 ± 0.13 mmol/L; $p = 0.04$) and chloride (eutocic = 114.27 ± 2.24 mmol/L, dystocic = 109.25 ± 3.30 mmol/L; $p = 0.02$) were significantly lower in dystocic ewes. Additionally, TCO_2 (eutocic = 23.64 ± 1.21 mmol/L, dystocic = 27.75 ± 2.99 mmol/L; $p = 0.03$), creatinine (eutocic = 0.76 ± 0.10 mg/dL, dystocic = 1.00 ± 0.082 mg/dL; $p = 0.001$), base excess (eutocic = -0.18 ± 1.33 mmol/L, dystocic = 5.75 ± 4.86 mmol/L; $p = 0.05$) and HCO_3 (eutocic = 23.20 ± 1.60 mmol/L, dystocic = 28.90 ± 4.80 mmol/L; $p = 0.05$) were all significantly higher in dystocic ewes. In the second cohort, 10 ewes were sampled 2-4 h after birth, and again at 24 h after birth from eutocic (n = 7; 6 single/ 1 twin births) and dystocic (n = 3; 3 single/ 0 twin births) lambing events. In the third cohort, 12 ewes were sampled 2-4 h after birth, and again at 24 h after birth from eutocic (n = 10; 6 single/ 4 twin births) and dystocic (n = 2; 1 single/ 1 twin births) lambing events. When data from cohorts 2 and 3 were analysed together (17 eutocic ewes and 5 dystocic ewes), no differences were seen between eutocic and dystocic ewes at the first time point 2-4 h after birth for any blood parameters measured. At the second time point 24 h after birth, sO_2 (eutocic = 83.65 ± 8.04, dystocic = 89.90 ± 3.42; *p* = 0.01) was significantly higher in dystocic ewes.

Cohorts 2 and 3 lambed in late winter/early spring and late spring, respectively, of the Australian New England climate. To address the question whether environmental conditions had an effect on the obtained results, data obtained for cohorts 2 and 3 were also analysed separately. When analysed separately, at the first time point for cohort 2, creatinine (eutocic = 0.64 ± 0.098 mg/dL, dystocic = 0.47 ± 0.058 mg/dL; *p* = 0.005) was significantly lower in dystocic ewes; however, glucose (eutocic = 129.57 ± 28.57 mg/dL, dystocic = 90.33 ± 35.73 mg/dL; *p* = 0.09) and potassium (eutocic = 4.37 ± 0.37 mmol/L, dystocic = 3.70 ± 0.56 mmol/L; *p* = 0.08) were not significantly different. At 24 h after birth, TCO_2 (eutocic = 25.00 ± 2.00 mmol/L, dystocic = 28.00 ± 2.00 mmol/L; *p* = 0.05) was significantly higher in dystocic ewes.

At the first time point of cohort 3, calcium (eutocic = 1.16 ± 0.12 mmol/L, dystocic = 1.29 ± 0.042 mmol/L; *p* = 0.02) was significantly higher in dystocic ewes. At 24 h after birth, chloride (eutocic = 109.70 ± 3.23 mmol/L, dystocic = 112.50 ± 0.71 mmol/L; *p* = 0.02) and sO_2 (eutocic = 79.2 ± 5.63%, dystocic = 87.00 ± 2.83%; *p* = 0.03) were significantly higher, and calcium (eutocic = 1.16 ± 0.18 mmol/L, dystocic = 0.95 ± 0.028 mmol/L; *p* = 0.003), TCO_2 (eutocic = 24.50 ± 2.27 mmol/L, dystocic = 22.50 ± 0.71 mmol/L; *p* = 0.03), pCO_2 (eutocic = 33.62 ± 3.59 mmHg, dystocic = 28.95 ± 1.06 mmHg; *p* = 0.006), base excess (eutocic = 1.40 ± 3.44 mmol/L, dystocic = -1.00 ± 0.00 mmol/L; *p* = 0.03) and HCO_3 (eutocic = 24.83 ± 3.06 mmol/L, dystocic = 22.40 ± 0.42 mmol/L; *p* = 0.02) were significantly lower in dystocic ewes.

Within each cohort, there were parameters differentiating between eutocic and dystocic ewes. There was no unifying trend across these cohorts due to the small sample sizes.

Time course

During pregnancy, all 100 ewes of the larger cohort which the ewes involved in the time course experiment belonged to, had blood glucose measured twice by test strip method; once at 80 d gestation, and again at 140 d gestation (mid- and late-gestation respectively). Glucose was significantly higher at mid-gestation compared to late-gestation (mid = 4.20 ± 0.75, late = 3.45 ± 1.05; $p < 0.001$). There was no difference found between dystocic and eutocic ewes.

All other results reported here were derived from iStat cartridge analysis (Table 2). Complete data sets were obtained from 6 out of the selected 12 ewes for the time course experiment. Anion gap were decreased at late-gestation, returned to mid-gestation at birth, decreased again from 4 h and remained lower than at mid-gestation up to 48 h. Potassium increased by 8 h after birth but returned to mid-pregnancy levels thereafter. Urea decreased at birth, remained lower than mid-pregnancy at 4 h after birth and then returned to mid-pregnancy level. Chloride increased at late-gestation and birth, returned to mid-pregnancy level at 4 h but increased again at 8 h and 24 h (Figure 1a). Glucose was higher at birth and nearly doubled compared to mid-gestation level. After birth, glucose dropped and remained significantly lower than mid-gestation for the remainder of the observation period (Figure 1b).

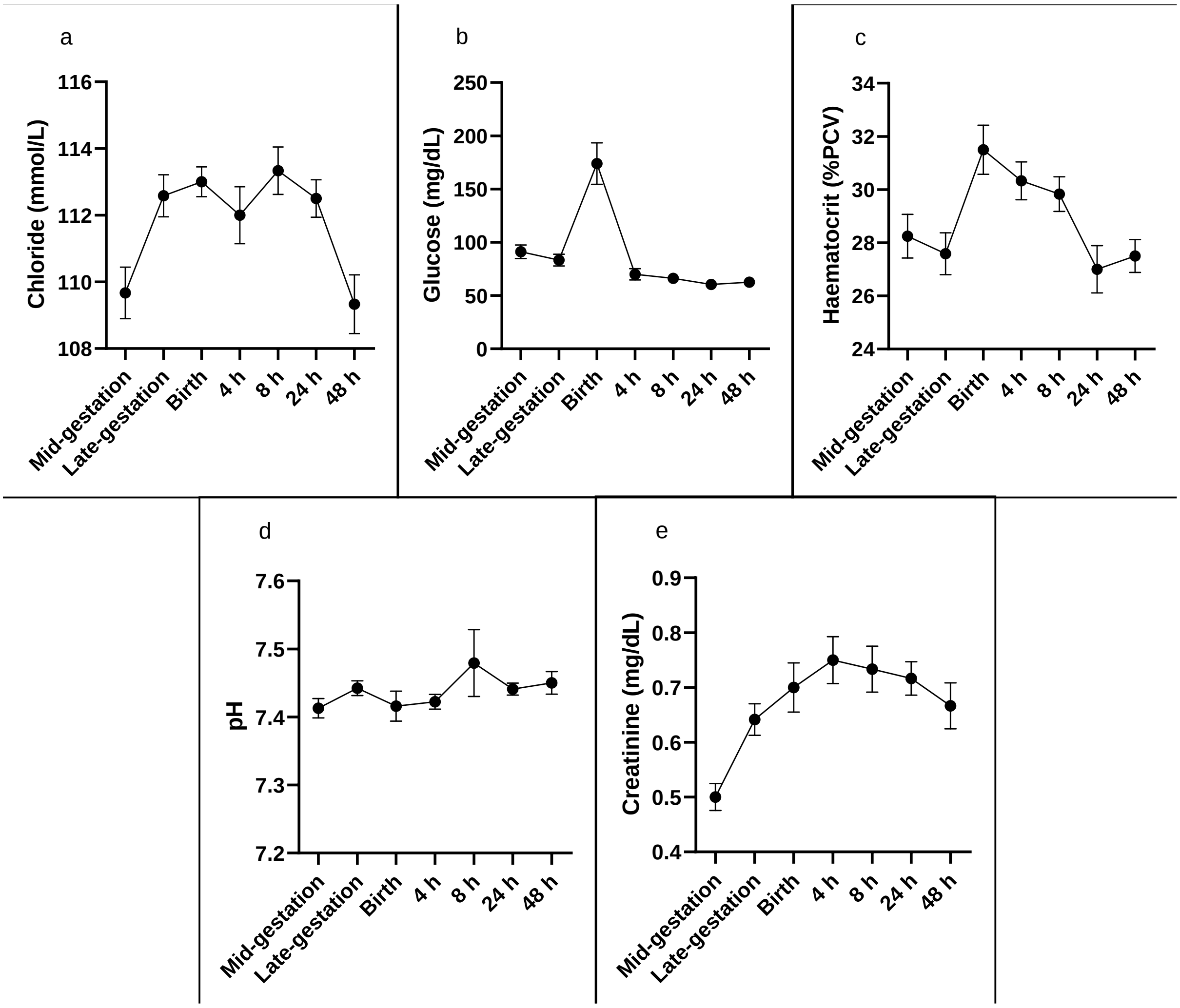

Figure 1 Average values (s.d. error bars) of 6 single bearing ewes at two time points before birth and five time points after birth for selected blood markers; a) Average chloride (mmol/L), b) Average glucose (mg/dL), c) Average haematocrit (%PCV), d) Average pH, e) Average creatinine (mg/dL).

Haemoglobin and haematocrit (Figure 1c) were higher by 11% at birth but were not different at any other time point. pH (Figure 1d) and PCO_2 remained unchanged throughout the entire observation period.

Creatinine concentration was higher at every time point compared to mid-gestation. It reached a peak at 4 h post-birth and declined thereafter but remained elevated for the entire observation period (Figure 1e). Sodium, TCO_2, PCO_2, HCO_3, base excess and pH (Figure 1d) and PCO_2 remained unchanged throughout the entire observation period (Table 2).

Table 2 Average (s.d.) values for investigated blood parameters measured at mid-gestation, late-gestation, birth and 4 h, 8 h, 24 h, 48 h post-birth. Comparisons are between mid-gestation and subsequent time points; *p < 0.05, ** p < 0.01, ***p < 0.001.

	Mid-gestation	Late-gestation	Birth	4h	8h	24h	48h
Sodium (mmol/L)	149.67 (1.67)	150.00 (1.21)	150.17 (1.72)	149.83 (2.40)	148.83 (1.33)	149.50 (2.43)	149.67 (2.07)
Potassium (mmol/L)	4.04 (0.40)	3.66 (0.28)	4.15 (0.37)	4.30 (0.45)	4.77 (0.36)**	4.40 (0.22)	4.23 (0.39)
Chloride (mmol/L)	109.67 (2.67)	112.58 (2.19)*	113.00 (1.10)**	112.00 (2.10)	113.33 (1.75)**	112.50 (1.38)*	112.19 (2.16)
Anion Gap (mmol/L)	20.33 (1.61)	17.17 (1.99)**	19.83 (2.71)	17.83 (1.33)**	17.00 (1.55)***	16.83 (1.60)***	18.17 (0.84)**
Glucose (mg/dL)	91.17 (21.87)	83.33 (19.54)	173.83 (47.94)***	70.00 (13.01)*	66.17 (3.43)**	60.33 (5.57)**	62.50 (10.09)**
Urea (BUN) (mmol/L)	28.42 (4.68)	29.58 (3.73)	19.83 (3.60)***	22.50 (4.32)**	25.50 (3.51)	25.33 (2.94)	25.19 (4.23)
Haematocrit (% PCV)	28.25 (2.86)	27.58 (2.75)	31.50 (2.26)*	30.33 (1.75)	29.83 (1.60)	27.00 (2.19)	29.09 (1.52)
Haemoglobin (g/dL)	9.61 (0.98)	9.33 (0.97)	10.72 (0.76) *	10.30 (0.58)	10.13 (0.55)	9.18 (0.73)	9.87 (0.53)
pH (standard unit)	7.41 (0.05)	7.44 (0.04)	7.42 (0.05)	7.42 (0.03)	7.48 (0.12)	7.44 (0.02)	7.44 (0.04)
pCO_2 (mmHg)	37.59 (4.81)	34.87 (2.53)	34.05 (8.21)	37.32 (3.75)	34.82 (3.06)	36.28 (2.65)	35.83 (3.89)
TCO_2 (mmol/L)	25.00 (2.22)	24.92 (2.19)	22.67 (4.80)	25.50 (2.26)	24.17 (1.83)	25.67 (1.21)	24.65 (1.17)
HCO_3 (mmol/L)	23.88 (2.08)	23.83 (2.27)	21.70 (4.34)	24.33 (2.11)	22.97 (1.82)	24.67 (0.96)	23.56 (1.28)
Base Excess (mmol/L)	-0.83 (2.41)	-0.25 (2.67)	-2.83 (4.26)	0.00 (2.45)	-1.33 (1.75)	0.67 (0.82)	-0.76 (1.72)
Creatinine (mg/dL)	0.50 (0.085)	0.64 (0.097)**	0.70 (0.11)***	0.75 (0.10)***	0.73 (0.10)***	0.72 (0.075)***	0.67 (0.10)**

Main trial

For this trial, samples were collected from all ewes (n = 100) at 80 d and 140 d gestation for peripheral blood glucose determination. No differences were observed neither was a difference observed between singleton or twin-bearing ewes and ewes which would later experience eutocic or dystocic birth.

The full set of blood parameters was measured approximately one week before parturition for all ewes, except for seven individuals (n = 93; including 84 eutocic (54 single/30 twin births) and 9 dystocic (6 single/ 3 twin births) events); five ewes were affected by 'stargazing' disease/thiamine deficiency (see above; one from each group), one ewe died before sampling (Group 4) and one ewe was excluded as she was the only ewe delivering triplets (Group 2). At birth, blood samples were collected within 3 h of birth (n = 27) including 19 eutocic (11 single/8 twin births) and 8 dystocic (6 single/2 twin births) events. Samples from the day following birth were collected within 16-26 h from a total of 30 ewes, including 21 eutocic events (21 single/13 twin births) with samples collected at 16 h 25 min-25 h 42 min post-partum (1224 ± 163 minutes) and 9 dystocic events (6 single/3 twin births) events with samples collected at 16 h 10 min-26 h 04 min post-partum (1253 ± 231 minutes).

Twin-bearing ewes were represented in 35% (33/93) of pre-birth samples (30/84 or 36% of eutocic, and 3/9 or 33% of dystocic samples); 37% (10/27) of samples collected at birth (8/19 or 42% of eutocic and 2/8 or 25% dystocic ewes) and 37% (16/43) of samples collected at 16-26 h post birth (13/34 or 38% of eutocic and 3/9 or 33% of dystocic samples). Hence, twin-and singleton-bearing ewes were represented consistent with the overall average in pre-birth and post-birth samples. At birth, twin-bearing ewes were slightly underrepresented in the total sample set as one dystocic twin-bearing ewe lambed outside of the daily observation period. Overall, twin-bearing ewes were not overrepresented in the samples and therefore, in the analysis of blood sample data, birth type (singleton or twin) was not further considered as a factor.

Before lambing, dystocic ewes had a significantly lower chloride concentration compared to eutocic ewes (eutocic = 113.84 ± 2.43 mmol/L, dystocic = 111.63 ± 1.77 mmol/L; $p = 0.014$). Additionally, dystocic ewes had a higher haematocrit (eutocic = 27.42 ± 2.69 %PCV, dystocic = 29.88 ± 3.87 %PCV; $p = 0.035$) and a higher haemoglobin concentration (eutocic = 9.32 ± 0.91 g/dL, dystocic = 10.16 ± 1.31 g/dL; $p = 0.033$) compared to eutocic ewes.

Blood glucose concentration was higher at birth for eutocic ewes compared to dystocic ewes but the difference was not significant (eutocic = 165.37 ± 44.17 mg/dL, dystocic = 127.75 ± 54.74 mg/dL; p =

0.07). Blood creatinine concentration was significantly higher at birth for dystocic ewes to eutocic ewes (eutocic = 0.79 ± 0.14 mmol/L, dystocic = 0.93 ± 0.18 mmol/L; p = 0.02).

In the sampling period 16-26 h after birth, glucose and creatinine concentrations were not significantly different between eutocic and dystocic ewes; the values were comparable to pre-birth concentrations. Dystocic ewes had a lower pH (7.41 ± 0.089) than eutocic ewes (7.47 ± 0.079) dystocic with a corresponding difference in base excess between eutocic (-1.38 ± 2.80 mmol/L) and dystocic ewes (-3.44 ± 2.55 mmol/L).

In summary, before birth, dystocic ewes presented with significantly lower chloride and higher haematocrit and haemoglobin compared to eutocic ewes. Within 3 h of birth, blood glucose was significantly lower, and creatinine was significantly higher in dystocic ewes. In the 16-26 h after birth, pH and base excess parameters were significantly lower in dystocic ewes.

Discussion

During the pilot trial, we aimed to establish whether we could observe indicators promising to distinguish between dystocic or eutocic lambing events in blood samples collected from ewes in the peri-natal time period and analysed with penside diagnostics.

In the first cohort, we found that potassium and chloride were lower in dystocic ewes. It is well documented that potassium and chloride are key ions involved with myocyte excitation and uterine contractility, allowing the proper progression of labour (Dunford et al., 2020). Disturbances in the concentrations and activity of these ions may lead to adverse labour events, including dystocia (Brainard et al., 2007). The disturbances we found in chloride and potassium may have existed before birth, and influenced birth difficulty, or they may have been an effect of dystocia itself. Without pre-birth sampling, as suggested by (Ghoneim et al., 2016) this question could not be addressed in the pilot trial.

Maternal acid-base function and dysfunction is well documented in humans (see reviews by (Arrowsmith et al., 2014; Omo-Aghoja, 2014). Several studies including by (Musaba et al., 2019;

Quenby et al., 2004) have shown that prolonged or dystocic labours have a greater risk of metabolic disturbance, notably decreased blood pH and oxygen saturation, as well as increased lactate (i.e. acidosis). In cohort 1 we found that dystocic ewes presented with higher TCO_2, base excess and HCO_3 levels than eutocic ewes. This could be a compensatory effect, counteracting the acidosis that may have occurred during labour. Additionally, creatinine was higher in dystocic ewes. This was expected as creatinine is a by-product of muscle and protein metabolism (Salazar, 2014). Elevations in blood creatinine concentration were described by (Ali et al., 2016; Ghoneim et al., 2016) in dystocic camels; however, their results were not significant.

When analysed separately, cohorts 2 and 3 returned conflicting results. This may be due to seasonal differences in nutrition, as cohort 2 lambed in late winter and cohort 3 in mid-spring, or small sample size in each cohort. When the data of cohorts 2 and 3 were combined and analysed together, there were no significant differences between eutocic and dystocic lambing events for any blood parameters analysed for samples taken 2-4 h after birth. The adaptive stress response, especially for acute stressors – including birth – is a fast-acting system that keeps the body systems in eustasis (Dhabhar, 2018) and it is possible that the changes in blood parameters seen at birth in the first cohort were resolved by the time samples were collected in the subsequent two cohorts with its slightly modified sampling protocol. In sheep, (Comline & Silver, 1972) described changes in maternal glucose, lactic acid and free fatty acids occurring at birth, but quickly resolving in the following 2-4 h.

In summary, we concluded from the pilot trial, that it may be possible to identify pen-side indicators of dystocia within experimental cohorts.

To refine our sampling protocol, a time course analysis was next undertaken. We aimed to establish a suitable time window for the first blood sample collected after birth, considering the need to allow establishing of ewe-lamb bond and anticipating feasible collection protocols for the main trial. We also aimed to establish the potential value of a blood sample collected within 48 h which can be realistic in field conditions. Blood samples were collected from 12 Merino ewes carrying single

Merino lambs at two time points before birth, at approximately 80 d and 140 d gestation, and of six of these ewes that had a normal birth, within 2 h of birth and then at 4 h, 8 h, 24 h and 48 h after birth.

A pronounced elevation in glucose was seen in all ewes at birth, followed by a rapid decrease back to pre-birth concentration by the time the 4 h sample was collected. A fundamental study by (Comline & Silver, 1972) described the changes in blood markers of ewes over time in late pregnancy, at parturition, and in the hours following birth. Similarly, they described glucose to be the most obvious change, with very pronounced hyperglycaemia occurring at birth. Additionally, they reported little change in pCO_2 and pH, which was also observed in the results presented here. Creatinine is a by-product of protein metabolism, and can increase as a result of muscle work or damage (Salazar, 2014), thus it is expected to rise after an intense period of work such as parturition. In the time course results, we saw a gradual increase in blood creatinine from mid-gestation, through birth and a peak at 4 h after birth. Creatinine then declined slowly to the end of the sampling protocol. These results mimic those reported by (El-Sherif & Assad, 2001) during pregnancy and lactation of ewes, and (Simões et al., 2016) during the early postpartum period of bitches. It should be noted that all time points fell within the normal range of blood creatinine concentration for sheep (Desco et al., 1989); periparturient creatinine concentrations changed but were not abnormal.

An increase at birth was also observed in haematocrit, with PCV slowly returning to pre-birth levels which mirrors the trend described by (Simões et al., 2016) in bitches.

From the time course and from our pilot trial, we determined that a pre-birth sample was necessary to capture the blood profile before lambing, and any possible indicators of birth difficulty could be recognised. A sample collected within 2-3 h of birth would capture the disturbance caused by birth, and any irregularities caused by a difficult birth should be present and identifiable. Finally, a sample should be taken 16-26 h after birth, as some important markers such as creatinine take longer to normalise, and differences may still be observable as a result of a dystocia. This relatively wide time range was chosen to avoid exclusion of sample sets for which a narrower time range would have precluded collection of the second time point for logistical reasons.

In the main trial, pre-birth blood samples were collected from all ewes around one week before lambing to serve as a pre-birth baseline. We also collected as many blood samples as possible within 3 h of birth, and again 16-26 h after birth.

At 140 days of pregnancy, dystocic ewes had significantly lower chloride than eutocic ewes. This result indicates that pre-birth disturbance or insufficiency of chloride may have an effect on birth difficulty. As chloride is essential for myometrial excitation, contractility and labour progression, a decrease in available chloride may decrease the strength of contractions and increase the risk of adverse labour events (Bernstein et al., 2014; Dunford et al., 2020; K. Jones et al., 2004).

Dystocic ewes had significantly higher haematocrit and haemoglobin levels. Increased blood volume and a smaller increase in PCV (haemodilution) during gestation is to be expected, as maternal body weight increases, plasma volume increases (Frayne & Pinchon, 2018). This phenomenon has been seen in many species and was described in goats well by (Iriadam, 2007). Higher PCV in dystocic cows just before surgical intervention was seen by (Tiwari et al., 2020). They also described a more pronounced increase in haemoglobin in dystocic cows. They hypothesised dehydration and excitation (releasing catecholamines) were the driving mechanisms behind these differences.

At birth, blood glucose concentration lower in dystocic ewes but the difference was not significant. Larger group sizes and an earlier collection time will be required to address whether the observed difference has significance. Based on our time course results, the chosen at-birth collection timepoint may have missed the period of perinatal elevation which may show differences between dystocic and eutocic ewes and epistatic mechanisms which tightly control blood glucose levels may have adjusted this difference already, if it had been there. Glucose appears to be the main energy source for myometrial cells (Rizzo et al., 2011; Steingrímsdóttir et al., 1993). During birth, glucose is mobilised via increased cortisol and adrenaline as a result of pain and stress (Vannucchi et al., 2015), ensuring the myometrium has enough energy to fuel the intense activity of birth. Generally, the peak in glucose is at birth, or soon thereafter (Chew & Rinard, 1979; Comline & Silver, 1972). Interestingly, many studies have reported an increase in glucose in dams that have experienced dystocia (Amin et al., 2020; Ghoneim et al., 2016; Simões et al., 2016; Vannucchi et al., 2015); however, many, if not all had surgical or manual intervention after a certain threshold whereas the ewes in this study were able to successfully lamb unassisted within the time boundaries set for the study.

Creatinine concentration was significantly higher in dystocic ewes compared with eutocic ewes. Creatinine levels rise during and after exercise as a result of exercise-induced muscle breakdown (Bongers et al., 2018), and parturition is an event characterised by intense myometrial activity, so it is reasonable to assume that creatinine would be elevated after birth. Prolonged or dystocic births are extended periods of myometrial activity and therefore creatinine concentrations would be elevated further. Across several studies and species such as the camel (Ali et al., 2016; Ghoneim et al., 2016), buffalo (Amin et al., 2020) and dog (Simões et al., 2016), creatinine appears trend higher in dystocic dams; however, their results were not statistically significant. Again, in this study, dystocic ewes were ultimately able to successfully lamb unassisted whereas in other studies intervention may have precluded elevation of creatinine.

By the time blood samples were collected at 16-26 h post-birth, most blood parameters had normalised, so there were no significant differences between eutocic and dystocic ewes. This further supports the idea expressed earlier that the acute adaptive stress response quickly regulates the body back to eustasis (Dhabhar, 2018). An exception to this were pH and base excess which were lower for dystocic ewes. This result matched the result found earlier in our first pilot trial cohort. As discussed above, acid-base balance may be affected by lactic acidosis as a result of anaerobic energy creation in the muscle during the physical exertion of prolonged labour. More work will be required to confirm whether this parameter can be used reliably to identify dystocic ewes.

For creatinine which remains overall elevated for an extended time period after birth, we could not establish differences between eutocic and dystocic ewes. A more narrowly defined time window for collection of the second post-birth sample may have allowed such differentiation and more work will be required to address this possibility.

In this study, we have restricted the investigated parameters to those readily assessable through pen-side diagnostics. To get a more complete understanding of differences in the physiology of dystocic and eutocic ewes, analysis of additional, metabolic markers such as beta-hydroxy-butyrate and Non-Esterised Fatty Acids may be of interest (González-García et al., 2015). Further studies will be needed to confirm the results presented here in repeat large scale studies.

Conclusion

In this series of trials, we aimed to use penside analysis techniques to collect information on blood parameters in pregnant and periparturient Merino ewes to further understand the effect of dystocia on the physiology of the ewe. Pilot study results indicated that observable difference can be found between dystocic and eutocic ewes. Observed differences in the pilot study were dependent on cohorts, pointing to strong influence of environmental conditions on individual parameters.

The main trial confirmed observable differences between normal and difficult lambing events in the pre- and postpartum periods. Dystocic ewes presented in late pregnancy with significantly lower chloride and higher haematocrit and haemoglobin, compared to eutocic ewes. At birth, creatinine was significantly higher in dystocic ewes. On the day following birth, acid-base balance was affected in dystocic ewes. The observed differences may have potential as diagnostic markers.

This study has demonstrated potential benefits of pen-side diagnostics to predict and identify dystocic ewes. More research will be needed to confirm these results and to further our understanding of the underlying factors contributing to incidences of dystocia in sheep production systems.

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Institutional Review Board Statement: The study was conducted in accordance with 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 Ethics Approval numbers 18/11 and 19/10.

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This series of manuscripts has investigated the role of behaviour and physiology in the identification and detection of dystocia in lambing ewes, and aimed to address the question of which underlying mechanisms may play a role in connecting dystocia with lamb survival. Dystocia is a main cause of lamb loss and is therefore of major importance in sheep production systems. Full appreciation of the scale of the problem is; however, relatively recent and has been an outcome of the concerted effort of the multi-year and multi-site research of the Sheep CRC. Research into lamb survival has existed for decades, although research has been largely focused on the outcome of lamb survival rather than investigating causes, mainly due to the practical limitations to allow direct observations on large numbers of lambing events. Where research has included dystocia, this has been through the inclusion of lamb *post mortems* to investigate causes of lamb mortality. Hence, dystocic events not resulting in lamb loss, such as mild cases of dystocia during otherwise favourable weather conditions, would have gone unrecorded in most studies. Measurements of phenotype are most useful in a constant environment. For lamb survival, where lambing outcomes are highly dependent on the weather at the time of lambing, it will be beneficial to describe a phenotype based on dystocia rather than on survival of the lamb. Recent advances in supporting technology including improved resolution of day and night cameras and the arrival of on-animal movement sensors are helping uncover the causes of dystocia and will help shape subsequent preventions for this insidious issue in the future.

As an important contributor to neonatal lamb mortality, dystocia directly accounts for half of all lamb deaths in the first few days of life, and may indirectly account for another 30% (Jacobson et al., 2020; Refshauge et al., 2016). Dystocia is influenced by many factors including nutrition (Dwyer et al., 2003; Mulvaney et al., 2008), ewe breed (Everett-Hincks et al., 2014; Holst & Allan, 1992; Holst et al., 2002), ewe parity (Alexander et al., 1993; N. McHugh et al., 2016), litter size (Everett-Hincks et al., 2005; Holst et al., 2002; McHugh et al., 2016; Schreurs et al., 2010), parturition length (Darwish &

Ashmawy, 2011; Dutra & Banchero, 2011), and has adverse effects on maternal behaviour (Dwyer, 2003; Dwyer & Lawrence, 2005; Nowak, 2000; Pickup & Dwyer, 2011) and lamb vigour (Darwish & Ashmawy, 2011; Nowak, 2000; Nowak & Poindron, 2006). These factors are intertwined and therefore cannot be investigated independently (Figure 1).

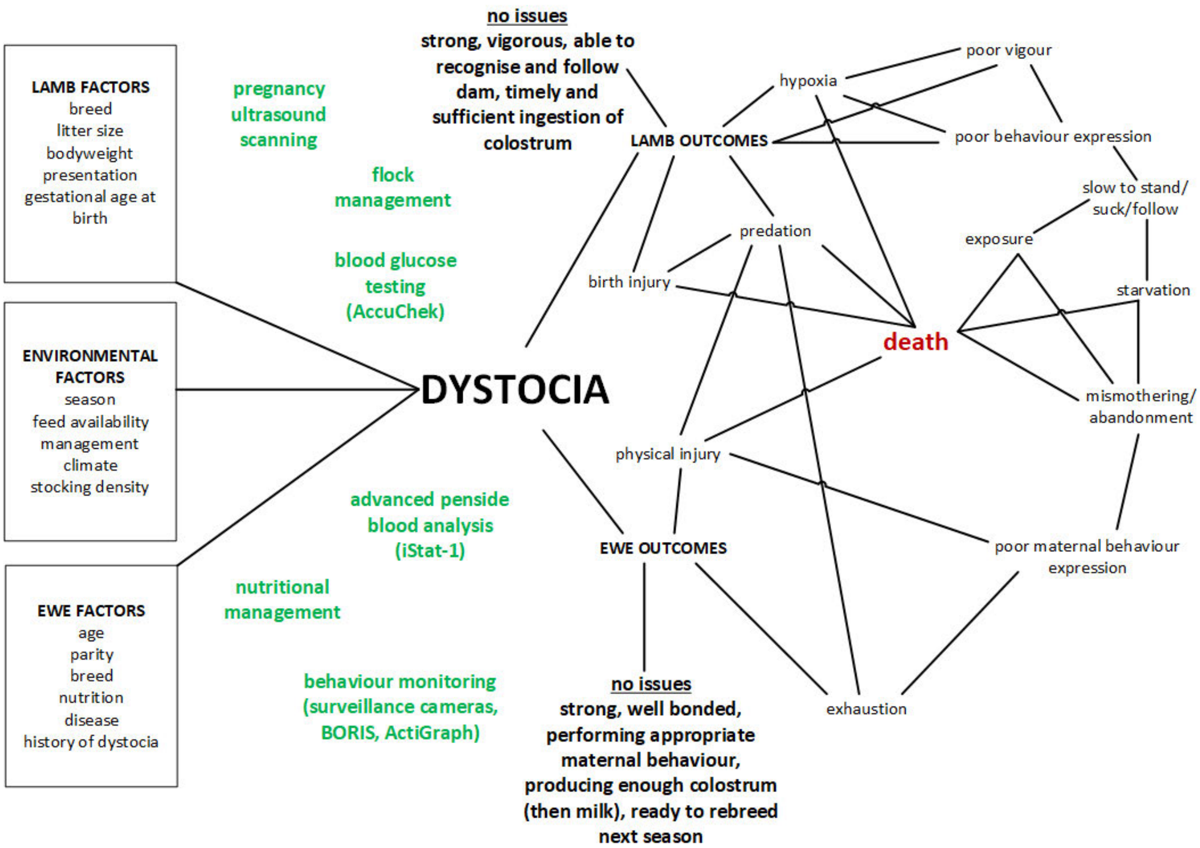

Figure 1 The interconnected and multifactorial nature of dystocia. Contributing lamb, environmental and ewe factors on the left; analytical methods in bold green; ewe and lamb outcomes on the right. This diagram is thorough, but not exhaustive.

Previous research has focused on identifying causative factors of lamb survival, and of dystocia leading to lamb loss; however, the causes for dystocia are multifaceted – explored in the review by Jacobson et al. (2020) – there will be no simple fix that will remove every possibility of the occurrence of dystocia. It is important that we shift our research focus from outcomes such as number of lambs dead – heavily influenced by environmental factors – to the underlying causes of dystocia.

In this thesis, I aimed to understand the characteristics of a dystocic birth. Labour consists of three phases: phase 1 – cervical dilation and movement of the foetus into the birth canal; phase 2 – active labour, pronounced abdominal straining and expulsion of the foetus, and; 3 – expulsion of the placenta. Typically, dystocia is characterised as a prolonged phase 2 of parturition, but there is no singular identifier of dystocia. Rather it is a subjective and varied experience, dependant on the individual ewe. I was interested in the possibility of behavioural identifiers and physiological markers that may be used to identify, and in turn, predict dystocic events. This was explored in Chapter 2.

One method explored in this thesis was to identify dystocic lambing events through behavioural observation. Behaviour is an important indicator of animal health and welfare status, and is being used more and more frequently to assist in animal management decision making (Nawroth et al., 2019). Additionally, behaviour assessment is a non-invasive option, and with an experienced observer can be an effective animal monitoring tool.

Sheep are grazing animals, and therefore spend the majority of their time grazing and resting (Alvarenga et al., 2016). Much like the study conducted with cows by Miedema et al. (2011), there is a noticeable change in behaviour when the ewe prepares for parturition (Waters et al., 2021). Time spent grazing and walking decreases, the frequency of standing and lying bouts increases, the ewe generally isolates herself, and performs nesting behaviours such as pawing at the ground and circling (Alexander, 1988; Dwyer & Lawrence, 2005). In our first study, these behaviours were common between eutocic and dystocic events, but the frequency at which behaviours were performed was not. We found that in an observation period of 5.5 h before lambing, on average, dystocic ewes performed 754 behaviour transitions, whereas eutocic ewes performed 398 behaviour transitions. These findings are similar to increases in postural changes and reported 'restlessness' in dystocic cows described by Barrier et al. (2012) and Proudfoot et al. (2009). Although total behaviour transitions were higher in dystocic ewes, we saw that they had lower behavioural transitions in the final 30 minutes of observation. When the observation period was broken down into 30 minute blocks and plotted on a graph (Chapter 2; Figure 2) we were able to visualise that the behaviour transition patterns over time for eutocic and dystocic ewes were quite different. Furthermore, when the peak of behaviour transitions were lined up, and the curves compared (Chapter 2; Figure 3) we noticed there were similarities (and no significant difference) at each time point. We suggest there is a link between behaviour transitions and the progress of parturition. The peak in behaviour transitions indicates the point at which birth should occur; however, dystocic events extend past this point causing over-exertion in the ewe and a noticeable decrease in behaviour transitions. From this chapter we were able to address our first research question: are there observable differences in ewe behaviour between eutocic and dystocic lambing events?

As dystocia influenced ewe behaviour before birth, this led us to investigate the effect of dystocia on ewe behaviour after birth in Chapter 3. Compared to prenatal behaviour, there have been more articles published on postnatal behaviour. As for many mammals, the survival of the neonatal lamb relies heavily on the provision of sufficient and appropriate care from their dam. Maternal behaviour includes licking the lamb, which helps dry and stimulate the lamb (Alexander, 1988) and allows the ewe to learn the scent of her lamb, forming a strong and exclusive bond (Dwyer, 2014; Dwyer et al., 2003; Nowak et al., 2007). The ewe will stand still allowing the lamb to locate the udder, and will stand in a "suckling posture" to enable sucking (Alexander, 1988; R Nowak et al., 2000). Maternal behaviour dysfunction without human intervention leads to a breakdown of the ewe-lamb bond, increasing the risk of death of the lamb by starvation, exposure or mismothering (Darwish & Ashmawy, 2011; R Nowak & Poindron, 2006). A common cause of maternal behaviour dysfunction is dystocia, manifesting as maternal exhaustion, disinterest, injury or death (Alexander, 1988; Bickell et al., 2010; Darwish & Ashmawy, 2011; El-Hamamy & Arulkumaran, 2005).

In contrast to previous studies, we performed continuous behaviour annotations of ewes for 2 h immediately following birth. We aimed to identify the differences in total behaviour transitions, and the differences between specific behaviours performed by eutocic and dystocic ewes, to answer our second research question: how does dystocia affect the behaviour of ewes after birth? We observed significant differences in behaviour after birth between eutocic and dystocic ewes. Firstly, on average dystocic ewes performed significantly fewer behaviour transitions in the 2 h after birth compared with eutocic ewes. Specifically, dystocic ewes performed fewer bouts of maternal behaviour (including grooming, licking, bonding and circling), and a shorter duration of these bouts. Additionally, dystocic ewes performed more bouts of avoidance-type behaviours (including walking and standing) and spent more time performing these behaviours. With these results, we were able to see that dystocia had a marked effect on the ability of the ewes to provide the same amount of care to her lamb(s) as eutocic ewes.

From these two investigations, the effect of dystocia on pre-lambing behaviour and post-lambing maternal behaviour were explored and differences were identified. The next step was to look at the possibility of using remote sensing technology to capture lambing activity, and whether it can be used to distinguish between eutocic and dystocic events.

Parturition detection in livestock using animal-mounted devices is a relatively recent development to already established animal monitoring systems – predominantly cattle – and is discussed in depth by Chang et al. (2020) and Saint-Dizier & Chastant-Maillard (2015). Neck-, ear- and tail-mounted accelerometers and inclinometers have been used to detect activity changes, belts to monitor uterine contractions, and intravaginal devices to monitor temperature changes, amniotic membrane rupture or birth of the neonate, some of which may be broken upon expulsion. There are some challenges faced when appropriating these devices for use in sheep, as they are much smaller in size, usually have some wool during lambing that may interfere with contraction detection, and in Australia, most sheep are tail-docked, rendering tail-mounted sensors incompatible. In our study, we deployed accelerometers mounted on elasticated straps, and attached them around the ewes' necks. This made application and removal quick and simple, and devices were reusable without any intermediate steps. There did not appear to be any discomfort while wearing the accelerometers, and they stayed in place for the duration of deployment. Accelerometer data was collected on ewes enrolled in previous observation trials described in Chapters 2 and 3. Behavioural differences between eutocic and dystocic ewes had been identified through direct observation, and through the parallel deployment of accelerometers, the question of whether this difference could be observed through movement data alone could be addressed. In the 5.5 h before lambing, we saw that dystocic ewes spent significantly more time active and less time resting than eutocic ewes (Chapter 2; Figure 4). Additionally, dystocic ewes spent equivalent time resting and active, while eutocic ewes spent more time resting than active in the hours before birth. A recent study by Gurule et al. (2021) assessed the use of ear-tag accelerometers to detect parturition-related behaviour in ewes. Similar to the findings of Smith et al (2020), they were able to use activity and inactivity to identify the day and the hour of lambing; however, they found it difficult to identify specific behaviours. Our results show promise for the possibility of detection of dystocia using remote-sensing devices. The fact that a difference in activity is able to distinguish between eutocic and dystocic events means that the investment into the development of this technology is worthwhile. Accelerometers and similar emerging technologies provide promising alternatives to traditional behaviour research, increasing the quantity and decreasing the labour investments associated with animal behaviour studies. It may be possible to predict dystocic events from behavioural signatures, enabling timely interventions and a subsequent increase in survival. Here we addressed the third research question: can accelerometers be used to capture the differences in lambing activity between eutocic and dystocic ewes?

In another line of enquiry to detect dystocia before birth, in Chapter 4 we used penside blood analysis technology in a series of lambing cohorts to gather information about blood markers around lambing for eutocic and dystocic events. It has been established that maternal nutrition contributes to the risk of dystocia (Dwyer et al., 2003; Holst & Allan, 1992; Jacobson et al., 2020), but to date no studies have looked at blood markers before birth as indicators or possible predictors of dystocia. We chose assays based on handheld devices (AccuChek glucometer and iStat-1 analyser) as assays performed in the lab are time consuming, require extra materials and a skilled operator. In this trial, we were able to address the fourth and final research question: can penside blood analysis be used to identify and/or predict dystocic events in the periparturient period?

Information was collected on blood parameters in pregnant and periparturient ewes to further understand the effect of dystocia on the physiology of the ewe. In the pilot study, results indicated that observable differences can be found between dystocic and eutocic ewes including lower potassium, chloride and glucose, elevated creatinine and disturbed blood gas values in dystocic ewes; however, observed differences in the pilot study were dependent on cohorts, pointing to a strong influence of environmental conditions on individual parameters, and the time at which samples were collected.

The second phase was a time course trial to refine our sampling protocol. From this phase, we determined that collecting a blood sample between birth and 3 h post-birth will show the disturbances caused by the birth event itself, and collecting a sample 12-24 h later may show lingering changes to markers that take longer to normalise, including creatinine (Chapter 4; Figure 1). Finally, we recognised the importance of a pre-birth sample to provide context to each ewe's baseline, and to identify possible predictors of dystocia.

In the main trial, we collected samples approximately one week before lambing, within 3 h of lambing, and a third sample 16-26 h after lambing. We confirmed observable differences between eutocic and dystocic lambing events in the pre- and postpartum periods. Dystocic ewes presented in late pregnancy with significantly lower chloride and higher haematocrit and haemoglobin, compared to eutocic ewes. At birth, creatinine was significantly higher in dystocic ewes. By 16-26 h after lambing, no differences were seen between eutocic and dystocic ewes, and markers were comparable with pre-birth concentrations.

From the experiments conducted to complete this thesis, the characteristics of dystocia were explored and described. There is a significant difference in the behaviour and activity of dystocic ewes in the 5.5 h leading up to birth, which is able to be observed through traditional behaviour annotation and with the use of animal-mounted accelerometers. Additionally, dystocia significantly impacts the expression of maternal behaviour in the 2 h following birth. Finally, penside blood analysis showed differences in blood markers between eutocic and dystocic ewes immediately post-birth, and potential physiological indicators of dystocia in the week leading up to birth. These differences however, were cohort dependant and marked seasonal differences were seen.

As with any experiment, there are limitations to this thesis. The biggest limitation was sample size; in Chapters 2 and 3, eutocic vs dystocic numbers were small (and uneven in Chapter 2). There was an element of luck for capturing dystocic lambing events under surveillance – firstly, the ewe had to be in the lambing enclosure to be recorded, and the video recordings had to be clear and complete. As there were other points of variation (litter size, lamb breed), it was difficult to make any generalised conclusions about the effect that litter size and lamb breed may have had on birth difficulty. There are other studies that have greater group numbers, so should be referred to over ours. If these experiments were to be designed again, perhaps we would use one sire breed per experiment rather than two, to reduce the number of potentially confounding factors.

Dystocia must continue to be researched in order to find feasible solutions and practical interventions that can be applied in any sheep production setting, whether in a research facility, a small holder property or a commercial farm. The use of sensor-based behaviour monitoring is a very attractive research avenue, as it may be able to replace direct behaviour observation and increase the traditionally small sample sizes used, and reduce the number of hours needed to collect data in behaviour research. Further improvement could lead to the refinement of a device that is commercially available to detect parturition behaviour and in turn, dystocic events in sheep. This may be the most relevant and viable option for further research, as it is less expensive, invasive and technically difficult compared with blood analysis methods described in Chapter 4.

The use of pen side blood analysis technology such as the iStat-1 used in this thesis, could be further investigated and refined in order to identify the most relevant blood markers, and continue to improve sampling time and technique for future research.

Ideally, dystocia should be approached as a multifaceted issue rather than targeting each factor individually. Improvements should be made in animal management, data collection, behaviour observation and physiological monitoring in order to improve animal outcomes, welfare standards and reduce animal and economic wastage.

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