

The Tail of a Lamb: The Welfare and Behavior Implications of Tail Docking Sheep

by

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## DEDICATION

*This dissertation is dedicated to  
My husband, Eddy Birth, for your endless support,  
My faithful companions, Sushi and Morel,  
And the billions of animals used each year to better the lives of humans. I hope this research is  
one small step toward a better future for the animals in our care.*



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## ABSTRACT

Each year, millions of sheep are tail docked globally, but we know remarkably little about how they recover from this procedure and how it may influence their behavioral development. To address some of these knowledge gaps, I conducted three experiments following a cohort of female twin sets from birth through their first pregnancy. My objectives were to: 1) characterize the healing progression of tail docking wounds and how this procedure may affect healing of wounds from ear tagging, another common painful procedure (Chapter 2); 2) determine how tail docking ewes as neonates affected ewe and ram mating behavior between 7 to 8 months of age (Chapter 3); and 3) evaluate whether tail docking ewe lambs impacted their maternal behavior and the behavior of their lambs (Chapter 4). Within 28 Polypay twin ewe lamb pairs, one twin was randomly selected to be docked at 24-36 hours of age using a rubber ring while her sister was left undocked (28 docked and 28 undocked). On average, tail wounds took  $43 \pm 9$  days to heal (range: 30-60 days), and pus, a sign of infection, was present at least once in 89% of wounds and was associated with delayed healing (Chapter 2). Tail docking did not affect the healing rates of ear tag wounds (Chapter 2). When a subset of 36 ewe lambs ( $n=18$ /treatment) were 7-8 months of age, they were exposed to virgin rams (2 twin pairs per ram), and their behavior was recorded for the first two hours of exposure (Chapter 3). Undocked ewes tended to be mounted sooner by the ram and, when mounted, tended to accept more mounts compared to docked ewes, suggesting that tail docking may reduce female sexual attractiveness and receptivity (Chapter 3). Finally, I recorded the behavior of 21 ewes (11 docked, 10 undocked) who were approximately 13 months of age and gave birth to singleton ( $n=6$  ewes) or twin ( $n=15$  ewes) lambs. Behaviors were recorded in the two hours following lambing and in response to a brief lamb-dam separation (Chapter 4). I did not find evidence that neonatal tail docking impaired the

ewe's ability to bond with her lambs later in life. Regardless of tail docking status, the ewes showed signs of distress when separated from their lambs and increased lamb-directed behaviors upon reunion (Chapter 4). Overall, these studies provide insight into the welfare implications of neonatal tail docking of lambs. The duration of healing and presence of infection in tail wounds are a welfare concern and warrant further investigation into alternatives to tail docking with a rubber ring, such as hot iron docking or raising undocked sheep. While maternal behavior appears unaffected, possible disruptions to sexual behavior in docked ewes underscore the need for further research on the long-term behavioral and welfare implications of tail docking. Future studies should investigate how tail docking may influence other aspects of social behavior long-term, including relationships among flock mates and potential transgenerational effects.

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## LIST OF ABBREVIATIONS

ARRIVE	Animal Research: Reporting of <i>In Vivo</i> Experiments
FOV	Field of View
HPA	Hypothalamic-Pituitary-Adrenal
LPS	Lipopolysaccharide
RFID	Radio Frequency Identification
USDA	United States Department of Agriculture

## CHAPTER 1: Literature review

The ethical concerns surrounding animal welfare can be delineated within three schools of thought: biological functioning, natural living, and affective state (Fraser et al., 1997). Those concerned with the animal's physiological state and whether their biological needs are met fall into the biological functioning school of thought. Natural living prioritizes an animal's ability to express species-specific behaviors and live in an environment reflective of their native habitat. Finally, the school of affective state believes that an animal's welfare depends on the absence of suffering and the presence of positive emotions. As the field of animal welfare has evolved, it is generally accepted that assessing an animal's affective state is the priority, as changes to an animal's affective state are reflected in metrics pertinent to each school of thought (Duncan, 2005). As such, there is a strong emphasis on reducing animal suffering through the alleviation of negative affective states which last a long time, such as pain and anxiety.

Across industries, animals in human care are exposed to situations that evoke suffering. Suffering is particularly prevalent in animal agriculture, where animals may be exposed to social isolation, maternal-offspring separation, suboptimal environments, poor handling, and painful procedures. In farm animals, painful procedures such as beak trimming in laying hens, castration in goats, sheep, pigs, and cows, and tail docking in pigs and sheep are commonplace. Each of these procedures elicits an affective response indicative of pain, but this response varies depending on numerous factors, such as the animal's age at the time of the procedure, the severity of the procedure, and an animal's previous experience. Most of these procedures occur early in life, which may alter the physiological and psychological development of animals, possibly resulting in abnormal behavior as adults.

### **Introduction to tail docking**

Each year, millions of sheep worldwide have a portion of their tail removed, a procedure known as tail docking (Orihuela and Ungerfeld, 2019). As of 2011, over 80% of the 3.5 million lambs born yearly in the United States were tail docked (USDA, 2014). Typically, lambs' tails are docked at varying lengths within the first two months of life by surgically shortening the tail with a knife or hot iron or by applying a constrictive rubber ring, which causes ischemic necrosis, eventually causing the distal end of the tail to fall off (Orihuela and Ungerfeld, 2019). In the United States, sheep are typically docked between the distal end and the "V" of the caudal folds (USDA, 2014).

The most cited reason for tail docking is to reduce fecal and urine soiling of the wool, a risk factor for disease and parasitism (Broughan and Wall, 2007), which can be lethal. External parasitism from blowflies, also known as ovine cutaneous myiasis, occurs when blowfly larvae hatch from eggs laid on the sheep's wool and feed upon the flesh of the sheep, resulting in lesions and death if left untreated (Lihou and Wall, 2019). Myiasis is a considerable economic and welfare concern worldwide, but its pervasiveness varies considerably based on location, climate, breed, and flock management, with the largest reported prevalence in the United Kingdom, Australia, and New Zealand (Lihou and Wall, 2019).

Myiasis is estimated to cost the Australian sheep industry 175 million dollars annually (Kotze and James, 2022). In Great Britain, 75% of producers reported at least one case of myiasis, with 1.4% and 2.8% of ewes and lambs being struck over one year, respectively (Bisdorff and Wall, 2008). However, in the United States, the prevalence of blowfly strikes is largely unknown. As of 2011, 41% of farmers across systems (e.g., open range, pasture, fenced) reportedly treated their sheep for any form of external parasite, including ticks, keds, fly larvae,

and mites (USDA, 2014). Further, a survey conducted in 2021 to assess the management and disease concerns of the United States sheep industry found that external parasitism was ranked lowest (10<sup>th</sup>) in the disease category (USDA, 2021).

Research to support the connection between tail docking and a reduction in fecal and urine soiling of the breech is scarce and conflicting. Fecal soiling is commonly evaluated using “dag scoring”, which is an ordinal scale usually from zero to five, indicating no fecal soiling or dense fecal soiling, respectively, over a specified proportion of the animal’s hind end. Using dag scoring, studies have found that fecal soiling was either greater (French et al., 1994b; Ware et al., 2000) or did not differ (Fisher and Gregory, 2007; Soriano et al., 2020) in undocked sheep compared to docked sheep. Additionally, studies that have compared different docking lengths have found conflicting results, with some reporting that longer tails had more dags (Fisher and Gregory, 2007), whereas others found no difference in the amount of dags between lengths (Fisher and Gregory, 2007; Scobie et al., 1999).

Similarly to cleanliness, there is no clear evidence to support a reduction in flystrike when sheep are docked. Studies have reported that the risk of blowfly strike in docked ewes is either lower (French et al., 1994b; Ware et al., 2000), not different (French et al., 1994a; Ware et al., 2000), or even greater (Barros et al., 2024) than in undocked ewes. When comparing flystrike across different tail lengths, no difference was found between short and hock length docking (Scobie et al., 1999). Conversely, a greater risk in short-docked tails compared to undocked ones has also been reported (Watts et al., 1979). Given the lack of clear evidence that tail docking reduces fecal soiling and blowfly strike, it is important to consider other outcomes of this procedure, such as wound healing progression.

## Wounding

Most painful procedures result in external wounds, which typically follow four sequential, overlapping stages of wound healing: hemostasis, inflammation, proliferation, and remodeling (Reinke and Sorg, 2012). Hemostasis is the process of ceasing bleeding, or sanguineous exudate, after the wound is inflicted by creating a platelet plug, which eventually forms a scab or crust (Yu and Zhong, 2021). The inflammation process occurs concurrently with hemostasis. As part of this process, capillaries dilate and become more permeable, leaking serous exudate, which supplies moisture and essential cells (e.g., leukocytes, growth factors, enzymes, nutrients) to the wound bed to support healing (Forss, 2022). In the proliferation stage, the provisional wound matrix formed during hemostasis is repaired with granulation tissue, which consists of a high density of fibroblasts, granulocytes, macrophages, capillaries, and loosely organized collagen bundles (Reinke and Sorg, 2012). Finally, the remodeling stage begins when the development of granulation tissue ends, the active wound healing process slows, and the wound contracts (Landén et al., 2016). Many factors can delay the wound healing process, such as multiple simultaneous procedures (Schwacha et al., 2008) or exposure to stressors that elicit fear, anxiety, or pain (Guo and DiPietro, 2010).

Although tail docking is prevalent in the sheep industry worldwide, there has yet to be a formal evaluation of the healing progression of wounds resulting from the procedure. One study reporting early wound scores for tail docking found that 44% of lambs docked with a hot iron were fly stricken at the wound site 4 days after the procedure (Small et al., 2014). Contrary to its intended purpose, this finding suggests tail docking may attract blowfly strikes rather than prevent them, at least in the first few days after the procedure. Previous informal reports have also found that wound healing outcomes are potentially affected by the length of the docked tail;

lambs docked at shorter lengths had more severe wounds that took 2 weeks longer to heal, at about 5 to 6 weeks, compared to longer tails, which healed at 3 to 4 weeks (Johnstone, 1944; Watts et al., 1979). Shorter docking lengths have also been found to increase the risk of infection at the wound site (Watts et al., 1979) and bacterial joint infection (Lloyd et al., 2016) when compared to tails docked at longer lengths.

Other factors, such as the docking method used, may influence healing outcomes, although this has not been evaluated in sheep. One study in piglets reported that tail docking wounds were not healed when observations ended at 7 days, regardless of whether cold cutting or a surgical laser was used (Lou et al., 2022). It has been hypothesized that the hot iron method may reduce infection risks due to the sterilization and immediate cauterization of the wound (Sandercock et al., 2016). However, two studies that compared wound healing rates in piglets who were docked with blunt trauma cutters (cold clipping/cutting) or hot iron cautery over 2 weeks found either no difference (Marchant-Forde et al., 2009) or that wounds from hot iron cautery took longer to heal (Sutherland et al., 2009).

The presence of multiple simultaneous wounds may also influence healing rates. In humans and mice, it has been found that individuals who experience multiple wounds concurrently have a suppressed inflammatory response which delays healing compared to individuals with a single wound (Han and Ceilley, 2017; Schwacha et al., 2008). In sheep, tail docking and ear tagging are often performed within minutes of each other, but it is currently unknown how performing these procedures concurrently impacts healing rates.

## Pain

Potential pain experienced from procedures and during healing poses a welfare concern. Indeed, research on other painful procedures, such as disbudding in cattle, shows that pain is present throughout the healing process and possibly longer (Adcock and Tucker, 2018; Casoni et al., 2019). Pain is defined as an “adverse sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues” (Molony and Kent, 1997). An animal is thought to be capable of experiencing pain if it has nociceptors, pain-processing brain structures, connections between nociceptors and pain-processing brain structures, opioid receptors, responds to noxious stimuli and analgesic treatment, shows avoidance learning, and changes to normal behavior in response to pain (Sneddon et al., 2014).

Pain occurs when a noxious stimulus, either mechanical, thermal, or chemical, activates nociceptors localized at nerve endings. Once this occurs, nociceptors generate action potentials that travel to the spinal cord, releasing neurotransmitters that activate other neurons, mediating immediate unconscious withdrawal reflexes, sending inflammatory molecules to the injury site, and relaying nociceptive activity to the brain. The signals are registered and identified as pain once they reach the cerebral cortex. Acute pain sensitizes the nociceptors at the site of the wound, which decreases the threshold for nociceptor response, causing the animal to react consciously to the pain. Acute pain has a protective role and typically resolves with healing (reviewed in Viñuela-Fernández et al., 2018). However, long-lasting changes in pain pathways, known as neuronal plasticity, can lead to chronic pain. This occurs when there is sustained nociceptive input to the spinal cord, resulting in an exaggerated response to a noxious stimulus, hyperalgesia, or pain in the presence of a non-noxious stimulus, allodynia. Chronic pain is

usually a consequence of nerve injury or prolonged inflammation and persists after the wound is fully healed. Chronic pain is considered maladaptive (Molony and Kent, 1997).

### *Tail docking pain*

Tail docking is commonly performed in sheep without anesthesia or analgesia, even though there are clear changes in physiology and behavior indicating acute pain regardless of docking length, age, or method (Sutherland and Tucker, 2011). Lambs who were tail-docked using rubber rings had elevated cortisol levels after applying the ring for up to two hours following the procedure compared to handled controls (Graham et al., 1997; Kent et al., 1998). Across studies, it has been found that tail docking evokes a distinct behavioral response in the hours following the procedure, including increases in abnormal postures, vocalizations, and lying-standing bouts compared to handled control lambs (Futro et al., 2015; Grant, 2004; Molony et al., 1993; Molony and Kent, 1997). A study that compared the behavioral responses of lambs experiencing different painful procedures found that tail docking with a rubber ring evoked a stronger pain response than lambs who were only ear tagged, hot docked, or experienced hot docking, mulesing, and ear tagging concurrently (Grant, 2004). Further, the pain reaction of lambs tail docked with a rubber ring was the same as lambs who were castrated with rubber rings and those who experienced a combination of hot docking, rubber ring castration, mulesing, and ear tagging. However, there is some evidence that a social partner (Guesgen et al., 2014) or the dam (Hild et al., 2011) can buffer acute pain experienced from tail docking.

There is also substantial evidence that tail docking can cause chronic pain. Docked tail stumps are more sensitive than intact tails for at least 90 days post-procedure, indicating long-term hyperalgesia (Larrondo et al., 2019). Additionally, tail docking has resulted in traumatic neuroma development in sheep (Fisher and Gregory, 2007; Larrondo et al., 2019), as well as pigs

(Herskin et al., 2015; Sandercock et al., 2016), dairy cattle (Eicher et al., 2006), and dogs (Gross and Carr, 1990). Neuromas are bundles of thickened nerve stumps at the site of the amputation and can cause chronic pain (Larrondo et al., 2019; Sehrioglu et al., 2009). Beyond long-lasting pain at the site of the injury, early life painful procedures can lead to prolonged systemic changes in pain sensitivity (Adcock, 2021).

### *Neonatal pain*

Not long ago, it was believed that neonates across species could not perceive pain due to underdeveloped neurological pain processing systems. However, this has since been dispelled with research on the early development of pain pathways, finding that neurons responsible for detecting noxious stimuli innervate the dorsal horn as early as embryonic day 14 in rodents (Jackman and Fitzgerald, 2000) and between the 8<sup>th</sup> and 19<sup>th</sup> week of gestation in humans (Konstantinidou et al., 1995). Further, very young preterm infants show heightened behavioral responses such as facial grimacing, crying, and reflexive withdrawal to painful stimuli (Abdulkader et al., 2008; Goubet et al., 2001). Additionally, there is some evidence that neonates may experience pain more intensely due to rapid conscious awareness immediately after birth, which, in lambs, may begin to decline between two to four weeks of age (reviewed in Mellor and Stafford, 2004).

Early life pain not only causes an immediate physiological and behavioral response in neonates, but it may alter neural pain pathways, leading to increased sensitivity, also known as hyperalgesia, to acute pain later in life (reviewed in Adcock, 2021). For example, lambs castrated earlier in life had a stronger reaction to tail docking later in life with increased rolling, standing unsteadily, and abnormal standing postures, compared to lambs who were tail docked at the same age but castrated at older ages (McCracken et al., 2010). Further, docked ewes expressed more

pain-related behavior, such as increased contractions and posture changes, when lambing, a naturally occurring painful experience, than undocked ewes, suggesting tail docking may induce long-lasting changes in pain sensitivity (Clark et al., 2014).

#### *Long-term behavioral outcomes*

Besides long-term changes in pain sensitivity, early activation of the HPA axis in response to early life stressors, such as pain, has been found to result in other long-term adverse effects in rodents, including abnormal behavior as adults (Mooney-Leber and Brummelte, 2017). Early life inflammation may harm behavioral development and is an essential consideration for animal (and human) welfare. Preterm babies in the neonatal intensive care unit experience many early life stressors, including painful procedures like heel lancing. Babies exposed to early life pain have a higher risk of developing mental disorders and cognitive deficits such as anxiety, autism, depression, and others, which alter their ability to cultivate appropriate social skills through development and adulthood (Mooney-Leber and Brummelte, 2017; Victoria and Murphy, 2016). Rats exposed to early life inflammation have reduced social interactions and experience more social rejection as adults (Connors et al., 2014; Mychasiuk et al., 2014). Male and female rats exposed to immune challenges, specifically lipopolysaccharide-induced (LPS) inflammation, as neonates have impaired sexual development and reproductive success as adults (Walker et al., 2011). Further, LPS-exposed female and male neonate rats showed reduced receptive behavior and mounting attempts as adults, respectively, compared to control rats (Walker et al., 2011). In addition to reproductive behavior, early life pain can negatively impact maternal behavior across species (Mooney-Leber and Brummelte, 2017). Female rats experiencing an LPS injection as neonates showed reduced maternal care and increased offspring mortality in adulthood (Sominsky et al., 2012; Walker et al., 2012). As such, it is possible that

sheep exposed to early-life pain from tail docking may have impaired reproductive and maternal behavior later in life.

### **Role of the tail**

It is important to note that across studies that examine the influence of tail docking on sheep behavior, the alteration to the tail itself, resulting in a nonfunctional tail stump with compromised movements, may also play a role. The tail is believed to be important in intraspecific communication, although this has been scarcely studied. Wild and domesticated sheep live in flocks and regularly communicate with conspecifics about threats of predators, sexual proceptivity, food availability, and care and protection of young (Dwyer, 2017). Sheep use a multitude of senses to communicate, including auditory, visual, and olfactory cues, as well as physical touch. Sheep can distinguish between photos of faces of familiar and unfamiliar sheep and retain the memory for at least 2 years, suggesting that visual cues are important for individual recognition (Kendrick et al., 2001). Immediately after birth, ewes rely on their sense of smell to bond with their lamb, but within a week, they primarily use visual and auditory cues (Dwyer, 2017). As such, the tail may play an important role in visual communication throughout key relationships in a sheep's life.

Tail posture and movement are well-known indicators of emotional state in ungulates (e.g., pigs: Camerlink and Ursinus, 2020; cattle: Schwartzkopf-Genswein et al., 1997). For example, lambs waggle their tails when suckling from the dam, a positive experience, and sheep elevate their tails when separated from their flock, a negative experience (Kiley-Worthington, 1976; Reefmann et al., 2009). The function of tail postures and movements for communication in sheep is poorly understood, but it is possible that docking the tail may impede a sheep's ability to communicate with conspecifics (Fierros-García et al., 2018), which has been found to be

detrimental to social interactions in tail docked dogs (Mellor, 2018). Indeed, past research suggests that tail wagging in female ruminants may function as a behavior of proceptivity and receptivity that increases sexual attractivity to males (Fierros-García et al., 2018; Haulenbeek and Katz, 2011).

The tail may also be essential for communication between mothers and their offspring, but this has been scarcely considered. There is some evidence in sheep (Futro et al., 2015), cattle (reviewed in González-Mariscal, 2022), and white-tailed deer (Townsend and Bailey, 1975) that tail wagging by the offspring occurs simultaneously with suckling. There are competing, untested theories for this phenomenon, such that tail wagging may attract siblings to the udder (Townsend and Bailey, 1975), indicate a reduction in milk flow (González-Mariscal, 2022), or draw the dam's attention to the offspring's perineal region for grooming (Kiley-Worthington, 1976).

We currently lack information on how the potential for impaired communication from tail docking influences key social relationships throughout a sheep's life – particularly her interactions with potential mates and offspring. Further, the physiological effects of neonatal tail docking in lambs, such as altered pain sensitivity and chronic pain, cannot be disentangled from the lack of tail, which may be essential for intraspecific communication. The goal of my research is not to isolate these mechanisms but to acknowledge that both physiological and communicative factors may contribute to observed behavioral changes after docking. As such, all mechanisms are considered throughout this work.

### **Objectives**

My dissertation assesses the welfare and behavior implications of tail docking ewe lambs with rubber rings at 24-36 hours of age. First, I will formally characterize the healing process of

tail docking wounds and assess whether this procedure influences healing of wounds caused by ear tagging, another routine procedure often performed concurrently with tail docking (Chapter 2). I will then evaluate whether the ewe's tail docking status affects her own or the ram's mating behavior at 8 months of age (Chapter 3). Finally, I will assess whether neonatal tail docking impacts her maternal behavior at 13 months of age or her offspring's behavior after lambing, as well as their behavioral and physiological response to separation (Chapter 4). The outcomes of this research will provide valuable insights into the short- and long-term consequences of tail docking sheep as neonates, which can be used to inform future recommendations for the practice.

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## CHAPTER 2: Healing progression of tail docking and ear tag wounds in lambs

### **Preface**

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Formatting and reference style were changed for consistency throughout the thesis.

### Abstract

Tail docking and ear tagging are common husbandry practices in lambs, but little is known about subsequent wound healing or how it may be affected by performing both procedures concurrently. Our objectives were to: 1) describe wound healing following tail docking and ear tagging, and 2) compare healing of ear wounds in docked and undocked lambs. Within 28 female Polypay twin pairs, one lamb was docked using a rubber ring between 1 to 2 days of age and the other lamb's tail was left intact. Tags were attached to both ears of all lambs. We photographed tail and ear wounds twice weekly until weaning (mean  $\pm$  SD;  $64 \pm 5$  days of age). Tail wounds took  $43 \pm 9$  days to heal (range: 30-60 days). Pus, a sign of infection, was present at least once in 89% of tail wounds and was associated with delayed healing. Only 49% of ear wounds had healed by weaning, and tail docking did not predict the probability of healing. Pus and sanguineous exudate (i.e., bleeding) were present at least once in 21% and 96% of ear wounds, respectively. Pus was not associated with the probability of ear wound healing, but ears with more frequent bleeding were less likely to have healed by weaning. The duration of healing and likelihood of infection following both procedures raise welfare concerns and suggest refinements or alternatives are warranted.

## Introduction

Tail docking sheep occurs on a large scale globally (Orihuela and Ungerfeld, 2019), often within the first two months of age (French and Morgan, 1992). Tail docking can be performed by surgically shortening the tail with a knife, scalpel, or hot iron, or by applying a constrictive rubber ring which leads to ischemic necrosis, eventually causing the distal end of the tail to fall off. The length of the docked tail varies but in the United States, docking typically occurs between the distal end and the “V” of the caudal folds (USDA, 2014). Tail docking is done primarily to reduce fecal and urine soiling of the wool, which is a risk factor for flystrike. However, research to support the connection between tail docking and cleanliness of the hind end (Fisher and Gregory, 2007; Scobie et al., 1999; Soriano et al., 2020; Ware et al., 2000) or a reduction in flystrike (French et al., 1994b; Scobie et al., 1999; Ware et al., 2000) is scarce and conflicting. Conversely, there is unequivocal evidence that tail docking causes acute pain (Grant, 2004; Marchewka et al., 2016) which can become chronic (Fisher and Gregory, 2007; Larrondo et al., 2019), regardless of docking age or method.

In addition to causing pain, tail docking wounds may become infected. Following tail docking with a scalpel, 82% of lambs developed signs of infection within 23 days (Ferrer et al., 2020). Signs of infection were also reported in tail wounds assessed 4 and 7 days after hot-iron docking, with 44% of the lambs’ tail wounds struck by blowflies on the fourth day (Small et al., 2014), a noteworthy finding given that tail docking is performed to prevent blowfly strikes. Untreated infections can prolong inflammation, resulting in systemic infection and delayed or failed wound healing, with potentially lethal consequences (Guo and DiPietro, 2010). Documentation of the healing progression of tail docking wounds in sheep or other commonly docked species (e.g., pigs, cows, dogs) is lacking. A study published in 1944 compared

infections and healing of tail docking wounds in lambs from cold knife cutting at 3 different lengths, but the time to heal was not reported (Johnstone, 1944). Similarly, a 1979 study comparing docking lengths found that shorter tails took about 5 to 6 weeks to heal, 2 weeks longer than other lengths, but no formal evaluation of wound healing was described (Watts et al., 1979).

Ear tagging is another common husbandry procedure performed across farmed animals. In many countries, sheep are required to have one form of identification, typically ear tags, before leaving the farm or being sold (APHIS, 2023). Ear tags vary in shape, size, material, and functionality with some serving as visual identifiers and others using radiofrequency technology (RFID) to identify the individual. Ear tagging causes acute pain in sheep (Karakuş and Karakuş, 2017), pigs (Leslie et al., 2010), and dairy cattle (Stewart et al., 2013) but research on the resulting wounds is scarce and findings are inconsistent. Two studies in sheep found greatly different rates of ear tag infections at 8% (9 infected, n=120) (Karakuş and Karakuş, 2017) and 65% (78 infected, n=120) (Karakuş et al., 2015). Healing rates reported for ear tag wounds in sheep are similarly variable, ranging from 6 (Karakuş and Karakuş, 2017) to 20 (Edwards et al., 2001) weeks. However, these studies did not describe a formal system for evaluating wound healing, limiting the interpretation and generalizability of these results.

It is common for ear tagging and tail docking to occur simultaneously, but whether and how concurrent wounds may impact healing is rarely considered (Adcock and Tucker, 2018a). In humans, it has been found that multiple simultaneous wounds delayed healing in comparison to subjects with only one wound (Han and Ceilley, 2017). Additionally, mice with excisional wounds in conjunction with thermal burns had a suppressed inflammatory response and delayed healing when compared to mice with a single excisional wound (Schwacha et al., 2008).

Psychological stressors resulting in fear, pain, or anxiety may also delay wound healing (Guo and DiPietro, 2010). Thus, additional stress incurred from performing multiple painful procedures simultaneously may impair the lamb's ability to heal.

The objectives of this study were to: 1) describe wound healing following tail docking and ear tagging, and 2) determine if multiple wounds impact healing rates by comparing the healing of ear tag wounds in docked and undocked lambs. We predicted that complications including infection and prolonged bleeding would be associated with delayed healing. We also predicted that docked lambs would have longer-lasting ear tag wounds compared to undocked lambs, due to the additional physical and psychological stress tail docking would have on the immune system.

### **Materials and methods**

This study was conducted from February to May 2022 at the University of Wisconsin-Madison's Arlington Sheep Unit. All experiments in this study were performed in accordance with the relevant guidelines and regulations. The University of Wisconsin-Madison's Institutional Animal Care and Use Committee approved all procedures and daily husbandry practices (Protocol #A006423). Study procedures followed ARRIVE guidelines.

#### *Animals and housing*

We enrolled 56 twin ewe lambs born from 28 multiparous Polypay ewes (mean  $\pm$  SD age in years:  $4 \pm 2$ , range: 2-10; parity:  $5 \pm 2$ , 2-12) between February 16 and March 14, 2022. Sample size was determined by flock availability. Before lambing, ewes were housed in an indoor barn (29 x 11.8 m) with access to a dirt-floor outdoor pen (30.3 x 24.5 m) that was evenly split into three group pens. One to two hours after the second lamb was born, each ewe and her twin lambs were moved from the group pen to an individual maternity pen (1.5 x 1.5 m) in an

adjacent room of the barn. Maternity pens were bedded with fresh straw. Upon entering the maternity pen, each lamb received 2cc of an oral vitamin supplement (Baby Lamb Strength, Premier 1 Supplies) and 2cc of an oral *E. coli* prevention supplement (SpectoGard Scour-Chek, Bimeda). Her umbilical cord was cut and 7% iodine was applied to the stump per standard farm practice. The ewe and lambs were then left alone for 24 to 36 hours to ensure bonding.

Between 24 to 36 hours of age, each lamb was weighed and assigned to a treatment group. One lamb from each twin pair (n=28) was randomly assigned to be docked while her sister remained undocked, but within this approach, assignments were balanced by weight (docked:  $5.62 \pm 1.12$  kg, undocked:  $5.63 \pm 0.63$  kg). Lambs were docked using the constrictive rubber ring method, in which the ring is expanded with an elastrator and placed on the tail at the distal end of the caudal folds (Figure 2.1). Analgesics were not administered following farm protocol and standard industry practice. Author JMW performed all tail docking for consistency. All lambs (docked and undocked) were ear-tagged with plastic-tipped tags (USDA Official Scrapie Set Tags, Shearwell North America, Inc.) immediately before tail docking. Rubber rings and ear tags were disinfected with chlorohexidine 2% prior to application, per standard farm practice. Orange visual identification and gray USDA 840 RFID tags (dimensions: 2.54 x 1.43 cm, hole diameter: 1.5 cm, weight: 1.5 g) were placed on the lamb's left and right ear, respectively, with an applicator by author JMW (Set Tag Applicator, Shearwell North America, Inc.). The ear tags were placed between the ribs at the midpoint between the base and tip of the ear (Figure 2.1). After ear tagging and tail docking (if docked), each lamb received a second dose of 2cc of the oral vitamin supplement (Baby Lamb Strength, Premier 1 Supplies) and 2cc of the oral *E. coli* prevention supplement (SpectoGard Scour-Chek, Bimeda), in addition to 5cc

subcutaneous shot of BO-SE (Selenium, Vitamin E, Merck & Co., Inc) to prevent White Muscle Disease.

Between 2 to 4 days of age, the lambs and their dam were moved into a communal pen bedded with straw with other lamb-dam groups included in the study. For the entirety of the study, half the group (14 ewes and 28 lambs) was housed in an 11.8 x 8.9 m indoor pen with a 24.3 x 10.5 m dirt-floored outdoor pen and the other half was housed in an adjacent 11.8 x 9.7 m indoor pen with a 24.5 x 10.6 m outdoor pen. Both groups were given access to the outdoor pens during the daytime when the weather permitted.

Prior to lambing, each ewe was fed 0.23 kg of whole shell corn and had free choice alfalfa bailage. For the first 1 to 2 days after lambing, ewes received 0.91-1.36 kg of hay. On the second or third day after lambing ewes received 0.23 kg of whole shell corn and 1.36-1.81 kg of hay. Beginning 3 to 4 days after lambing and until 10 days before weaning, ewes received 0.68 kg of whole shell corn per head and had free access to alfalfa bailage within the group pens. 10 days before weaning, ewes were no longer given corn to help reduce milk production. Within the group pens, lambs had *ad libitum* access to 19% crude protein pelleted feed medicated with decoquinatate (Deccox, Premier 1 Supplies) within a creep pen, which restricts ewe access to the feed. Both ewes and lambs had *ad libitum* access to water throughout the study. Lambs were weaned at  $64 \pm 5$  (range: 57-76) days of age. At weaning, ewes and lambs were separated into group pens within the same barn and were not given contact with each other.

#### *Digital photography of wounds*

Photographs of the lambs' ear and tail wounds (docked individuals only) were taken twice weekly on Tuesday and Friday beginning at 1 or 2 days of age when they were ear tagged (and docked) until they were weaned between 57-76 days of age. On observation days, lambs

were separated from their dams into a creep pen to take photos. For ear tag photos taken between 1-2 weeks of age, the handler held the lamb in their arms while steadying the head with their hands. After 2 weeks of age, the lamb was placed between the handler's legs with the head held in place between their hands. Photos were taken for both ears at 6 different angles: aerial view of tag (anterior and posterior ear), left side of tag (anterior and posterior ear), and right side of tag (anterior and posterior ear). To provide a clear view of the wound, the handler used gloved fingers to pull back the wool surrounding the tag as much as possible without disturbing the wound.

Tail photos were taken with the handler holding the lamb in their lap with the lamb's ventral side exposed and pulling all the lamb's legs toward the handler, exposing the tail and breech. Before the tail fell off, 4 photos were taken of the tail at each observation: dorsal, ventral, left, and right sides. To take the dorsal photo, the observer gently moved the tail up towards the lamb's stomach. After the tail fell off, a closeup of the wound stump, looking head-on, was taken. All photos were taken approximately 5-10 cm from the wounds on an Apple iPhone 13 Pro Max 5G, Model MLQJ3LL/A by author JMW. Data collection resulted in 1,978 tail wound and 14,584 ear tag wound photos. Lambs were photographed for a total of 16 to 22 observation days depending on when they were weaned.

### *Wound scoring systems*

We developed scoring systems to evaluate tail docking and ear tag wounds, adapted from a scoring system for disbudding wounds in dairy calves (Adcock and Tucker, 2018b). The presence or absence of three external tissues (slough, granulation, and crust) and three fluid types (serous exudate, sanguineous exudate, and pus) were evaluated in tail wounds for each observation day (Figure 2.2). Tails were additionally scored as attached (1) or fallen off (0) on

each observation day. For ear tag wounds, we scored slough, pus, and sanguineous exudate as potential indicators of prolonged inflammation, infection, and trauma, respectively (Figure 2.3). The presence or absence of each tissue/fluid type was determined after observing all photo angles for each wound. Ear tag and tail wounds were determined to have healed when none of the tissue/fluid types were present and the wound was fully contracted (Figures 2.2 & 2.3). A subset of the tail (n=186) and ear tag (n=36) photos were scored by both authors to determine inter-observer reliability for all tissue and fluid types (Cohen's Kappa for tail and ear wounds > 0.75). The remaining photos were scored by author JMW. The observer was not blind to lamb ID or date but was blind to the docking status when scoring ear tag wounds.

### *Statistical analysis*

Data analyses were performed using the statistical software program R, v4.3.3 (R Core Team, 2024). To determine whether the tail wound healing rate was affected by the prevalence of tissues or fluids indicating prolonged inflammation (slough), infection (pus), or trauma (sanguineous exudate), we fit a linear model (*lm* function, base R) with the number of days it took the wound to heal as the outcome variable and the number of observations that each tissue/fluid type was present as fixed effects. The model assumption of normality was assessed with QQ plots and Shapiro Wilks test (*plot*, *residuals*, and *shapiro.test* functions, base R). The assumption was not met so we moved to a generalized linear model with a Gamma distribution using the *glm* function in base R. The model assumptions of homogeneity of variance and no multicollinearity were assessed with plots of residuals versus fitted values (*plot* and *residuals* functions, base R), and variance inflation factors (VIF<2, *vif* function, *car* package v3.1-2, Fox and Weisberg, 2019), respectively. All assumptions were met. The data and associated R script and output are in Supplementary Files S1 and S2.

We determined whether tail docking or the presence of pus or sanguineous exudate influenced ear wound healing by fitting a generalized linear mixed model with a binomial distribution (*glmmTMB* function, *glmmTMB* package v1.1.9, Brooks et al., 2017). One undocked lamb was excluded from the model due to an injury sustained on her stomach. Due to the high number of unhealed ear wounds still present at the end of the observation period, we evaluated healing as a binary outcome variable (healed = 1, not healed = 0). Tail docking status (docked or undocked), tag type (gray or orange), the number of observations with pus and sanguineous exudate, and the interaction between tail docking status and tag type were fitted as fixed effects and lamb ID (n=55) as a random effect. The model was not overdispersed as assessed using the *testDispersion* function from the *DHARMA* package (Hartig, 2022) v0.4.6. The data and associated R script and output are in Supplementary Files S3 and S4.

## Results

### *Tail docking wounds*

Tails took  $19 \pm 2$  days (mean  $\pm$  SD; range: 16-25 days) to fall off and  $43 \pm 9$  days (30-60 days; Figure 2.4) to heal after the rubber ring was applied. Crust was the most prevalent tissue and was present in 45% of observations for each tail on average, followed by granulation, serous exudate, pus, slough, and sanguineous exudate (Table 2.1 and Figure 2.4). Slough or pus were present at least once in 96% (27/28) of tail wounds and were present mainly in the first few weeks after docking before the tail had fallen off (Figure 2.4). Supplemental File S5 shows the proportion of observations each tissue or fluid was present for each lamb. Tail wound healing time increased as the number of observations that had pus present increased ( $X^2 = 5.55$ ,  $p = 0.02$ ) but was not affected by the presence of slough ( $X^2 = 0.27$ ,  $p = 0.60$ ) or sanguineous exudate ( $X^2 = 0.62$ ,  $p = 0.43$ ).

One tail docked lamb developed edema of the distal tail 4 days after the rubber ring was applied (Figure 2.5). When her condition was discovered, the tail distal to the rubber ring was removed with a hot cauterizing knife.

#### *Ear tag wounds*

Only 49% (55/112) of the ear tag wounds were healed by the time lambs were weaned between 57-76 days of age (Figure 2.6). The probability of the wounds being healed was not predicted by the number of observations with pus ( $X^2 = 0.41, p = 0.52$ ), tail docking ( $X^2 = 2.54, p = 0.11$ ), tag type ( $X^2 = 1.60, p = 0.21$ ), nor the interaction between tail docking and tag type ( $X^2 = 0.001, p = 0.97$ ). However, the probability of a healed ear tag wound decreased as the number of observations with sanguineous exudate increased ( $X^2 = 5.03, p = 0.02$ ). Of the healed ear tag wounds, 32 and 23 belonged to docked ( $n=21$ ) and undocked lambs ( $n=17$ ), respectively (Figure 2.6).

Of the total ear wounds ( $n=112$ ), 21% ( $n=23$ ) and 96% ( $n=107$ ) had pus and sanguineous exudate present at least once, respectively. Slough was observed in only one ear on one observation day. Sanguineous exudate and pus were present on average in 15% and 2% of observations for each ear, respectively (Table 2.1). Supplemental File S6 contains the proportion of observations each tissue or fluid was present for each ear. Sanguineous exudate was observed in nearly all wounds immediately after ear tagging and gradually declined throughout the observation period (Figure 2.7). Pus was present at low rates throughout the observation period (Figure 2.7).

## Discussion

The first aim of our study was to describe wound healing following tail docking and ear tagging. We found, on average, the tail took almost 3 weeks to fall off and 6 weeks to heal after the rubber ring was applied. Only 49% of the ear tag wounds were healed when observations ended between 8 and 11 weeks after ear tagging. Our second objective was to determine if simultaneous wounding from tail docking and ear tagging would influence healing rates. Contrary to our predictions, we observed no influence of docking status on the probability that the ear wounds had healed by the end of the observation period.

Pus, a sign of infection, was present at least once in 89% of the tail wounds and was associated with longer healing times. Pus is an exudate composed of live and dead neutrophil polymorphs and indicates localized infection due to the invasion of microorganisms (Barer, 2012). When infection is present, wound healing is stalled and remains in the inflammatory stage until the infection is cleared (Barer, 2012). Infected wounds are a risk factor for other morbidities. For example, infected wounds following tail docking have been implicated in the development of embolic pneumonia in lambs (Giadinis et al., 2009). The length of the docked tail influences infection risk, with shorter tails at greater risk of flystrike (Watts et al., 1979), infection at the wound site (Watts et al., 1979), and bacterial joint infection (Lloyd et al., 2016) when compared to tails docked at longer lengths. Previous informal reports on tail docking wounds in lambs found that shorter tails, similar to the length in our study, had more severe wounds and took longer to heal at about 5 to 6 weeks than longer tails (Johnstone, 1944; Watts et al., 1979). Thus, docking at longer lengths is recommended to reduce the risk of infection and promote healing. It has also been hypothesized that the hot iron method may reduce infection risks due to the sterilization and immediate cauterization of the wound (Sandercock et al., 2016).

However, docking methods have not been compared in sheep, and only to a limited extent in pigs (Lou et al., 2022; Marchant-Forde et al., 2009; Sutherland et al., 2009). Future studies of the complete healing progression of tail docking wounds across methods are needed to inform recommendations for this procedure.

Slough, a tissue suggested to be a byproduct of prolonged wound inflammation, was present at least once in 93% of tail wounds. Slough is a devitalized tissue that overlays the wound bed and acts as a reservoir for bacteria (Townsend et al., 2024). In human wound care, it is recommended to debride wounds with slough as its presence can delay wound healing and lead to chronic infection (Angel, 2019). However, in some instances, human wounds with slough have healed without intervention (Townsend et al., 2024). We saw similar results in our study, as slough did not lengthen the healing process of tail wounds.

We observed edema in one docked tail, likely due to a faulty ring which was not tight enough to restrict blood flow, allowing buildup of fluid at the distal end of the tail. Tail docking with a rubber ring restricts blood flow to the distal end, which leads to ischemic necrosis and eventually causes the tail to fall off. Using infrared thermography, the presence of ischemia, or the reduction in blood flow, was seen within 15 minutes of tail docking lambs with a rubber ring (Moore et al., 2024). Complications during the ischemic process may arise from incorrect placement or faulty rubber rings. Edema has also been observed after tail docking in puppies and is associated with severe pain, behavioral changes, and systemic infection (Eyarefe and Oguntoye, 2016).

The occurrence of infections and healing complications in tail wounds raise additional welfare concerns about the procedure. Conversely, leaving sheep undocked may pose a welfare concern through increased risk of blowfly strike, though the literature does not sufficiently

substantiate this association. Studies have reported that the risk of blowfly strike in docked ewes is either not different (French et al., 1994a; Ware et al., 2000), lower (French et al., 1994b; Ware et al., 2000), or greater (Barros et al., 2024) than in undocked ewes. Considering the equivocal evidence to support tail docking in sheep, the potential benefits of leaving the tail intact deserve consideration. For instance, undocked ewes had fewer vaginal biota present during copulation when compared to docked ewes, suggesting that the tail may protect the vaginal environment (Orihuela et al., 2019). Additionally, rams preferred to court and mount undocked ewes over docked ewes, suggesting potential advantages of leaving the tail intact for natural service breeding (Orihuela et al., 2018). Given these considerations, the need for tail docking should be evaluated on a flock-by-flock basis. Painless and non-invasive alternatives such as genetic selection for shorter tails also warrant consideration in cases where docking is deemed necessary (James, 2006).

Only 49% of the ear wounds were healed when observations ended between 8 and 11 weeks after ear tagging. Previously reported healing rates for ear tags in sheep ranged from 6 weeks (Karakuş and Karakuş, 2017) to as long as 20 weeks (Edwards et al., 2001), similar to dairy calves whose ear tag wounds took at least 12 weeks to heal (Harmon et al., 2023). These healing times are as long or longer than those reported for other routine painful procedures in livestock, including tail docking piglets (Sandercock et al., 2016), disbudding dairy calves (Adcock and Tucker, 2018b; Drwencke et al., 2023) and goat kids (Alvarez et al., 2019), and castrating lambs (Sutherland et al., 2000). It has been speculated that repeated disturbance of ear tag wounds from human handling, rubbing on enclosures, or interacting with conspecifics may delay healing (Harmon et al., 2023; Hayer et al., 2022; Sherwin, 1990). In our study, ear wounds with more occurrences of sanguineous exudate (i.e., bleeding) were less likely to have healed by

the end of the observation period. Overall, sanguineous exudate was observed in 78% of the ear wounds and was seen in some wounds up to 60 days after tagging, suggesting that the wounds may have experienced repeated trauma that prolonged healing (Bertone, 1989).

Of the total ear wounds, 21% had pus present at least once during the observation period. In humans, infections from ear cartilage piercings can result in ear deformities and hospitalizations (Sosin et al., 2015). Two studies have confirmed that ear tag infections are a risk factor for infectious arthritis in lambs as these infections provide a port of entry for *Streptococcus dysgalactiae dysgalactiae* (Smistad et al., 2021, 2020). It is possible that repeated trauma of the ear wounds could have introduced infection, further prolonging healing. Documenting the behavior of animals following painful procedures would provide insight into the influence of wound disturbance on infection and healing rates. Additionally, identifying the microbiota composition and abundance of immune cells in ear tag wounds could provide insight into the source and severity of infection (Menke et al., 2007; Townsend et al., 2024).

Contrary to our predictions, we did not find a difference in the healing rates of ear wounds between docked and undocked lambs. We predicted that experiencing multiple procedures concurrently may delay healing by overwhelming the animal's ability to mount an effective immune response across multiple wound sites (Schwacha et al., 2008). A previous study in mice reported delayed healing of an excisional wound following severe thermal burns that covered 25% of their body (Schwacha et al., 2008). Thus, it is possible that the inflammatory response to tail docking was not extreme enough to impair an immune response at the ear tag sites. However, other outcomes not measured in the current study, such as pain, may be differentially affected by performing procedures concurrently rather than at separate time points. For example, concurrent wounding may diffuse pain through a phenomenon known as diffuse

noxious inhibitory control, in which the original pain signal is dampened by a new noxious stimulus in another part of the body (Le Bars et al., 1979). Thus, the industry standard of performing procedures concurrently could be better from a pain-mitigation perspective than performing them sequentially over an extended period, although studies are needed to explore this possibility in livestock (Mosher et al., 2013).

### **Conclusions**

Tail wounds took six weeks to heal on average, and half of the ear tag wounds were still not healed when observations ended at two months. Pus, a sign of infection, was present in tail docking and ear tag wounds, indicating the possibility for chronic infection and long-term health risks. Experiencing both procedures concurrently did not delay wound healing, suggesting that the inflammatory response from tail docking was not extreme enough to overwhelm the response to ear tagging. The long healing times and prevalence of infections raise welfare concerns; refinements or alternatives to both procedures should be explored.

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### Tables and figures

**Table 2.1** The mean proportion of observations (out of 16-22 total observations per lamb) in which each tissue or fluid was present in the tail (n=28) and ear tag (n=112) wounds following the procedures. The standard deviation, minimum, and maximum are also shown.

Procedure	Tissue or fluid	Mean proportion of observations with tissue or fluid present	Standard deviation	Min	Max
Tail docking	Crust	0.45	0.13	0.27	0.76
	Granulation	0.19	0.14	0.00	0.47
	Pus	0.10	0.07	0.00	0.24
	Sanguineous exudate	0.08	0.06	0.00	0.19
	Serous exudate	0.15	0.11	0.00	0.44
	Slough	0.09	0.05	0.00	0.19
Ear tagging	Pus	0.02	0.04	0.00	0.18
	Sanguineous exudate	0.15	0.10	0.00	0.50
	Slough	0.0005	0.005	0.00	0.06



**Figure 2.1** Three images showing, from left to right, the rubber ring placed at the distal end of the caudal folds for tail docking, the anterior side of a lamb's left ear with an orange ear tag placed between the ribs at the midpoint between the base and tip of the ear, and the posterior side of a lamb's right ear with a gray ear tag.

Example	Tissue or fluid	Description
	Serous exudate	Clear gel or liquid that can have a yellow tint
	Sanguineous exudate	Red gel or liquid – not dried
	Pus	Milky, thick exudate
	Slough	White or yellow necrotic tissue that is stringy or clumped, loosely or firmly attached to the wound bed
	Granulation	Pale pink to bright, beefy red, opaque, granular tissue
	Crust	Dried blood or exudate, bumpy, not flaky. Above the plane and attached to the wound bed. Can be tan, dark red, or black.










Healed

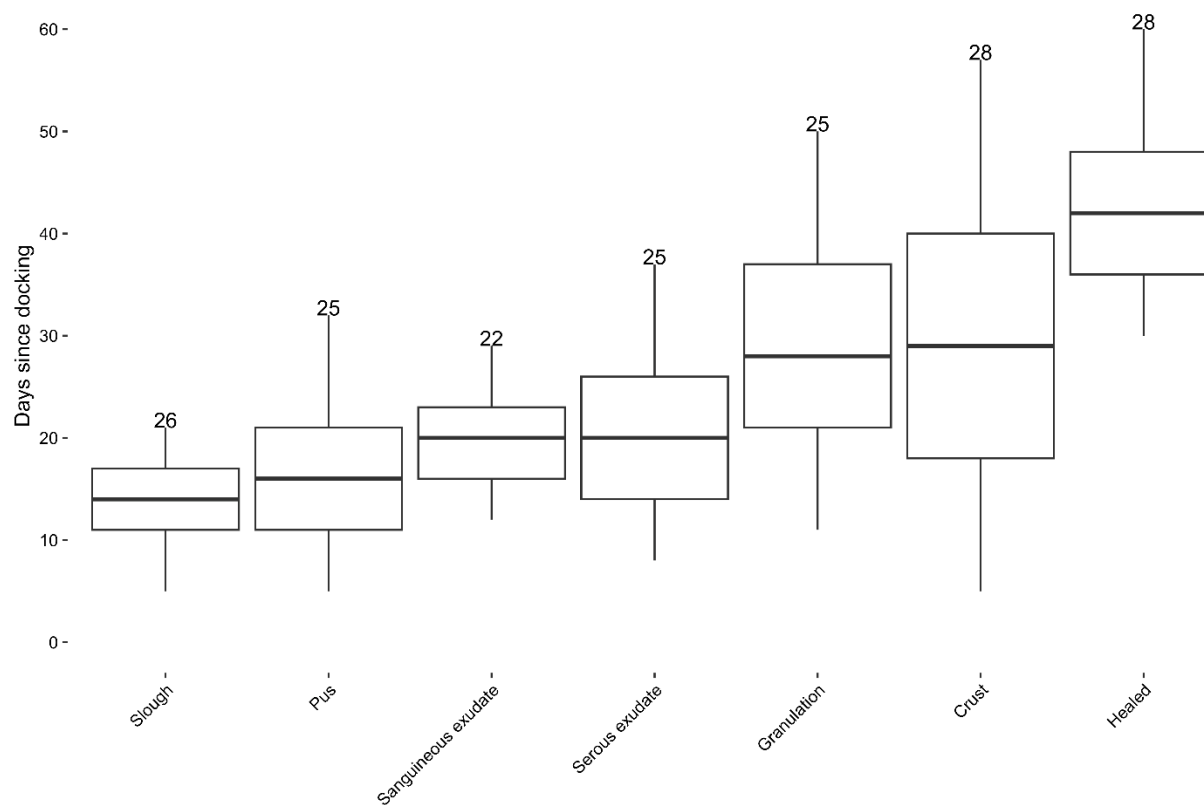
Wound is fully contracted; scar line may be visible; other tissues or fluids are absent

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**Figure 2.2** The scoring system used to evaluate the tissue and fluid types present or absent (1/0) in tail docking wounds. Each tissue/fluid was scored from photos taken twice weekly until weaning, resulting in 16-22 observation days/lamb.

Example		Tissue or fluid		Description
Posterior	Anterior			
		Sanguineous exudate		Red gel or liquid
		Pus		Milky, thick exudate
		Slough		White or yellow necrotic tissue that is stringy or clumped
		Healed		No crust, granulation, exudates, pus, or slough are present on the anterior and posterior ear

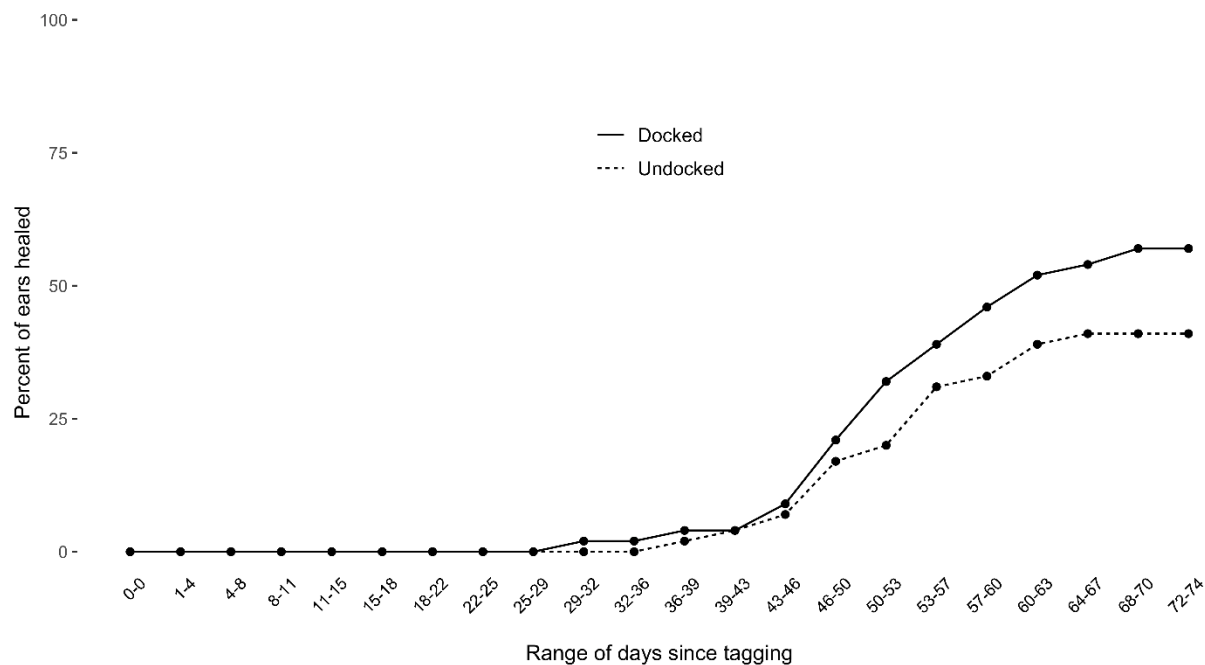
**Figure 2.3** The scoring system used to evaluate the tissue and fluid types present or absent (1/0) in ear tag wounds. Orange visual identification and gray RFID tags were placed on the lamb's left and right ear, respectively. Photos of the anterior and posterior sides of each ear were taken twice weekly until weaning, resulting in 16-22 observation days/lamb.



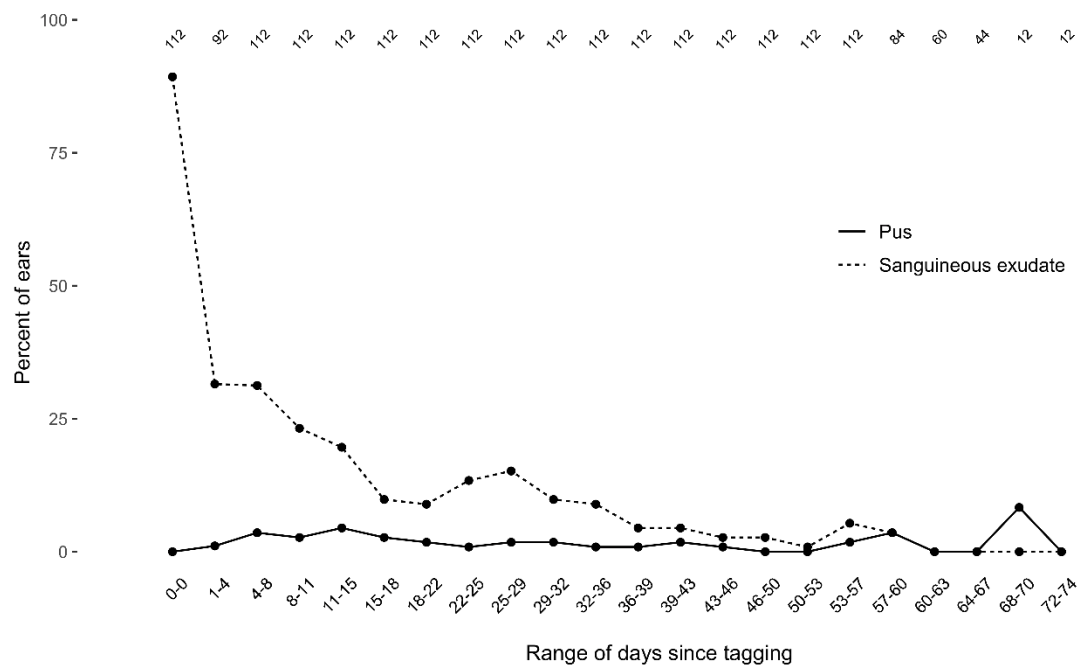
**Figure 2.4** Boxplot displaying the days that each tissue or fluid was observed in the tail wounds relative to docking. The median, 75th quartile, and 25th quartile are indicated by the midline, and top and bottom edges of the boxes, respectively. Whiskers indicate the minimum and maximum. The number of lambs (out of 28) in which the tissue or fluid was observed at least once over the course of the study is displayed above each box.



**Figure 2.5** Tail photos of the lamb who developed edema 4 days after the application of the rubber ring. The photo on the left and in the middle show a view of the ventral and left side, respectively. The photo on the right shows the tail 3 days after cutting off the portion below the ring with a hot cautery knife.



**Figure 2.6** The percent of ear tag wounds (n=110) healed in docked (solid line; n=28) and undocked (dashed line; n=27) lambs relative to days since ear tagging. 53 wounds were healed when observations ended between 55-74 days after ear tagging. One undocked lamb was excluded from this analysis due to an injury sustained on her stomach.



**Figure 2.7** Line plot displaying the percent of ears with pus (solid line) and sanguineous exudate (dashed line) by the days since ear tagging. The numbers above the plot indicate the number of ears that were sampled for each observation day.

CHAPTER 3: Sexual attractivity and receptivity in tailed and docked ewes

**Preface**

This chapter is currently under review in *Applied Animal Behavior Science*

### Abstract

Removing a portion of the tail, also known as tail docking, is commonly performed in sheep. However, there is evidence that females of small ruminant species use their tails to communicate in sexual contexts. The objective of this study was to test whether a ewe's tail status affected the mating behavior of ewes and rams. Within 18 Polypay female twin pairs, one lamb was docked between 24-36 hours of age by placing a constrictive rubber ring on the tail while her sister's tail was left undocked (n=18 lambs/treatment). The estrous cycles of the ewes were synchronized when they reached 7-8 months of age, and 9 groups of 4 ewes (2 twin pairs/group) were each exposed to a single unfamiliar virgin ram (n=9; 7-8 months of age) for 48 hours. Behavioral interactions were video recorded over the first 2 hours and analyzed to determine the ram's latency to investigate each ewe's perineal region and to mount her, the duration of perineal investigation, the number of headbutts and mounting attempts towards the ewe, and the proportion of mounts that the ewe accepted out of the total attempted mounts. We used real-time location sensors to record the *x, y* location of ewes and rams every second, from which we determined each ewe's total distance traveled and average distance to the ram. Undocked ewes tended to be mounted sooner by the ram and, when mounted, tended to accept more mounts compared to docked ewes. No differences were observed between docked and undocked ewes in any of the other behaviors. These findings suggest that tail docking neonatal ewe lambs may reduce sexual attractivity and receptivity later in life, with potential implications for reproductive success.

## Introduction

The practice of removing a portion of the tail, known as tail docking, is commonly performed in sheep worldwide (Orihuela and Ungerfeld, 2019), typically within the first two months of age (French and Morgan, 1992). In the United States, for instance, over 80% of the 3.5 million lambs born yearly are tail docked (USDA, 2014). Tail docking is thought to reduce fecal and urine soiling of the wool, a risk factor for flystrike, which can be lethal. However, there is mixed evidence that tail docking improves the cleanliness of the hind end (Fisher and Gregory, 2007; Scobie et al., 1999; Soriano et al., 2020; Ware et al., 2000) or reduces flystrike (Barros et al., 2024; French et al., 1994; Scobie et al., 1999; Ware et al., 2000). On the other hand, arguments against tail docking include that it hinders the tail's role in social communication (Fierros-García et al., 2018; Orihuela et al., 2018), causes acute and chronic pain, including heightened pain sensitivity (Clark et al., 2014; Fisher and Gregory, 2007; Larrondo et al., 2019), and may alter behavioral development when performed during the neonatal period (Adcock, 2021).

Tail posture and movement are well-known indicators of emotional state in ungulates (e.g., pigs: Camerlink and Ursinus, 2020; cattle: Schwartzkopf-Genswein et al., 1997). For example, lambs waggle their tails when suckling from the dam, a positive experience, and sheep elevate their tails when separated from their flock, a negative experience (Kiley-Worthington, 1976; Reefmann et al., 2009). The function of tail postures and movements for communication have received less attention; however, studies suggest they play an important role in female sexual attractivity, proceptivity, and receptivity (Beach, 1976). Sexual attractivity is the female's ability to stimulate interest from the male. Proceptive behaviors are appetitive actions by the

female that initiate or maintain the sexual interaction with the male. Finally, receptive behaviors are actions by the female that allow the male to achieve intravaginal ejaculation (Beach, 1976).

Evidence suggests that tail wagging in female ruminants may function as a proceptive behavior that increases attractivity. In goats, male sexual arousal increases with the frequency of female tail wagging, as indicated by faster mounting, more ejaculations, and decreased inter-ejaculatory intervals (Haulenbeek and Katz, 2011). In sheep, ewes in estrus have been observed elevating their tails after physical contact with a ram but before copulation, possibly indicating receptivity (Fierros-García et al., 2018). When given the choice between a restrained docked and undocked ewe, rams sniffed, nudged, and mounted the undocked ewes more often, suggesting they found ewes with tails more attractive (Orihuela et al., 2018). However, as ewes were restrained in previous studies, it is unknown whether tail docking affects mating behavior when ewes are unhindered in their sexual interactions.

In addition to potentially impeding sexual communication, tail docking causes acute and chronic pain, including long-term sensitivity in the tail stump (Fisher and Gregory, 2007; Larrondo et al., 2019), regardless of docking length, age, or method used (Grant, 2004; Marchewka et al., 2016; Mellor and Stafford, 2000). Docked ewes expressed more pain-related behavior when lambing at 2 years of age, including contractions and postural changes, compared to undocked ewes, potentially due to an upregulation of inflammatory reactions in pain processing pathways or alterations in neuroimmune responses (Clark et al., 2014). Ongoing heightened pain sensitivity in docked ewes may also alter mating behavior, particularly if pressure is applied to the tail stump during mounting.

In addition to long-term pain sensitivity, tail docking may alter the development of neurobiological pathways. In rodents, early life inflammatory events can alter physical and

behavioral development, including suppressing sexual behavior (reviewed in Adcock, 2021). For example, adult female rats exposed to lipopolysaccharide-induced (LPS) inflammation as neonates were less receptive to males, engaged in fewer proceptive behaviors, and were mounted less than uninjured controls, possibly due to changes in neuroendocrine functioning, including decreased progesterone activity (Mayila et al., 2020; Walker et al., 2011). Since tail docking is an early life inflammatory event (Ferrer et al., 2020), it may similarly suppress sexual behavior as seen in rats following early life inflammation.

We investigated whether tail docking ewe lambs shortly after birth affected ewe and ram mating behavior between 7 to 8 months of age. We predicted that rams would display more mating behaviors toward undocked than docked ewes due to the tail's role in sexual communication. Very little is reported on headbutting by domestic ungulates in a breeding context, but it has been suggested that it indicates sexual interest (Ungerfeld and Alexander, 2024). As such, we predicted that undocked ewes would receive more headbutts compared to docked ewes. Additionally, we predicted that docked ewes would be less sexually receptive than undocked ewes due to chronic pain or impaired sexual development and, thus, would accept fewer mounts and move more as they attempted to avoid the ram and maintain greater distances from him.

### **Materials and methods**

This study was conducted at the University of Wisconsin-Madison's Arlington Sheep Unit from February to October 2022. The University of Wisconsin-Madison's Institutional Animal Care and Use Committee approved all procedures (Protocol #A006423).

#### *Animals and housing*

We enrolled 36 sexually naïve Polypay twin ewe lambs born between February and March 2022. The sample size was determined by flock availability. One ewe within each twin pair was randomly assigned to have her tail docked between 24 to 36 hours after birth while her sister remained undocked. Treatment assignments were adjusted as needed to ensure they were balanced by birth weight (docked:  $5.62 \pm 1.12$  kg, undocked:  $5.63 \pm 0.63$  kg). The ewe lambs were docked by author JMW using the constrictive rubber ring method, where the ring is expanded with an elastrator and then placed on the tail at the distal end of the caudal folds. Rubber rings were disinfected with chlorohexidine 2% before application. Analgesics were not administered in accordance with farm protocol and standard industry practice.

Between 2 to 4 days of age, the lambs and their dam were moved to a communal pen bedded with straw. Lambs were weaned from their dam at  $65 \pm 6$  (range: 57-81) days of age. All ewe lambs were housed together until the current study and were given outdoor access during the daytime when the weather permitted. Lambs were fed a 16% crude protein pelleted feed medicated with decoquinatone *ad libitum* for approximately 30 days after weaning or until they weighed at least 45 kg. They were then switched to a 13% crude protein pelleted feed medicated with decoquinatone and free choice alfalfa bailage until the current study. All sheep had *ad libitum* access to water throughout the study.

### *Experimental design*

We enrolled 9 sexually naïve Polypay rams (mean  $\pm$  SD age in months:  $7.4 \pm 0.3$ , range: 6.9-7.7; weight:  $68.9 \pm 4.5$  kg, range: 63.0-76.2 kg) born between March and April 2022. The farm manager docked all rams following the same procedure as the ewe lambs between 24 and 36 hours after birth. Ram lambs were raised with other rams not enrolled in this study and were housed separately from the ewe lambs from birth.

When ewe lambs were  $8.0 \pm 0.2$  months of age (mean  $\pm$  SD; range = 7.6-8.3), two twin ewe sets (n=4 ewes) were assigned to each ram based on pedigree and balancing for ewe weight within treatment (docked:  $60.45 \pm 7.26$  kg, undocked:  $61.51 \pm 6.35$  kg). Each group (4 ewes, 1 ram) was randomly assigned to one of three indoor test pens (Figure 3.1) on one of three test days (October 24, 26, or 28, 2022). Three groups were tested each day and remained with the ram for 48 hours. Pens did not provide visual access to the neighboring pen. Rams were moved to singly housed pens (1.5 x 1.5 m) 24 hours before their test day without visual access to the test pens. Ewes remained in their original group in a separate barn until testing.

11 days prior to ram exposure, estrus was synchronized using an intravaginal controlled internal drug release (CIDR) insert impregnated with 0.3 g of natural progesterone (EAZI-BREED CIDR Sheep Insert, Pfizer Inc.). Each ewe was restrained within a chute while the CIDR was inserted into the vulva with a lubricated applicator (CIDR Insert Applicator, Pfizer Inc.). After 10 days with the CIDR in place, ewes were again restrained in a chute and the CIDR was removed with a gloved hand by pulling the exposed nylon tail of the CIDR. At this time, each ewe's back was sprayed with orange, yellow, green, or purple paint dots to allow identification from video recordings (Figure 3.2). Each color was represented once within each group and balanced across docked and undocked ewes. The following day, the ewes were exposed to the ram in the test pen.

Rams were fitted with marking harnesses (Nylon Breeding Harness, Premier 1 Supplies) and moved to their respective pens between 6:00 and 8:00 am on their assigned test day. Once the rams were moved, each set of 4 ewes was moved as a group within ten minutes through the alleyway (Figure 3.1) to the pen with their assigned ram. During the test, they were fed 1.36-1.81 kg of hay per animal within a feed trough.

### *Video-based observations*

6 cameras (Varifocal Turret Network Camera; 8MP/4K IR 4mm lens; CMIP3382NW-M; LT Security Inc.) were positioned in the barn (2 cameras/pen, Figure 3.1) and mounted at an average height of 2.81 m (range: 2.08-3.51 m) above the ground to maximize the view of the pen and limit blind spots. The cameras had a 2.8 mm focal length lens with 107° horizontal field of view (FOV), 57° vertical FOV, and 128° diagonal FOV and a frame rate of 20 frames per second. A network video recorder (NVR; LTN8932H-P16 32 Channel Enterprise; LT Security Inc.) automatically saved the video recordings at 3840 x 2160 pixels. Videos were analyzed using BORIS (Behavioral Observation Research Interactive Software, Friard and Gamba, 2016). One trained observer scored behaviors (Table 3.1) during the first two hours of ram exposure for all 9 groups. The observer was not blind to the treatments as it was apparent which ewes were docked but she was blind to the predictions. Intra-observer reliability was calculated using one randomly selected 2 hour video (Intraclass correlation coefficient > 0.88 for all behaviors).

### *Real-time location data*

The facility was equipped with a real-time location system (TrackLab, Noldus Information Technology, Netherlands). This system uses ultra-wideband radio signals to triangulate the  $x,y$  location of wearable sensors using anchors fixed around the perimeter of the test area. Six anchors were mounted at an average height of 2.79 m (range: 2.49-2.92 m) around the perimeter of the barn (Figure 3.1). The anchors were connected and powered via ethernet cables that ran to a desktop computer for real-time tracking and download of data. We previously recorded a dynamic error of  $0.53 \pm 0.31$  m (mean  $\pm$  SD) when using this system as currently configured (Woods and Adcock, 2024).

On test days, rams and ewes were fitted with custom collars that held the sensors in a 3D-printed housing at the base of the sheep's neck (Figure 3.2). We used 30 sensors, which measured 5 x 4 x 1.5 cm and weighed 8 g. Sensors were set to sample at a rate of 10 Hz and to “sleep” if no motion was detected after two seconds to conserve battery life. The “sleep” setting resulted in gaps in the data until movement was detected. As a post-processing step, each second where gaps occurred was filled with the last known coordinate until the sensor was active again. To smooth out short-term fluctuations in the coordinates due to noise and rapid movements of the subject, a default smoothing window size of 9 samples was applied to the data per software recommendations. The smoothing filter calculated the position of the sensor at sample time  $t$  as the average of the coordinates of 9 samples centered on the focal sample.

We calculated the Euclidean distance between each ewe and her assigned ram each second over the first 2 hours, beginning when the first ewe from each group entered the pen with her assigned ram. We then averaged each ewe-ram pair's Euclidean distances to determine their average distance to each other over the observation period. Distance traveled by each ewe was calculated using the Euclidean distance between their consecutive locations each second over the first 2 hours. For each ewe, the Euclidean distances were summed to determine their total distance traveled over the 2-hour period:

$$\text{Distance traveled} = \sum \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2}$$

### *Statistical analysis*

Data analyses were performed using the statistical software program R, v4.4.0 (R Core Team, 2024). All data and associated R scripts are available in the Dryad repository: <https://doi.org/10.5061/dryad.qbzkh18t7>. On average, rams were not visible for only 0.22 minutes (SD: 0.27, range: 0.02-1.60 minutes) of the 120-minute video recordings. Therefore, we

analyzed raw counts and durations rather than converting them to frequencies and proportions. When a significant effect was detected, estimated marginal means and standard errors were obtained using the *emmeans* function from the *emmeans* package v.1.10.2 (Lenth, 2024).

To determine whether the rams' counts of headbutting and total mounts (sum of accepted and rejected mounts) toward the ewes were affected by the ewe's tail status, we fitted 2 generalized linear mixed models with negative binomial distributions. The count of each behavior by the ewe (n=36) was the outcome variable and the treatment of the ewe (docked or undocked) was a fixed effect. Twin ID (n=18) nested within ram ID (n=9) was included as a random effect in both models. To determine whether a ewe's treatment influenced whether she accepted or rejected an attempted mount, we fitted a generalized linear mixed model with a binomial distribution. The outcome was a binary variable indicating whether the ewe accepted (1) or rejected (0) the attempted mount and treatment was included as a fixed effect. Individual ewe ID (n=22) was nested within twin ID (n=14), which was nested within ram ID (n=9) as a random effect. The 14 ewes (6 docked, 8 undocked) who were not mounted over the 2-hour observation period were not included in the model. All models were fitted using the *glmmTMB* function in the *glmmTMB* package v1.1.9 (Brooks et al., 2017). None of the models were overdispersed as assessed using the *testDispersion* function from the *DHARMA* package v0.4.6 (Hartig, 2022).

We fitted a linear mixed model (*lmer* function, *lme4* package v1.1-35.3, Bates et al., 2015) to test whether the rams' total duration of investigation with each ewe's perineal region was affected by tail docking status (fixed effect) with twin ID (n=18) nested within ram ID (n=9) as a random effect. The model assumption of normality was assessed with QQ plots and the Shapiro Wilks test (*plot*, *residuals*, and *shapiro.test* functions, base R). The assumption was not

met so we moved to a generalized linear mixed model with a Gamma distribution using the *glmmTMB* function in the *glmmTMB* package v1.1.9 (Brooks et al., 2017). The model was not overdispersed as assessed using the *testDispersion* function from the *DHARMA* package v0.4.6 (Hartig, 2022).

To assess the ram's latency to investigate each ewe's perineal region and to mount, we used two mixed effects cox proportional hazards models (*coxme* function, *coxme* package v2.2-20, Therneau, 2024). The outcome variable for each model was the latency for each ewe with the ewe's treatment as a fixed effect and twin ID (n=18) nested within ram ID (n=9) as a random effect. The model assumption of proportional hazards, which states that the hazard ratios of the covariates are proportional over time, was assessed with the *cox.zph* function from the *coxme* package. The assumption was met for both models.

To test whether the ewe's tail docking status influenced their total distances traveled or average distance to their assigned ram over the first two hours, we fitted two linear mixed models (*lmer* function, *lme4* package v1.1-35.3, Bates et al., 2015). The outcome variables were either total distance traveled or average distance to the ram with docking status as a fixed effect, and twin ID nested within ram ID as a random effect. Due to faulty real-time location sensors, one ewe was removed from the distance traveled analysis and 5 ewes were removed from the distance to ram analysis. The model assumptions of normality and homogeneity of variance were assessed with QQ plots and plots of residuals versus fitted values, respectively (*plot*, *residuals*, and *shapiro.test* functions in base R). Both assumptions were met for each model.

## Results

Whether a ewe was docked or undocked did not affect the latency for a ram to investigate her perineal region after she had entered the pen (mean  $\pm$  SE hazard ratio; docked: 1.00  $\pm$  0.18,

undocked:  $1.00 \pm 0.17$ ;  $X^2 = 0.37$ ,  $P = 0.55$ ; Figure 3.3). However, rams tended ( $X^2 = 3.07$ ,  $P = 0.08$ ; Figure 3.4) to mount undocked ewes sooner (hazard ratio:  $1.57 \pm 0.40$ ) compared to docked ewes (hazard ratio:  $0.69 \pm 0.15$ ). By the end of the 2-hour observation, 12 docked (67%) and 10 undocked (56%) ewes had been mounted (Figure 3.4).

We did not observe a difference in the number of total mounts between docked and undocked ewes ( $X^2 = 0.15$ ,  $p = 0.70$ , Figure 3.5). However, when mounted, undocked ewes tended to be more likely to accept the mount compared to docked ewes (undocked:  $0.12 \pm 0.11$ , docked:  $0.01 \pm 0.01$  probability of accepting a mount when mounted;  $X^2 = 3.08$ ,  $P = 0.08$ ; Figure 3.5). The ewe's tail docking status did not impact the number of headbutts received from the ram ( $X^2 = 0.29$ ,  $P = 0.59$ ; Figure 3.5), the ram's duration of investigation with her perineal region ( $X^2 = 0.07$ ,  $P = 0.80$ ; Figure 3.6), her total distance traveled ( $X^2 = 2.18$ ,  $P = 0.14$ ; Figure 3.7), or her average distance from the ram ( $X^2 = 1.95$ ,  $P = 0.16$ ; Figure 3.7).

### Discussion

We investigated whether tail docking ewe lambs shortly after birth affected ewe and ram mating behavior later in life. Our results suggest that tail docking neonatal ewe lambs reduced their sexual attractiveness and receptivity at 7 to 8 months of age. Rams tended to mount undocked ewes before docked ewes. Further, undocked ewes tended to have a higher probability of accepting a mount compared to docked ewes. However, contrary to our predictions, tail docking did not affect the amount of headbutts and total mounts received from the ram, the ram's duration of investigation of the perineal region, nor the ewe's total distances traveled and distances to the ram.

Rams tended to mount undocked ewes before docked ewes, suggesting they were more sexually attracted to ewes with tails, in agreement with findings by Orihuela et al. (2018). These

authors suggested that rams were more attracted to undocked ewes because the latter could communicate with their tails. Tail wagging is a proceptive behavior that increases attractiveness in female goats (Haulenbeek and Katz, 2011) and is more common in estrous than non-estrous ewes (Fierros-García et al., 2018). It has been speculated that females may wag their tails to disperse olfactory cues about her estrous status as well as to serve as a visual cue for more distant males (Fierros-García et al., 2018; Orihuela et al., 2018; Rekwot et al., 2001). Since we did not assess tail behaviors in the current study, we cannot ascertain that tail wagging explains the ram's tendency to mount the undocked ewes faster than the docked ewes. Future research that focuses on the ewe's proceptive behavior, including her tail movements and efforts to follow the ram, would provide insight into the effect of tail docking on sexual attractiveness and proceptivity.

It is also possible that the rams were attracted to the novelty of the ewe's tail, as they were raised with docked individuals. However, Orihuela et al. (2018) observed a preference for undocked ewes in virgin rams raised with undocked sheep. Additionally, a study that cross-fostered the offspring of sheep and goats found that rams raised by goats preferred mating with female goats rather than sheep (Kendrick et al., 1998). This result suggests that the ram's attraction towards undocked ewes occurred despite, rather than because of, the novelty of the tail. Future research should investigate how early life experiences and subsequent sexual experiences with docked and undocked sheep may influence mating preferences.

We did not observe differences between undocked and docked ewes in the latency or duration of perineal investigation by the ram. Past research has demonstrated that rams will nose and sniff the anogenital region of ewes during the precopulatory phase to determine their estrous status and evaluate sexual attractiveness (Bernon and Shrestha, 1984; Stevens et al., 1982; Ungerfeld and Alexander, 2024). In our study, we recorded perineal investigation as a proxy for

sniffing the anogenital region. The lack of difference we observed suggests that the rams did not discriminate by tail status when deciding whether to investigate a ewe, perhaps because all four ewes were relatively close to the ram due to the pen size. It would be interesting to mimic a more intensive breeding scenario with more ewes to a single ram or a more extensive setting, which would require rams to be more selective in their choices.

Headbutting did not differ based on the ewe's tail status but was highly variable between rams, with only 5 of the 9 rams displaying the behavior. Two of these rams headbutted ewes at high frequencies (50 and 158 counts); 1 headbutted all 4 ewes in his group, while the other focused on a single undocked ewe. Rams usually began headbutting ewes after 20 minutes of exposure and after attempting to mount one or more of the ewes within the group, but whether it occurred after an accepted or rejected mount varied. Very little is reported on headbutting by domestic ungulates in a breeding context, but it has been suggested that it indicates sexual interest and may be coupled with kicking and vocalizations in sheep (Ungerfeld and Alexander, 2024). Headbutting and other agonistic acts toward ewes may also be related to the ram's age. One study of wild mouflon sheep suggested that behaviors such as headbutts, kicks, and blocking directed toward ewes could be a form of mate guarding by subordinate males, as these behaviors only occurred in males under 7 years of age (Bon et al., 1995). Future studies may elucidate whether headbutting indicates sexual attractivity or is more reflective of other components of sexual behavior (e.g., mate guarding).

When mounted, docked ewes tended to be less likely to accept the mount compared to undocked ewes, indicating reduced sexual receptivity (Banks, 1964). We speculate that lower sexual receptivity may reflect chronic pain experienced at the tail docking site. Docked sheep experienced increased sensitivity to mechanical stimulation of the tail stump for at least 90 days

after the procedure (Larrondo et al., 2019) and more painful parturitions at 2 years of age (Clark et al., 2014). Additionally, traumatic neuromas can occur at an amputation site in the form of misfiring nerve stumps (Larrondo et al., 2019; Sehirlioglu et al., 2009) and can cause chronic pain. These neuromas have been found in the tail stumps of docked sheep up to at least one year of age (Fisher and Gregory, 2007; Larrondo et al., 2019), as well as in pigs (Herskin et al., 2015; Sandercock et al., 2016), dairy cattle (Eicher et al., 2006), and dogs (Gross and Carr, 1990). Thus, if the ewe's tail stump is painful, the weight of a ram on her back and anogenital region may agitate the site and cause her to move away. Reduced sexual receptivity may also result from inflammation-induced alterations in neurobiological pathways in early development. In male and female rats, for example, neonatal inflammation can lower reproductive capacity and suppress sexual behavior due to altered metabolic and neuroendocrine functions that regulate sexual maturation (Fuller et al., 2017; Mayila et al., 2020, 2018; Sominsky et al., 2012; Walker et al., 2011; Wu et al., 2011).

Reductions in sexual attractivity and receptivity could translate into fewer successful breeding events, thereby lowering the productivity of flocks that are naturally serviced. We did not evaluate the effect of tail docking on reproductive performance, but this would be interesting to evaluate in the future. In fat-tailed sheep, docked ewes had higher lambing rates and litter sizes compared to undocked ewes (Shelton, 1990). Movement of the tail may be limited in fat-tailed breeds, impeding vaginal intromission (Kridli et al., 2008, 2007) for undocked ewes, and findings may differ in non-fat-tailed breeds with lighter tails. Fierros-García et al. (2018) found that, in a hair breed with a non-fat tail, more rams had anal intromissions when mounting docked ewes compared to undocked ewes, suggesting that the tail may protect against non-reproductive intromissions. In the current study, we were unable to assess the proportion of accepted mounts

that led to successful intromission and ejaculation. In addition, we used only docked rams, and it would be worth exploring the effects of tail docking ram lambs on their mating behavior and performance in future studies.

We predicted that docked ewes, who may be experiencing chronic pain, would travel more and have greater distances to the ram than undocked ewes to avoid mounting attempts, which could agitate their sensitive tail stump. However, we found no differences in either metric between docked or undocked ewes. Each group contained 2 twin pairs, and previous research has found that twins maintain close association patterns within a flock (Ozella et al., 2022). Thus, each ewe's movements may have been more influenced by her twin, who received the opposite treatment, than the ram. Positional data may be a more sensitive indicator of changes in mating behavior following tail docking when used in a different experimental design than ours.

### **Conclusions**

We found some evidence to suggest that tail docking decreases ewe sexual attractivity and receptivity. Three potentially overlapping mechanisms may explain the results: 1) undocked ewes can use their tails to visually and olfactorily stimulate rams during mating, 2) docked ewes are unable to withstand extended pressure on the anogenital region because of chronic pain at the docking site while undocked ewes had an absence of pain, or 3) early life stress experienced by tail docking disrupted docked ewe's behavioral development resulting in reduced sexual behavior. The results of this research suggest that tail docking ewes early in life may have lasting impacts on their mating behavior, with potential repercussions for flock welfare and performance. Given the existing welfare concerns with tail docking, the benefits and drawbacks of this practice warrant further evaluation.

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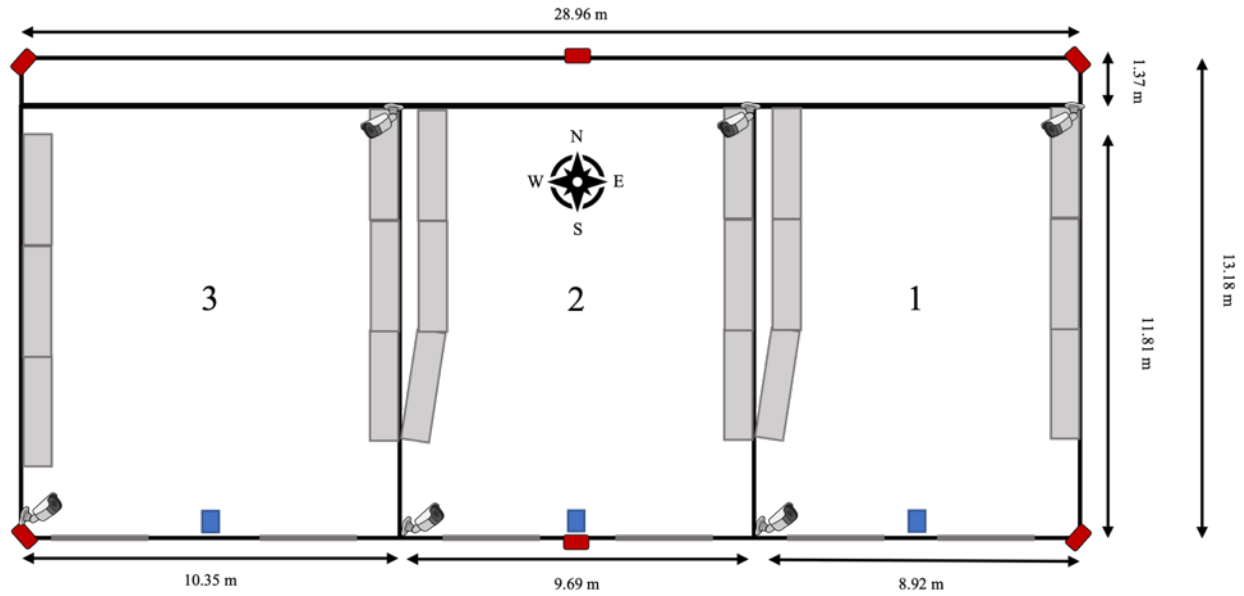
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### Tables and figures

**Table 3.1** Definitions used to score mating behaviors between rams and ewes with docked or undocked tails (n=4 ewes/ram). Behavioral observations began as soon as the ewes were moved into the pen with their assigned ram and lasted 2 hours.

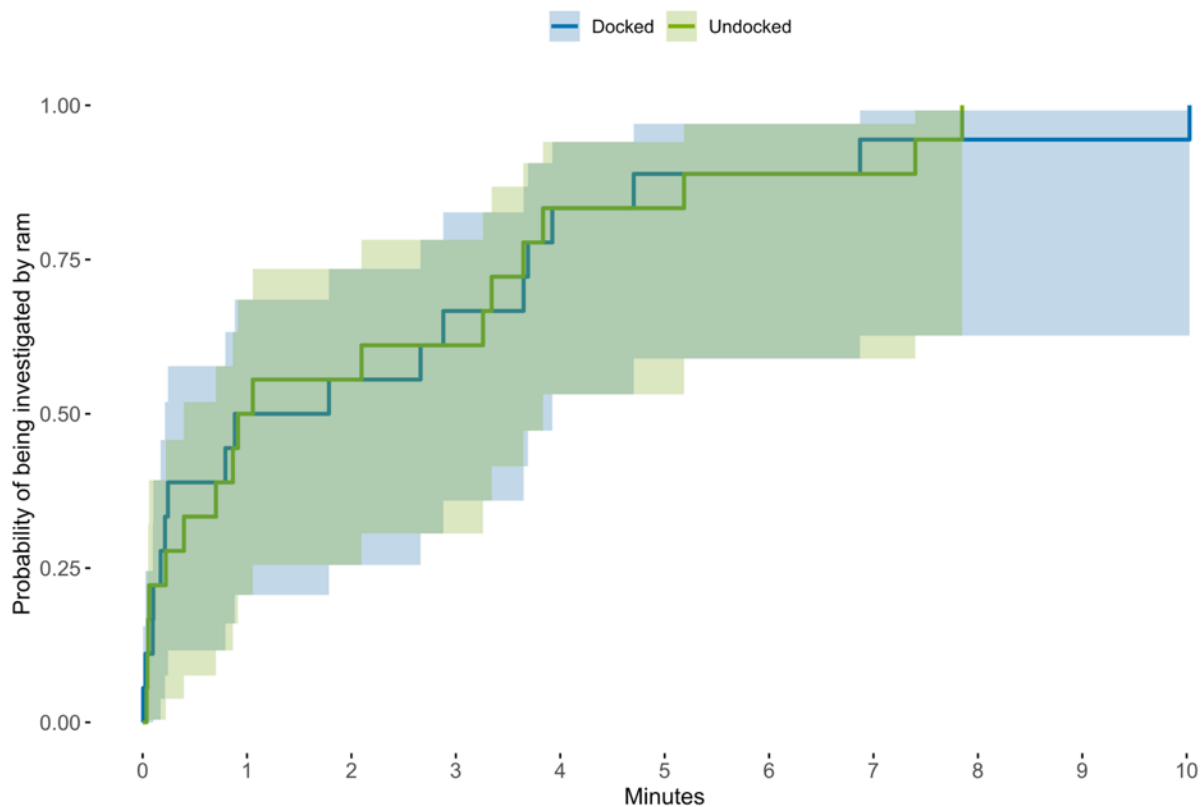
<b>Behavior</b>	<b>Type</b>	<b>Definitions</b>
Rejected mount	Count	Ram rears both front legs while standing behind the ewe and places one or both front legs onto her back. The ewe moves away from him causing his legs to return to the ground.
Accepted mount	Count	Ram climbs onto the ewe's back end and the ewe remains in place until the ram dismounts.
Headbutt	Count	Ram forcefully pushes the ewe with his head.
Perineal investigation	Duration	Ram's snout is in direct contact or within a head's length of the ewe's upper thigh to her genital region.
Not visible	Duration	The ram's head or rump is out of view.



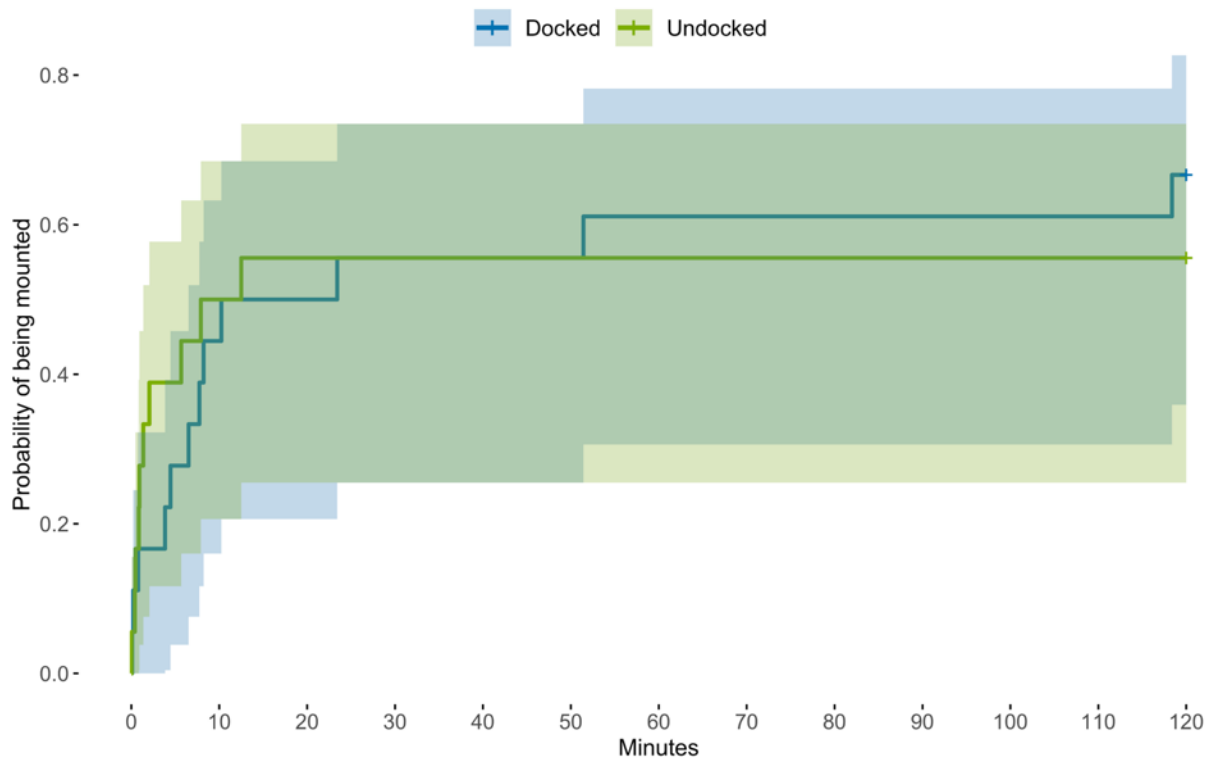
**Figure 3.1** Aerial map of the barn where the behavioral tests occurred. The barn was divided into 3 pens with an alleyway along the north wall. Behavior was recorded via video cameras and real-time location sensors. The 6 anchors, 6 video cameras, 3 water troughs, and 18 feed troughs are indicated by the small red rectangles, camera icons, blue squares, and long gray rectangles, respectively.



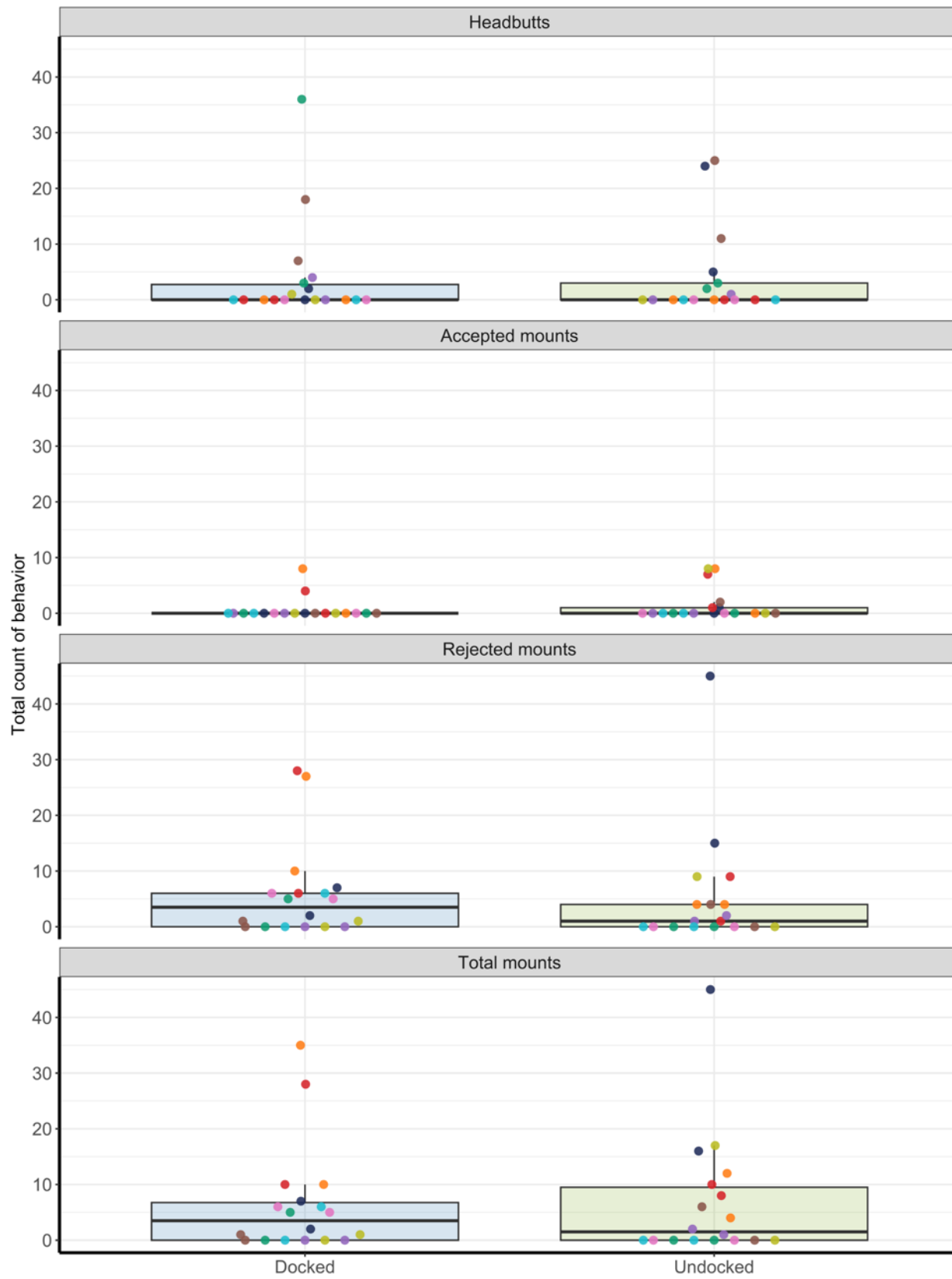
**Figure 3.2** Still frame from video recording showing 2 ewes and 1 ram wearing custom collars affixed with the real-time location sensors in 3D-printed housings. The ram wore a black marking harness, and each ewe's back was painted with dots to allow identification from the video.



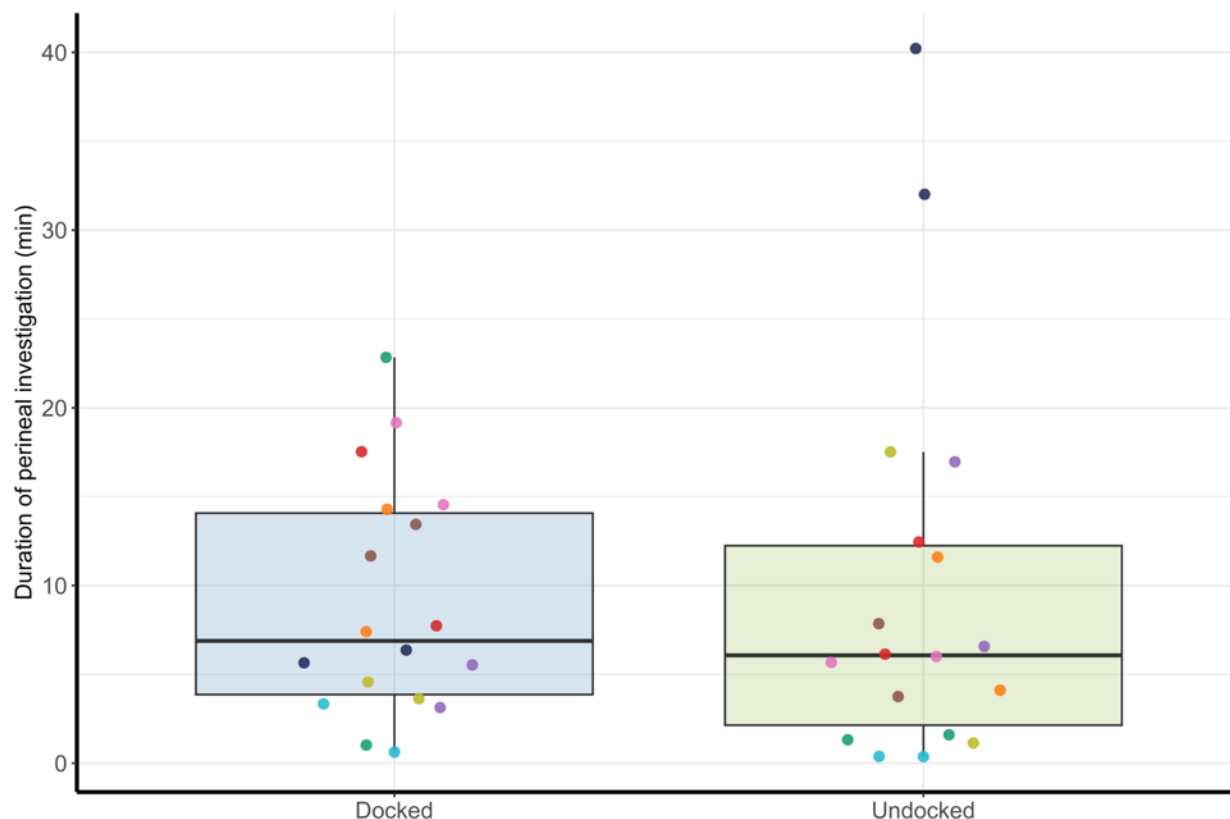
**Figure 3.3** The probability of a docked (blue, n=18) or undocked (green, n=18) ewe receiving perineal investigation from the ram relative to the minutes since entering the pen. Shaded areas indicate the 95% confidence intervals. All ewes were investigated within 10 minutes after entering the pen.



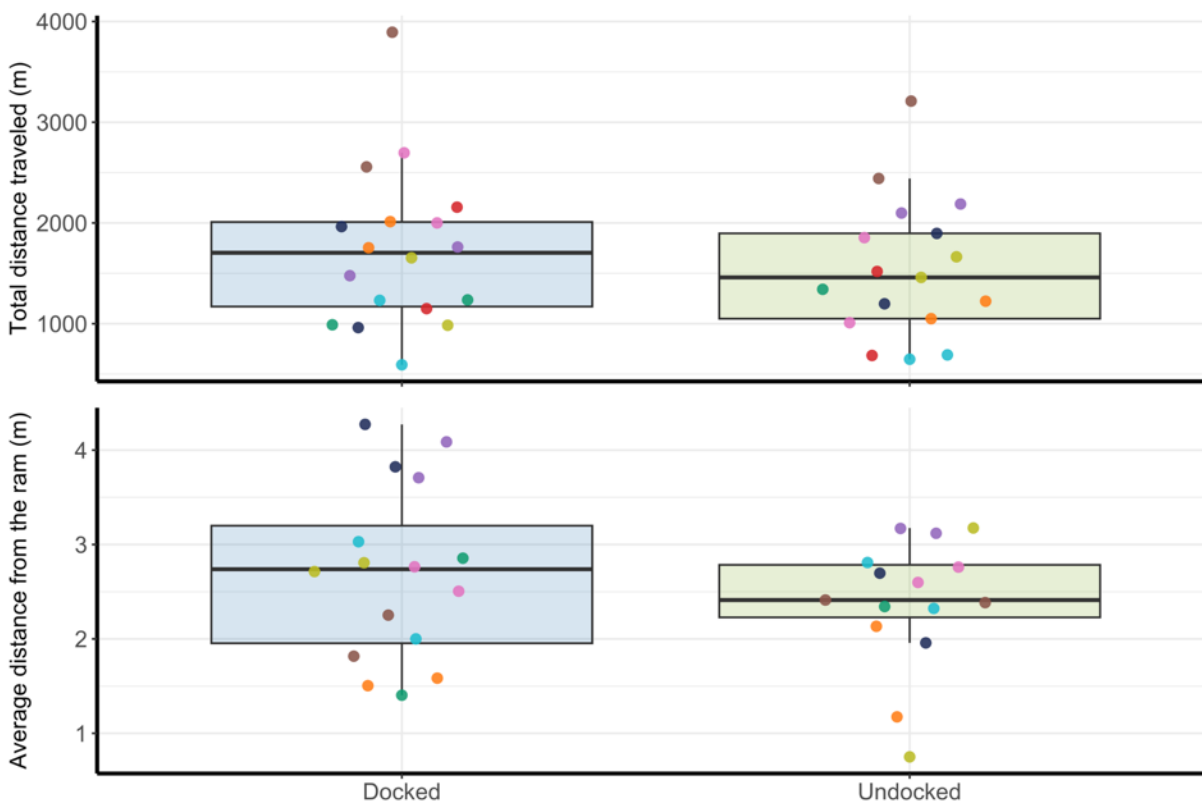
**Figure 3.4** The probability of a docked (blue, n=18) or undocked (green, n=18) ewe being mounted by the ram relative to the minutes since entering the pen. Shaded areas indicate the 95% confidence intervals.



**Figure 3.5** Boxplots showing the total number of times that docked (n=18) and undocked (n=18) ewes were head butted and mounted by the ram over the first 2 hours of interaction. Mounts are separated into the number of mounts that were accepted and rejected by the ewe. The median, 75th quartile, and 25th quartile are indicated by the midline and top and bottom edges of the boxplots, respectively. The boxplot whiskers indicate the lower and upper extremes calculated as  $Q1 - 1.5(Q3 - Q1)$  and  $Q3 + 1.5(Q3 - Q1)$ , respectively. Each dot represents an individual ewe (n=36). The color of the dot represents her assigned ram (n=9). Each ram was assigned to 4 ewes (2 docked, 2 undocked). One undocked ewe received 158 headbutts, which was excluded from this plot for visualization purposes.



**Figure 3.6** Boxplots showing the total duration that docked (n=18) and undocked (n=18) ewes received perineal investigation from the ram over the first 2 hours of interaction. The median, 75th quartile, and 25th quartile are indicated by the midline and top and bottom edges of the boxplots, respectively. The boxplot whiskers indicate the lower and upper extremes calculated as  $Q1 - 1.5(Q3 - Q1)$  and  $Q3 + 1.5(Q3 - Q1)$ , respectively. Each dot represents an individual ewe (n=36). The color of the dot represents her assigned ram (n=9). Each ram was assigned to 4 ewes (2 docked, 2 undocked).



**Figure 3.7** Boxplots displaying the total distance traveled (top) and the average distance from the ram (bottom) over the first 2 hours of exposure by tail docking treatment (top: 18 docked, 17 undocked; bottom: 16 docked, 15 undocked). The median, 75th quartile, and 25th quartile are indicated by the midline and top and bottom edges of the boxplots, respectively. The boxplot whiskers indicate the lower and upper extremes calculated as  $Q1 - 1.5(Q3 - Q1)$  and  $Q3 + 1.5(Q3 - Q1)$ , respectively. Each dot represents an individual ewe (top:  $n=35$ ; bottom:  $n=31$ ). The color of the dot represents her assigned ram (top:  $n=9$ ; bottom:  $n=8$ ). Each ram was assigned to 4 ewes (2 docked, 2 undocked).

CHAPTER 4: The maternal-offspring relationship in tailed and docked ewes and their lambs

**Preface**

This chapter is currently under review in *Applied Animal Behavior Science*

### Abstract

The hours after lambing are crucial for establishing the lamb-dam bond, which plays a key role in lamb development and survival. A dam's maternal behavior may be influenced by her early life experiences, which include routine painful procedures such as tail docking, potentially impairing bonding. We tested whether neonatal tail docking in ewe lambs altered (1) their maternal behavior and their offspring's behavior after lambing and (2) behavioral and physiological stress responses to separation. Polypay ewe lambs were tail docked using the rubber ring method between 24 to 36 hours of age ( $n=11$ ) or left undocked ( $n=10$ ). At approximately 13 months of age, they gave birth to singleton ( $n=6$ ) or twin ( $n=15$ ) litters. Between 30-60 minutes after the last lamb's birth, the ewe and her lamb(s) were moved to an individual maternity pen, where behaviors were video recorded for 2 hours. Between 6 to 12 hours after lambing, the lambs were separated from their dam for 15 minutes, with video recordings taken from 15 minutes before separation until 15 minutes after reunion. Ewe and lamb eye temperatures were assessed using infrared thermography immediately before and after separation. For all video recordings, we analyzed the duration the ewe spent eating, pacing, lying, and grooming her lamb(s), as well as the duration the lambs spent wagging their tail, nursing, and lying. Transitions from lying to standing were also recorded for ewes and lambs in the 2-hour bonding period. All models included the dam's tail docking status as a fixed effect with litter size as a covariate; separation models also included observation period (before, during, and after separation). We found no significant effect of the dam's tail docking status on maternal and offspring behavior following lambing or on their responses to separation. However, lambs of undocked ewes tended to spend more time lying in the bonding period compared to lambs of docked ewes. Regardless of docking status, ewes ate less while separated from their lambs and

spent 26% of this time pacing, a behavior not observed before or after separation. After separation, ewe-lamb interactions increased relative to baseline, with more grooming, nursing, and tail wagging, and less lying. No differences were observed in eye temperatures before and after separation. We did not find evidence to suggest that neonatal tail docking impairs the ewe's ability to bond with her lambs later in life.

## Introduction

A ewe's maternal responsiveness and ability to bond with her offspring are critical determinants of lamb health and survival (Dwyer, 2017; Nowak and Poindron, 2006). A weak or disrupted lamb-dam bond is a leading cause of lamb mortality, in some cases accounting for 25% of deaths – surpassing losses from stillbirths, birth injuries, and dystocia (Dwyer, 2017; Refshauge et al., 2016). Typically, in the hours following parturition, ewes show strong maternal responsiveness through grooming, nursing, and maintaining close proximity with their offspring, while also rejecting unrelated lambs as selective bonds form (Nowak et al., 1997). However, mismothering can also occur, with ewes ignoring, avoiding, or aggressing toward their offspring (Nowak and Poindron, 2006). Disturbances in ewe maternal behavior have been linked to multiple factors, including breed (Dwyer, 2014), parity (Dwyer and Lawrence, 2000), ewe and lamb health (Dutra and Banchemo, 2011; Refshauge et al., 2016), and litter size (Refshauge et al., 2016). Identifying factors that disrupt bonding is essential for flock productivity and ewe and lamb welfare.

A ewe's early life experience may shape her maternal behavior in adulthood. In rodent models, neonatal exposure to immune challenges, such as lipopolysaccharide-induced (LPS) inflammation, has been shown to reduce maternal care and increase offspring mortality in adulthood (Sominsky et al., 2012; Walker et al., 2012). This suggests that early-life stressors in lambs could have similar long-term consequences on maternal behavior. Tail docking, a common early-life procedure in sheep, is typically performed within the first two months of life and causes acute (Ferrer et al., 2020) and chronic pain (Larrondo et al., 2019), with wounds that take several weeks to heal (Woods and Adcock, 2025). Notably, ewes that were docked as neonates have been found to experience heightened pain during parturition, exhibiting more contractions

and posture changes compared to undocked ewes (Clark et al., 2014). Given that early-life inflammatory events can alter behavioral development in other species and may increase long-term pain sensitivity, it is possible that neonatal tail docking may affect maternal care in adulthood, though this has not yet been investigated in sheep.

We investigated whether dams who were tail docked shortly after birth displayed altered maternal behavior compared to undocked dams following parturition. Additionally, we examined docked and undocked dams' behavioral and physiological responses to temporary separation from their lambs using video observations and infrared thermography, respectively. We predicted that docked ewes would show reduced maternal care, evidenced by less grooming and nursing and more eating, lying, and lying-standing transitions due to pain than undocked ewes. Additionally, we predicted that the lambs of docked ewes would have more lying-standing transitions and display less lying and tail wagging, reflecting increased effort to nurse from a less cooperative dam. During lamb separation, we predicted that undocked ewes would have a stronger maternal response than docked ewes, evidenced by more pacing, escape behavior, and higher maximum eye temperatures indicative of a heightened stress response (Arfuso et al., 2022; Sutherland et al., 2020).

### **Materials and methods**

This study was conducted at the University of Wisconsin-Madison's Arlington Sheep Unit from February 2022 to March 2023. The University of Wisconsin-Madison's Institutional Animal Care and Use Committee approved all procedures (Protocol #A006423).

#### *Animals and housing*

We enrolled 36 Polypay twin ewes born between February and March 2022. The sample size was based on flock availability, and all animals free of clinical signs of disease were eligible

for enrollment. One ewe within each twin pair was randomly assigned to have her tail docked while her sister remained undocked. Treatment assignments were adjusted as needed to balance birth weights (docked:  $5.55 \pm 1.32$  kg, undocked:  $5.44 \pm 0.53$  kg). Tail docking was performed 24 to 36 hours after birth by author JMW using the constrictive rubber ring method. A rubber ring, disinfected with 2% chlorohexidine, was expanded with an elastrator and placed on the tail towards the distal end of the caudal folds. Analgesics were not administered, in accordance with farm protocol and standard industry practice.

Between 2 to 4 days of age, the twin ewe lambs and their dams were moved to a communal straw-bedded pen with outdoor access, weather permitting. Within the group pens, lambs had *ad libitum* access to 19% crude protein pelleted feed medicated with decoquinatate (Deccox, Premier 1 Supplies) within a creep pen, which restricts ewe access to the feed. Ewe lambs were weaned from their dam at  $63 \pm 5$  (range: 57-76) days of age. For approximately 30 days after weaning or until lambs weighed at least 45 kg, lambs were fed a 16% crude protein pelleted feed medicated with decoquinatate *ad libitum*. They were then switched to a 13% crude protein pelleted feed medicated with decoquinatate and free-choice alfalfa bialage until ram exposure. Water was available *ad libitum*.

Ewe lambs were exposed to a Polypay ram (n=9) at  $7.4 \pm 0.3$  months of age. Estrus was synchronized 11 days before ram exposure using an intravaginal controlled internal drug release (CIDR) containing 0.3 g of natural progesterone (EAZI-BREED CIDR Sheep Insert, Pfizer Inc.). The CIDR was removed the day before ram exposure (see Woods & Adcock in prep). Each ewe was exposed to a single ram for 48 hours and each twin pair was exposed to the same ram. After breeding, the ewes were moved into a communal straw-bedded pen and fed 0.23 kg whole-shell corn with free-choice alfalfa baleage until the study began. Ewes had outdoor access when

weather permitted. Shortly after breeding, one docked ewe died. The remaining 35 ewes were checked for pregnancy using ultrasounds 40-44 days after ram exposure. 11 docked and 15 undocked were confirmed pregnant (6 docked and 3 undocked were not pregnant). Non-pregnant ewes were not rebred.

On March 9, 2023, approximately two weeks before the first expected due date, ewes were assigned to one of two adjacent pens. Ewes were housed with their twin pair, except for the one undocked ewe whose docked twin died shortly after breeding. One pen (11.8 x 8.9 m) housed 16 ewes (8 docked, 8 undocked), while the other (11.8 x 9.7 m) housed 19 ewes (9 docked, 10 undocked). 26 ewes lambbed from March 19 to March 29, 2023. Only ewes with singleton or twin litters were included in this study. As such, three undocked ewes with triplets were excluded, as well as two undocked ewes due to severe dystocia and a lamb born with a birth defect which resulted in euthanasia, respectively. Additionally, the 9 ewes who did not lamb were excluded. Our final sample consisted of 21 ewes (11 docked and 10 undocked; age at lambing:  $13 \pm 0.2$  months, range: 12.6-13.3) and their 36 lambs. Docked ewes had 3 single lambs (2 male, 1 female) and 8 sets of twins (10 male, 6 female). Undocked ewes had 3 single lambs (1 male, 2 female) and 7 sets of twins (11 male, 3 female).

### *Experimental design*

Ewes were moved to individual straw-bedded maternity pens (1.5 x 1.5 m) in an adjacent room of the barn 30-60 minutes after their last lamb was born. Water was available *ad libitum*. Upon entering the maternity pen, each lamb received 2 mL of an oral vitamin supplement (Baby Lamb Strength, Premier 1 Supplies) and 2 mL of an oral *E. coli* prevention solution (SpectoGard Scour-Chek, Bimeda). Each lamb's umbilical cord was cut, and 7% iodine was applied to the stump per standard farm practice.

Between 6 to 12 hours after lambing, the separation trial was conducted, with one ewe-lamb family tested at a time. Upon entering the maternity pen, author JMW restrained the ewe by backing her into a corner and holding her head steady. A thermal imaging camera (FLIR E8-XT; Teledyne FLIR LLC) was positioned 30 cm away from the ewe's left eye, and JMW took a series of at least 2 thermal photos with the pupil centered in the frame. This procedure was repeated for the right eye. Next, the lamb(s)' thermal eye images were taken. Each lamb was picked up, and their head was held in place while images were taken following the same procedure as the ewe. Once all thermal images were captured, the lamb(s) were placed in a 56-liter rubber tub bedded with approximately 5-10 cm of straw in an adjacent room. This location prevented visual contact between the ewe and her lamb(s) but did not fully eliminate auditory cues. After 15 minutes of separation, the thermal imaging procedure was repeated for both the ewe and her lamb(s). Following image collection, the lamb(s) were reunited with their dam in the maternity pen.

#### Video-based observations

Seven cameras (Amcrest UltraHD Outdoor PoE Camera; 5MP; IP5M-B1186EW-28MM; Amcrest Inc.) were mounted approximately 3 m above each maternity pen (1 camera/pen) to maximize visibility and minimize blind spots. The cameras had a 2.8 mm focal length lens with 103° viewing angle and had a frame rate of 20 frames per second. The cameras continuously recorded and automatically saved videos at 2592 x 1994 pixels to an external hard drive.

Videos were analyzed using BORIS (Behavioral Observation Research Interactive Software, Friard and Gamba, 2016). Three trained observers scored ewe and lamb behaviors (Table 4.1) continuously for two hours immediately after the lambs were processed (oral administrations and umbilical cord cutting) in the maternity pen. Additionally, four trained

observers scored ewe and lamb behavior continuously for 15 minutes before and after the separation, as well as ewe behavior during separation. The observers were not blind to treatment, as the dam's tail docking status was visually apparent, but were blind to study predictions. Inter-observer reliability was calculated using videos from a randomly selected ewe-lamb family across all observation periods (Table 4.1).

#### Thermal image analysis

A total of 664 thermal images (245 ewe, 419 lamb) were taken before and after separation. After excluding photos where the animal was moving or the eye was not visible, 487 thermal images remained (188 ewe, 299 lamb). Each focal animal had at least 4 usable images: pre-separation (left and right eye) and post-separation (left and right eye), besides one lamb who was missing a photo of her left eye pre-separation. Most animals (88%) had multiple images of each eye during either pre-separation (left:  $2 \pm 0.7$  images, range: 1-4; right:  $2.1 \pm 0.7$  images, range: 1-4) or post-separation (left:  $2.3 \pm 0.6$  images, range: 1-4; right:  $2.1 \pm 0.6$  images, range: 1-4).

Using FLIR Thermal Studio software v2.0.2, a single observer manually outlined the lacrimal caruncle of the eye in each image using the polygon tool. The lacrimal caruncle was selected because it has been used in other studies with high reliability (Bakker et al., 2024; Comin et al., 2023). The maximum surface temperature of the lacrimal caruncle was then extracted and saved to a CSV file. The observer was blind to the treatments and predictions. Inter-observer reliability for maximum eye temperature was calculated against JMW as the gold standard using 35 randomly selected images (13 ewe, 22 lamb) (Intraclass correlation coefficient = 0.95). Visual inspection revealed no clear differences in the maximum surface temperature

between the left and right lacrimal caruncle in ewes or lambs. Therefore, maximum temperatures from both eyes were averaged for subsequent analysis.

### Statistical analysis

Data analyses were performed in R v4.4.2 (R Core Team, 2024). All data and associated R scripts are available in the Dryad repository: <https://doi.org/10.5061/dryad.m905qfvcc>.

Behaviors that were too rare for statistical analysis (self-grooming, spy hopping, and receiving headbutt) are presented descriptively. For all other behaviors, a two-column response variable was used: (1) The total number of minutes the behavior was performed; and (2) The number of minutes it was not performed, calculated as total visible time (observation length minus time not visible and human in pen) minus behavior duration. In the separation trial, individual lamb identities were not tracked across observation periods. Therefore, for twin litters we averaged the proportion of visible time each lamb spent performing the behavior (lying, nursing, tail wagging) to obtain litter-level proportions in each observation period. To retain the binomial structure of the response variable, we converted this proportion back into count data by multiplying it by the average visible time of both lambs in the litter.

When a significant effect was detected, estimated marginal means and standard errors were obtained using the *emmeans* function from the *emmeans* package v.1.10.2 (Lenth, 2024).

### Bonding period

For ewe behaviors (the number of minutes spent eating, grooming lamb, or lying), we fitted generalized linear models with a binomial distribution (*glm*, base R) to test the effect of tail docking status (fixed effect) with litter size (single or twins) as a covariate. All three models were overdispersed (*testDispersion*, DHARMA package v0.4.6; Hartig, 2022). Therefore, we moved to generalized linear models with a beta-binomial distribution (*vglm*, VGAM package

v1.1-13; Yee and Ma, 2024). The model assumption of homogeneity of variance was assessed with plots of residuals versus fitted values (*plot* and *residuals*, base R). The assumption was met.

For lamb behaviors (the number of minutes spent tail wagging, nursing, or lying) we fitted generalized linear mixed models with a binomial distribution including her dam's tail docking status (fixed effect), litter size (covariate), and ewe ID (random effect; n=21). The assumption of no overdispersion was met (*testDispersion*). One twin lamb of an undocked ewe was removed from the behavioral analysis in the bonding period because they were visible for less than 22% of the observation period. On average, the remaining lambs were visible for  $92\% \pm 7\%$  of the observation (range: 69-100%).

Lying-standing transitions were also analyzed for ewes and lambs in the bonding period. We used generalized linear models with a Poisson distribution (*glm* function) to test whether the total number of lying-standing transitions over the 2-h observation period was affected by tail docking status (fixed effect), with litter size as a covariate. For the lamb model, we included ewe ID as a random effect (n=21). The ewe model was overdispersed, so we moved to a generalized linear model with a negative binomial distribution.

#### Separation trial

We used generalized linear mixed models with binomial distributions (*glmmTMB* function; Brooks et al., 2017) to test whether the proportion of time ewes engaged in each behavior (eating, grooming lamb, pacing, and lying) before, during, and after separation was affected by her tail docking status, observation period (pre, during, or post), and their interaction. Litter size was included as a covariate and ewe ID (n=21) as a random effect. Overdispersion was evaluated using the *testDispersion* function. The assumptions were met for eating and grooming lamb. Due to model convergence issues for pacing and lying, we removed observation

periods where the behavior was never observed (pacing was only observed during separation, and ewe lying and grooming lamb were only observed pre-and post-separation). To test the effect of the observation period on the proportion of time spent pacing and lying (total duration divided by time visible), we performed Cochran's Q tests using the *CochransQTest* function from the *DescTools* package v.0.99.59 (Signorell, 2025).

For lamb behaviors (nursing, lying, and tail wagging), which were only recorded before and after separation, we fitted generalized linear mixed models with binomial distributions, including the ewe's tail docking status, observation period (pre-and post-separation), and their interaction, with litter size as a covariate and ewe ID (n=21) as a random effect. The assumption of no overdispersion was met for nursing and tail wagging but not lamb lying. Therefore, we moved to a generalized linear mixed model with a beta-binomial distribution for lamb lying (*glmmTMB*). The model assumption of homogeneity of variance was confirmed by visually inspecting the residuals versus fitted values plot.

Linear mixed models were used to assess whether the average maximum eye temperature of the ewes and lambs was influenced by the ewe's tail docking status, observation period (pre-or post-separation), and their interaction, with litter size as a covariate and ewe ID (n=21) as a random effect (*lmer* function, *lme4* package v.1.1-35.3). The model assumptions of normality and homogeneity of variance were assessed with QQ plots and plots of residuals versus fitted values, respectively. Both assumptions were met for the lamb model but not the ewe model. Therefore, we moved to a generalized linear mixed model with a Gamma distribution (*glmmTMB*) and confirmed the assumption of homogeneity of variance was met using a residuals versus fitted values plot.

## Results

There were no significant effects of the dam's tail docking status on her behavior or that of her lamb(s) during the 2-hour observation period after lambing (Table 4.2; Figures 4.1 and 4.2). However, lambs of undocked ewes tended to spend a greater proportion of time lying (estimated marginal mean  $\pm$  SE:  $0.51 \pm 0.09$ ) compared to lambs of docked ewes ( $0.32 \pm 0.07$ ). There was also a tendency for twins to have more lying-standing transitions ( $7.92 \pm 0.78$ ) than singletons ( $5.33 \pm 1.11$ ), but the number of transitions was not affected by their dam's tail docking status (docked:  $7.06 \pm 1.02$ , undocked:  $5.98 \pm 0.89$ ). The proportion of time lambs spent nursing was significantly affected by litter size, with singleton lambs nursing more ( $0.24 \pm 0.05$ ) than twin lambs ( $0.13 \pm 0.02$ ). The number of lying-standing transitions by the ewe was not affected by her tail docking status (docked:  $1.09 \pm 0.60$ , undocked:  $0.34 \pm 0.25$ ; Table 4.2) nor her litter size (single:  $0.48 \pm 0.40$ , twin:  $0.77 \pm 0.37$ ; Table 4.2). On average, docked and undocked ewes self-groomed 0.07% (SD, range: 0.15%, 0-0.52%) and 0.01% (0.44%, 0-0.14%) of the observable time, respectively. No lambs were headbutted by their dam during the bonding period.

There was no effect of the dam's tail docking status on her behavior or that of her lamb(s) during the separation trial (Table 4.3). However, many behaviors were affected by the observation period. Ewes spent a smaller proportion of time eating during separation ( $0.008 \pm 0.004$ ) than before ( $0.10 \pm 0.03$ ) or after ( $0.09 \pm 0.03$ ) separation (Figure 4.3). Ewes also spent a smaller proportion of time lying after ( $0.002 \pm 0.002$ ) compared to before ( $0.06 \pm 0.06$ ) separation and did not spend any time lying during the separation ( $Q = 12.6$ ,  $P = 0.002$ ; Figure 4.3). Ewes groomed their lambs more after ( $0.28 \pm 0.03$ ) compared to before ( $0.09 \pm 0.02$ ; Figure 4.4) separation. Ewes spent 26% of the time they were separated from their lambs pacing, a

behavior they did not display before or after separation ( $Q = 40.0, P < 0.001$ ; Figure 4.3). Spy hopping was only observed during separation with docked ewes spy hopping on average 2.4% of the time (SD, range: 4.5%, 0-11.9%) and undocked ewes spy hopping 3.2% of the time (3.6%, 0-9.6%; Figure 4.3). Self-grooming was rare, occurring only 0.05% of the observable time in docked ewes (0.15%, 0-0.56%) and 0.11% in undocked ewes (0.48%, 0-2.54%), respectively. Eating was affected by litter size, with ewes eating more when they had twins ( $0.08 \pm 0.02$ ) compared to single lambs ( $0.02 \pm 0.01$ ).

Lambs spent a greater proportion of time nursing ( $0.23 \pm 0.03$ ) and tail wagging ( $0.23 \pm 0.04$ ) after separation than before separation (nursing:  $0.04 \pm 0.01$ ; tail wagging:  $0.06 \pm 0.02$ ) (Figure 4.4). Further, lambs spent less time lying after ( $0.17 \pm 0.05$ ) than before separation ( $0.61 \pm 0.06$ ; Figure 4.4). Lamb lying was also influenced by litter size, with singletons spending a greater proportion of time lying ( $0.51 \pm 0.09$ ) compared to twins ( $0.23 \pm 0.05$ ). One lamb received a single headbutt from their undocked dam post-separation.

The average maximum surface temperature of the ewe's lacrimal caruncle was not affected by her docking status ( $X^2 = 0.25, P = 0.62$ ), the observation period ( $X^2 = 1.08, P = 0.30$ ), or their interaction ( $X^2 = 0.06, P = 0.81$ ; Figure 4.5). Similarly, the average maximum surface temperature of the lamb's lacrimal caruncle was not affected by her dam's docking status ( $X^2 = 0.003, P = 0.96$ ), the observation period ( $X^2 = 2.61, P = 0.11$ ), or their interaction ( $X^2 = 0.08, P = 0.78$ ; Figure 4.5). Litter size also had no effect on eye surface temperature of either ewes ( $X^2 = 0.01, P = 0.92$ ) or lambs ( $X^2 = 1.36, P = 0.24$ ).

## Discussion

This is the first study to investigate the effect of a dam's tail docking status on maternal and offspring behavior during the early postnatal bonding period and in response to dam-lamb

separation. We did not find evidence to suggest that tail docking 24-36 hours after birth using a rubber ring significantly affects these outcomes. Regardless of the dam's docking status, ewes showed behavioral signs of distress during separation, with ewe-lamb interactions increasing from baseline following separation.

We hypothesized that early life stress from tail docking might disrupt physiological and behavioral development as seen in rodent models (Sominsky et al., 2012; Walker et al., 2012), impairing their maternal behavior and perhaps the behavior of the next generation (Crews et al., 2012). Indeed, docked ewes have previously been shown to experience heightened pain during parturition compared to undocked ewes (Clark et al., 2014), which may subsequently interfere with their ability to provide maternal care (Dwyer et al., 2001). However, contrary to our predictions, tail docking did not influence maternal or offspring behavior in our study. Many factors may have mitigated potential long-term effects of tail docking, including the maternal care that all the docked ewes received as lambs. Research in mice has found that pups who received more maternal care after experiencing inflammation were less likely to have social impairments as adults compared to foster-reared pups (Hood et al., 2003). Since all the ewes in this study were dam-reared until weaning around 2 months of age, early social support may have protected them from the adverse consequences of early-life tail docking. Future research should explore environmental factors, such as rearing conditions, that may moderate the long-term effects of tail docking in sheep.

It is also possible that we did not capture an effect of tail docking because our observation window did not include the ewe's immediate maternal behavior. Immediately after birth, ewes display maternal responsiveness by licking their lamb(s), allowing them to identify their lamb(s) by smell and, eventually, selectively choose between their own lambs and alien

lambs (Nowak et al., 2011). Past research has found that most ewes, regardless of maternal experience, show selectivity toward their lamb(s) within 30 minutes of parturition (Keller et al., 2003). Our behavioral observations began 30-60 minutes after the last lamb was born, potentially missing important early interactions. Future research should examine the maternal behaviors displayed by docked and undocked ewes after birth to determine if this procedure impacts the onset of bonding.

There was a tendency for lambs of undocked ewes to spend more time lying in the bonding period (50%) compared to lambs of docked ewes (35%). However, lying time is difficult to interpret as an indicator of welfare without additional context. Depending on the circumstances, lying time can increase or decrease in response to disease, painful procedures, or stressors (reviewed in Richmond et al., 2017). Lambs that experienced difficult births spent more time lying in the following 3 days than lambs from unassisted deliveries, indicating reduced vigor (Dwyer, 2003). However, welfare-compromising situations have also been associated with reduced lying time in lambs, such as high nematode parasite loads (Högberg et al., 2021), temporary separation from their dam (Han et al., 2024), or hunger (Dwyer, 2003). Without additional behavioral changes, it remains unclear whether increased lying in lambs of undocked ewes reflects greater relaxation or reduced vigor, and thus whether it signals improved or compromised welfare.

Litter size affected some behaviors. Single lambs nursed twice as much during the bonding period (26%) and spent almost twice the amount of time lying in the separation trial (51%) than twins. Additionally, singletons tended to have fewer lying-standing transitions in the bonding period compared to twins. These results align with previous findings that ewes nurse singletons more and, in turn, may develop a stronger bond with them compared to dams of twin

lambs (Freitas-de-Melo et al., 2021). Further, we hypothesize single lambs may be afforded more time to rest compared to twin lambs, who spend additional time interacting with one another.

Twin-bearing ewes spent triple the amount of time eating during the separation trial compared to single-rearing ewes, potentially reflecting their higher nutritional demands during pregnancy and lactation (Young et al., 2016), although this effect was not observed during the bonding period.

A ewe's behavior in the hours after parturition, particularly grooming and nursing, is critical to lamb survival (Napolitano et al., 2008; Nowak and Poindron, 2006). Although specific time budgets for these behaviors during the bonding period are not well-described, one study tracking maternal behavior of ewes across parities for 3 hours postpartum found that lambs suckled between 8 and 14 minutes (~4-8% of 3 hours) and were groomed between 19 and 27 minutes (~11-15% of 3 hours) (Karaca et al., 2023). We observed that, on average, ewes nursed and groomed their lambs 17% and 31% of the time visible in the 2-hour bonding period, respectively. These higher proportions may reflect that our ewes were singly housed with their lambs, unlike the former study, which observed groups of 7 to 8 ewes and their lambs (Karaca et al., 2023). Group housing provides opportunities for non-filial social interactions, possibly reducing interactions between the dam and her offspring. We also observed that tail wagging often occurred concurrently with nursing, which may indicate a positive affective state, but very little is known about this behavior in sheep (Kiley-Worthington, 1976; Reefmann et al., 2009). Future research could investigate the potential communicative function of tail wagging in ewe-lamb interactions.

It is well known that social isolation, including lamb-dam separation, even for short periods of time, results in behavioral and physiological signs of distress in sheep (Cockram et al., 1993; Guesdon et al., 2015). In our study, ewes showed behavioral changes indicative of distress

in response to a 15-minute separation from their lambs. Ewes paced 26% of the time they were separated from their lambs but did not display this behavior pre- or post-separation. Across species, pacing is an indicator of a negative affective state (Mason, 2006), and sheep are known to display this behavior in response to early weaning (Freitas-de-Melo et al., 2021) and social isolation (Lauber et al., 2012), but to our knowledge it had not previously been reported in ewes in response to ewe-lamb separation. Spy-hopping, which occurred when ewes were bipedal and peering over the wall of their pen, was also only observed during separation and could reflect escape attempts, as described in previous ewe-lamb separation research (Barnard et al., 2015). Although we did not observe lamb behavior during separation, past research has demonstrated that ewe-lamb separation also results in behavioral and physiological changes indicative of distress in lambs (Han et al., 2024; Mora et al., 2017).

Following separation, ewe-lamb interactions, namely nursing and grooming, increased relative to baseline. These behaviors may serve to soothe distressed offspring, as maternal care has been found to buffer distress across species (Blass, 1994). Previous work has shown that lambs and dam reunite within seconds after brief separation and begin suckling quickly (Maldonado et al., 2015; Sales et al., 2020). It is possible that ewes were responding to their lamb(s) in distress (Futro et al., 2015; Hild et al., 2011). Future research could explore the effectiveness of a dam's maternal buffering on a lamb's physiological state following different routine stressors.

Despite behavioral signs of distress, we did not observe changes in eye temperature before and after separation in either ewes or lambs. Activation of the autonomic nervous system results in stress-induced hyperthermia, increasing body temperature. Eye regions, such as the lacrimal caruncle, are highly vascularized and innervated by the sympathetic nervous system,

making them sensitive indicators of changes in body temperature (Whittaker et al., 2023).

Thermal imaging of the eye has been used across species to measure stress-induced changes in body temperature, but findings have been inconsistent. Some studies report significant changes in response to stressors (sheep: Cannas et al., 2018; Comin et al., 2023; Menchetti et al., 2021), while others do not (dairy cows: Gómez et al., 2018; sheep: Harris et al., 2021; beef cows: Van Der Saag et al., 2019). Methodological differences, including the metric (e.g., maximum, minimum, average), the region of the eye used (Bakker et al., 2024), and the timing of image capture, may contribute to these discrepancies. One study in sheep found that the maximum temperature of the lacrimal caruncle increased 5 minutes after shearing, a stressful procedure, but returned to baseline within 60 minutes (Arfuso et al., 2022). Thus, it is possible we missed a transient increase in eye temperature. Additionally, ambient temperature likely influenced our measurements; our experiment was conducted in a colder climate, which may have contributed to the lower eye temperatures we observed compared to previous studies conducted in climate-controlled or warmer environments (Arfuso et al., 2022; Cannas et al., 2018; Comin et al., 2023; Menchetti et al., 2021). Taken together with the broader literature, our findings suggest that further investigation is warranted into the sensitivity and temporal dynamics of eye temperature as an indicator of stress.

### **Conclusions**

We did not find evidence to suggest that neonatal tail docking influenced the ewe's maternal behavior or behavior of her offspring during the early bonding period or in response to brief lamb-dam separation. Nonetheless, given the known welfare concerns with tail docking, the long-term effects of this practice merit further investigation. Our findings also underscore the impact of even brief lamb-dam separation. Ewes displayed clear behavioral signs of distress

when separated from their lambs, and increased maternal behaviors after reunion, highlighting the importance of minimizing early lamb-dam separation to support ewe and lamb welfare.

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## Tables and figures

**Table 4.1** Definitions used to score ewe and lamb behaviors during the first 2 hours after being moved to the maternity pen. Behaviors were also scored 15 minutes before and after a 15-minute ewe-lamb separation. During the separation, only the ewe's behaviors were scored. The mean, standard deviation, and range for the Intraclass correlation coefficient (ICC) are reported for each behavior. Asterisks indicate behaviors where the ICC was only calculated from the separation observations since they were either not present or too rare in the bonding period.

Behavior	Type	Focal	Definitions	Intraclass correlation coefficient
Eating	Duration	Ewe	The ewe is chewing for at least 3 seconds with her head over the feed trough	0.93 ± 0.11, 0.72-0.99
Grooming lamb	Duration	Ewe	The ewe's mouth is in contact with the lamb's wool for at least 2 seconds	0.91 ± 0.10, 0.78-1
Pacing	Duration	Ewe	The ewe rapidly walks in circles or back and forth for at least 3 seconds. Her head may be lifted with ears erect or lowered toward the ground.	0.99 ± 0.002, 0.99-1*
Self-grooming	Duration	Ewe	The ewe's mouth is in contact with her own wool for at least 2 seconds	--
Spy hopping	Duration	Ewe	The ewe raises up on hind legs with her head at or above the level of the pen wall	--
Receiving headbutt	Count	Lamb	The ewe forcefully strikes the focal lamb with her forehead	--
Nursing	Duration	Lamb	The lamb's head is under the ewe's udder for at least 3 seconds	0.99 ± 0.004, 0.99-1
Tail wagging	Duration	Lamb	The lamb's tail is raised and moves side to side, either rapidly or slowly	0.92 ± 0.09, 0.78-0.99
Lying	Duration	All	The focal animal is not bearing weight on any legs	0.99 ± 0.009, 0.98-1
Human in pen	Duration	NA	Any part of a human's body is in the maternity pen	0.99 ± 0.005, 0.99-1*
Not visible	Duration	All	The focal animal's behavior is obscured from view	0.91 ± 0.11, 0.75-1

**Table 4.2** Test statistics and p-values for each outcome variable (behaviors) and their respective predictors (treatment and litter size) analyzed in the bonding period over 2 hours after the last lamb was born. The focal column refers to the age of the focal animal for that behavior. Asterisks (\*) indicate significant effects ( $P < 0.05$ ) and daggers (†) indicate tendencies ( $P < 0.10$ ).

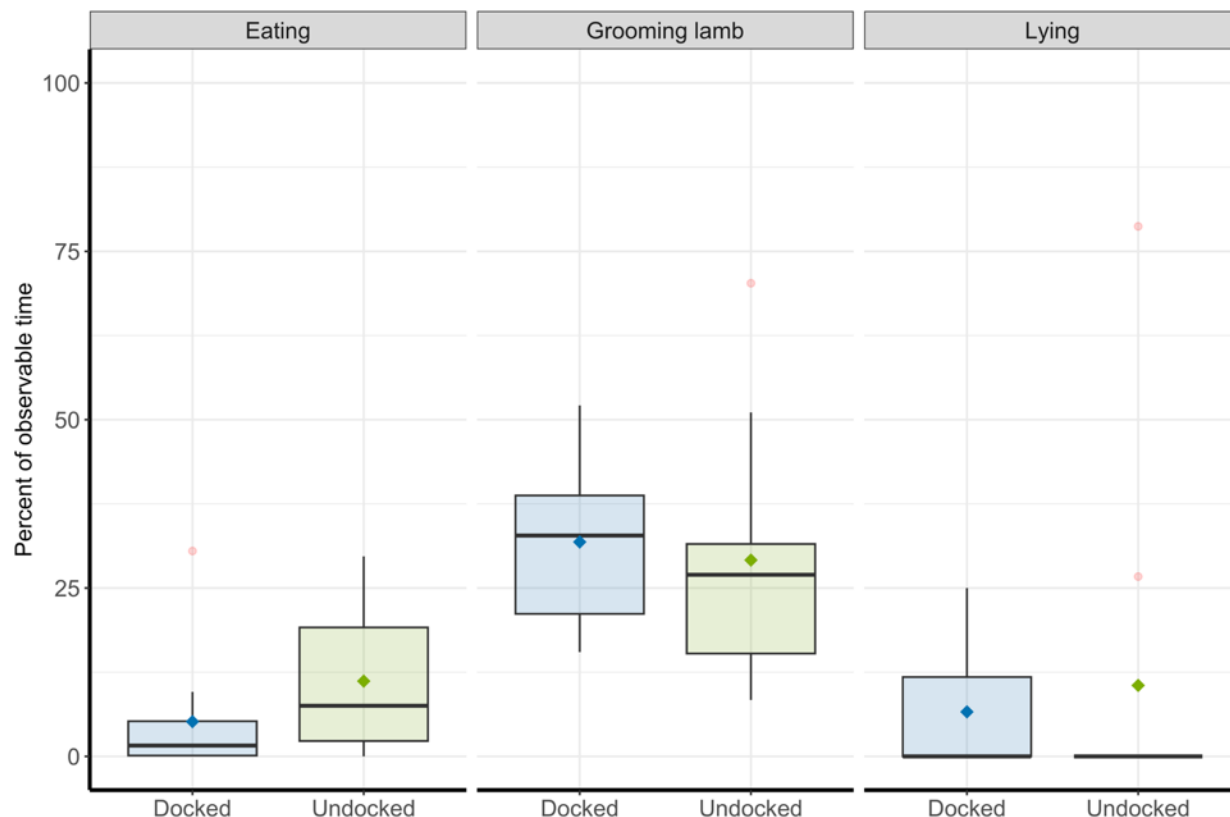
Focal	Outcome	Treatment	Litter size
Ewe	Eating	$\chi^2 = 2.03, P = 0.15$	$\chi^2 = 1.11, P = 0.29$
	Grooming lamb	$\chi^2 = 0.33, P = 0.57$	$\chi^2 = 0.68, P = 0.41$
	Lying	$\chi^2 = 1.30, P = 0.25$	$\chi^2 = 0.004, P = 0.95$
	Lying-standing transition	$\chi^2 = 1.89, P = 0.17$	$\chi^2 = 0.25, P = 0.62$
Lamb	Lying	$\chi^2 = 2.99, P = 0.08\dagger$	$\chi^2 = 0.008, P = 0.93$
	Lying-standing transition	$\chi^2 = 0.88, P = 0.35$	$\chi^2 = 2.98, P = 0.08\dagger$
	Nursing	$\chi^2 = 0.63, P = 0.43$	$\chi^2 = 4.95, P = 0.03^*$
	Tail wagging	$\chi^2 = 0.006, P = 0.94$	$\chi^2 = 0.10, P = 0.75$

**Table 4.3** Test statistics and p-values for each outcome variable (behaviors) and their respective predictors (treatment, observation period, treatment×observation period interaction, litter size) analyzed in the separation trial. The focal column refers to the age of the focal animal for that behavior. Asterisks (\*) indicate significant effects ( $P < 0.05$ ).

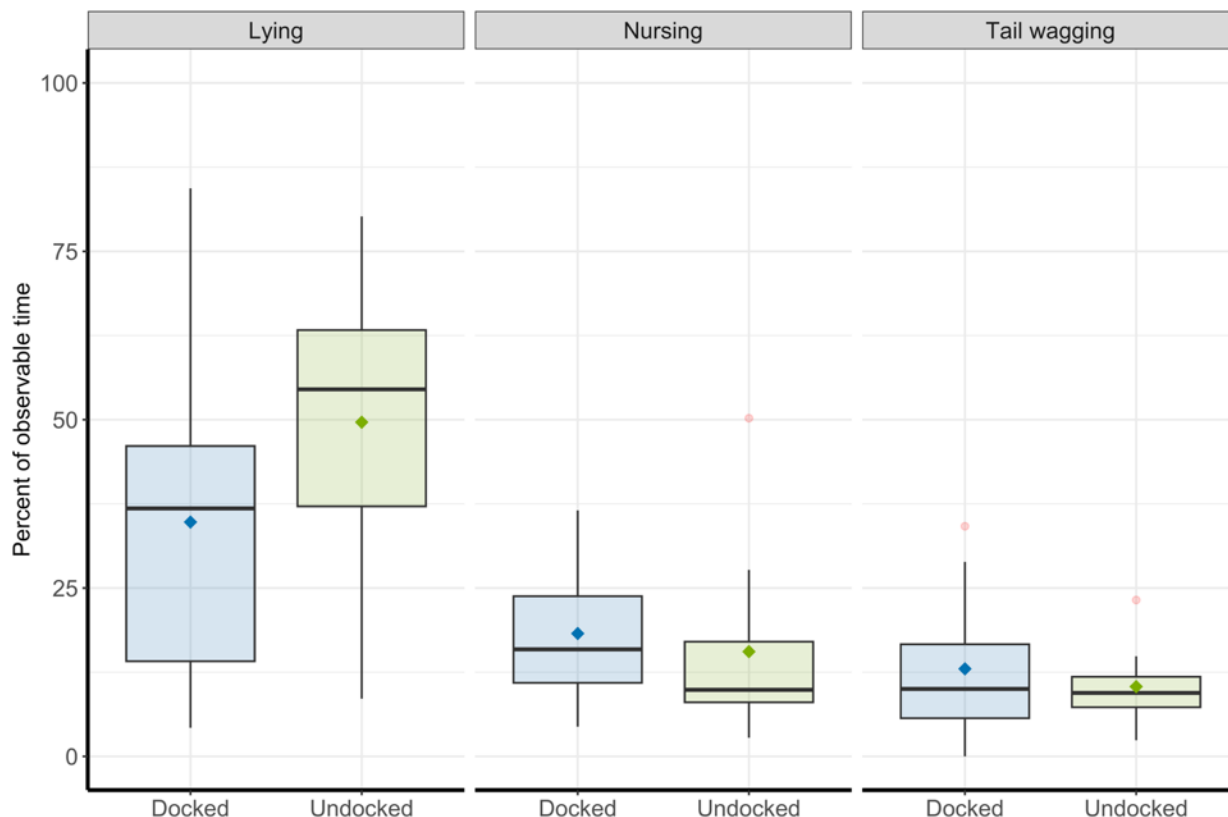
Focal	Outcome	Treatment	Observation period	Treatment×observation period interaction	Litter size
Ewe	Eating	$\chi^2 = 1.53, P = 0.22$	$\chi^2 = 18.50, P < 0.001^*$	$\chi^2 = 7.64, P = 0.02$	$\chi^2 = 6.26, P = 0.01^*$
	Grooming lamb <sup>1</sup>	$\chi^2 = 0.002, P = 0.99$	$\chi^2 = 15.55, P < 0.001^*$	$\chi^2 = 0.63, P = 0.43$	$\chi^2 = 0.15, P = 0.70$
	Lying <sup>1</sup>	$\chi^2 = 1.82, P = 0.18$	$\chi^2 = 18.46, P < 0.001^*$	$\chi^2 = 2.33, P = 0.13$	$\chi^2 = 2.33, P = 0.13$
	Pacing <sup>2</sup>	$\chi^2 = 0.002, P = 0.96$	--	--	$\chi^2 = 1.85, P = 0.17$
Lamb	Lying	$\chi^2 = 0.14, P = 0.71$	$\chi^2 = 11.50, P < 0.001^*$	$\chi^2 = 0.007, P = 0.94$	$\chi^2 = 8.10, P = 0.004^*$
	Nursing	$\chi^2 = 0.88, P = 0.35$	$\chi^2 = 23.41, P < 0.001^*$	$\chi^2 = 0.40, P = 0.53$	$\chi^2 = 0.87, P = 0.35$
	Tail wagging	$\chi^2 = 0.09, P = 0.77$	$\chi^2 = 17.11, P < 0.001^*$	$\chi^2 = 0.08, P = 0.78$	$\chi^2 = 0.03, P = 0.87$

<sup>1</sup>Only data from the pre-and post-separation periods were included in these models as the behavior did not occur during separation.

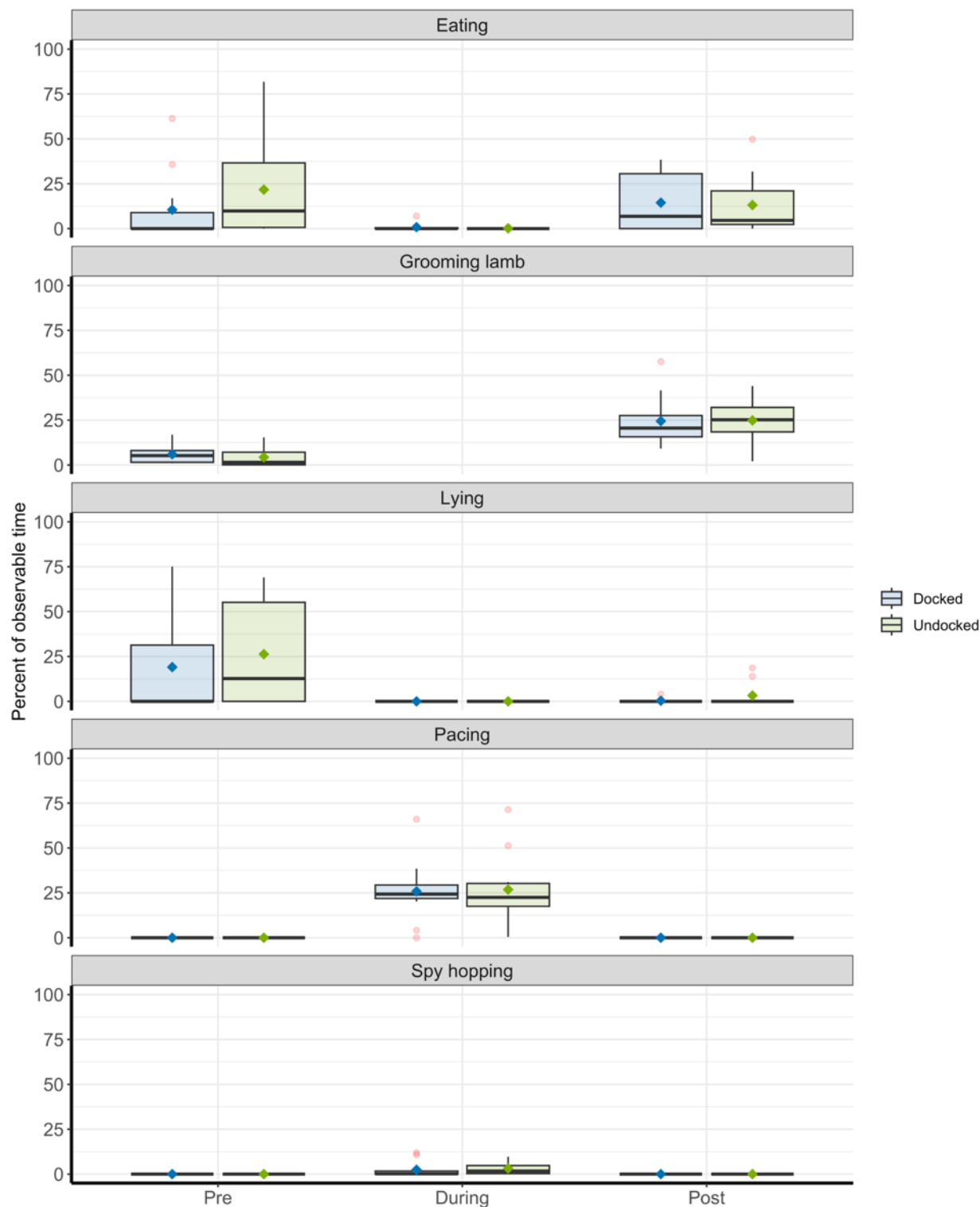
<sup>2</sup>Only data from during the separation were included in this model as the behavior did not occur before or after separation. The effect of the observation period was assessed with Cochran's Q test and reported in text.



**Figure 4.1** Boxplots showing the percent of observable time that docked (n=11) and undocked (n=10) ewes were eating, grooming their lamb, and lying in the 2 hours of the bonding period. The median, 75th quartile, and 25th quartile are indicated by the midline and top and bottom edges of the boxplots, respectively. The boxplot whiskers indicate the lower and upper extremes calculated as  $Q1 - 1.5(Q3 - Q1)$  and  $Q3 + 1.5(Q3 - Q1)$ , respectively. Red dots represent outliers, while treatment-colored dots within each box indicate the mean. One lamb of an undocked ewe was removed from the analysis as she was visible for less than 22% of the observation.

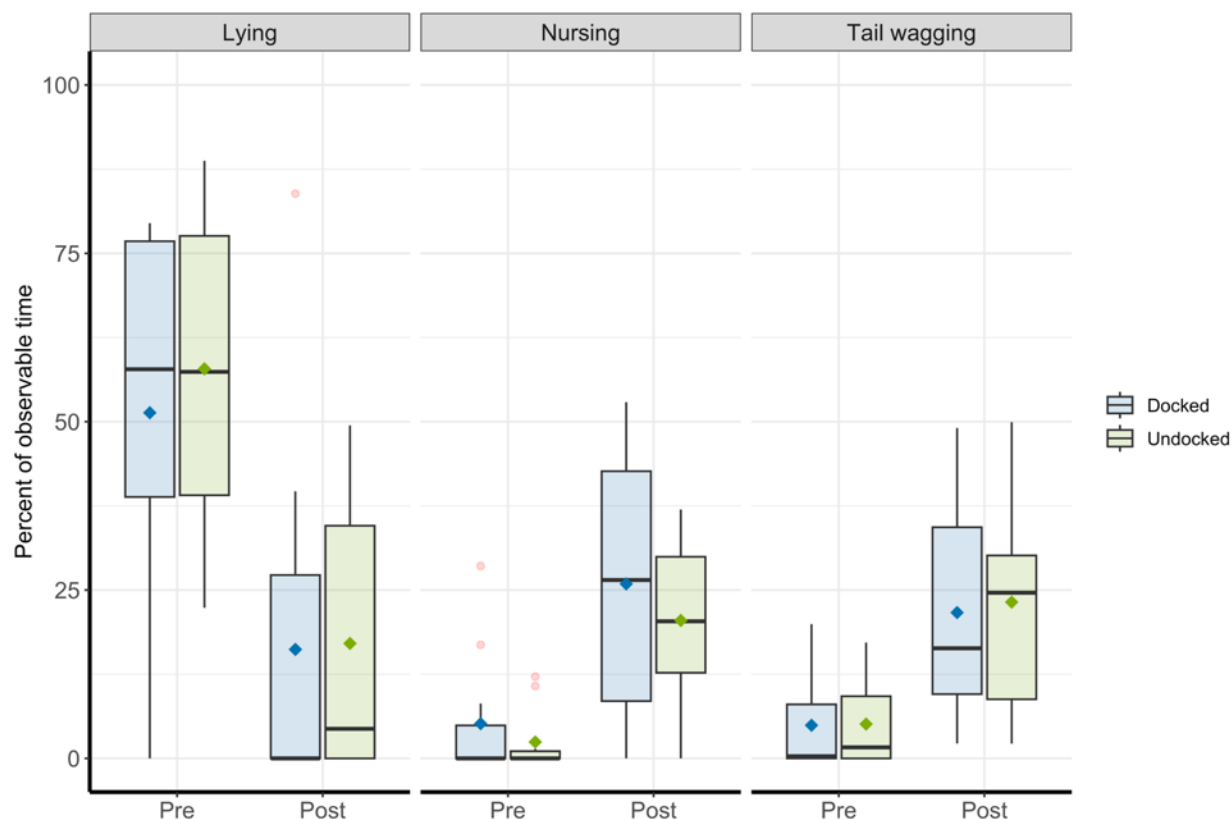


**Figure 4.2** Boxplots showing the percent of the observable time that lambs of docked (n=19) and undocked (n=16) ewes were lying, nursing, and tail wagging in the 2 hours of the bonding period. The median, 75th quartile, and 25th quartile are indicated by the midline and top and bottom edges of the boxplots, respectively. The boxplot whiskers indicate the lower and upper extremes calculated as  $Q1 - 1.5(Q3 - Q1)$  and  $Q3 + 1.5(Q3 - Q1)$ , respectively. Red dots represent outliers, while treatment-colored dots within each box indicate the mean. One lamb of an undocked ewe was removed from the analysis as she was visible for less than 22% of the observation.

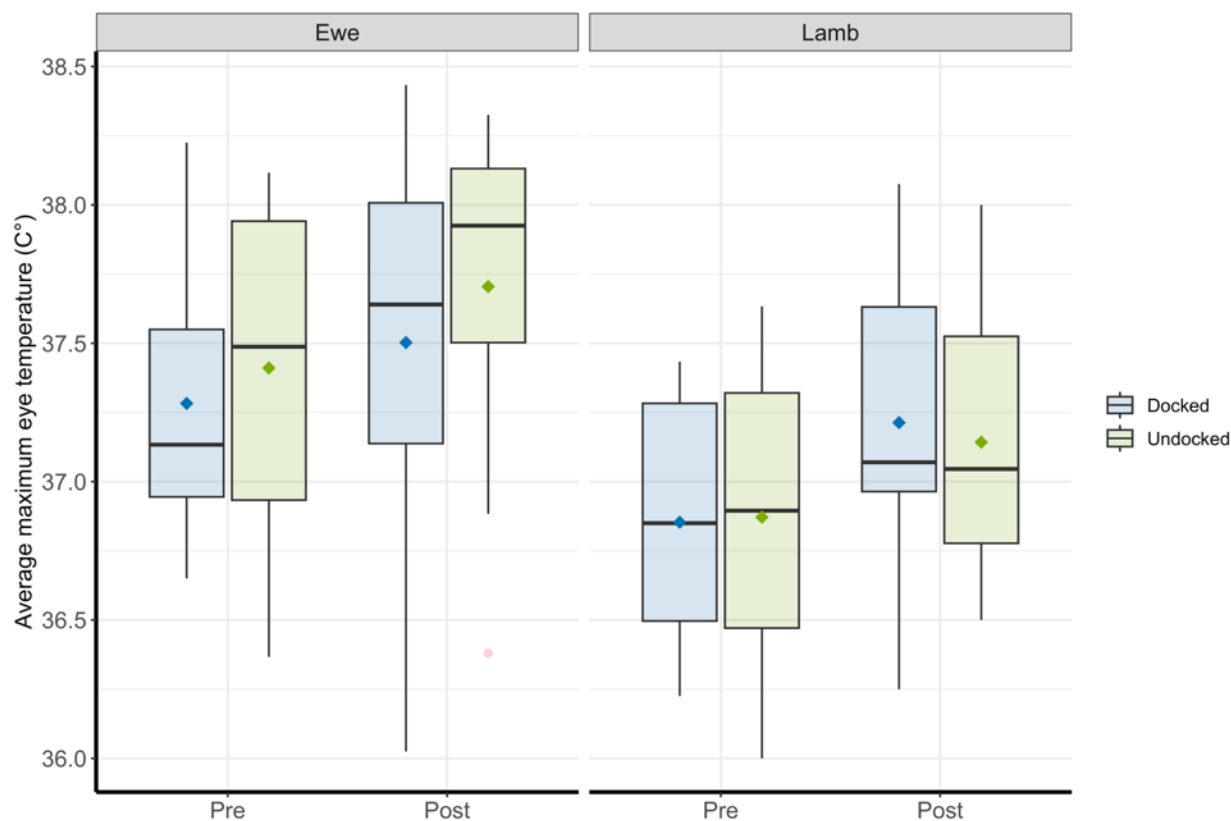


**Figure 4.3** Boxplots showing the percent of the observable time docked (n=11) and undocked (n=10) ewes were eating, grooming their lamb(s), lying, pacing, and spy hopping before, during, and after lamb-dam separation. The median, 75th quartile, and 25th quartile are indicated by the midline and top and bottom edges of the boxplots, respectively. The boxplot

whiskers indicate the lower and upper extremes calculated as  $Q1 - 1.5(Q3 - Q1)$  and  $Q3 + 1.5(Q3 - Q1)$ , respectively. Red dots represent outliers, while treatment-colored dots within each box indicate the mean.



**Figure 4.4** Boxplots showing the percent of the observable time lambs of docked (n=19) and undocked (n=17) ewes were lying, nursing, and tail wagging before, during, and after lamb-dam separation. The median, 75th quartile, and 25th quartile are indicated by the midline and top and bottom edges of the boxplots, respectively. The boxplot whiskers indicate the lower and upper extremes calculated as  $Q1 - 1.5(Q3 - Q1)$  and  $Q3 + 1.5(Q3 - Q1)$ , respectively. Red dots represent outliers, while treatment-colored dots within each box indicate the mean.



**Figure 4.5** Boxplots showing the average maximum eye temperature of ewes (right, n=21) and lambs (left, n=36) before and after lamb-dam separation. The median, 75th quartile, and 25th quartile are indicated by the midline and top and bottom edges of the boxplots, respectively. The boxplot whiskers indicate the lower and upper extremes calculated as  $Q1 - 1.5(Q3 - Q1)$  and  $Q3 + 1.5(Q3 - Q1)$ , respectively. Red dots indicate outliers, and dots corresponding to the treatment colors within each box indicate the mean.

## CHAPTER 5: Conclusions and future directions

### **The welfare and behavior implications of tail docking**

The public's increasing concern for animal welfare is reflected in legislation and consumer demand (Broom, 2011). The animal agriculture industry has implicit societal approval to conduct its business, also known as a social license to operate. However, with increasing concern and media attention, the industry is at greater risk of losing its social license to operate. If that happens, the industry could suffer financially and be under intense scrutiny (Hampton et al., 2020). Aside from being ethically important, proactively addressing public concerns and ensuring that farm animals have good welfare by providing them with the highest quality of life possible is essential to maintaining the industry's social license to operate. Painful procedures are a major animal welfare concern and evoke a strong reaction from members of the public (Weary et al., 2006). In addition, early life pain can result in short- and long-term consequences that negatively impact animal welfare, health, and productivity (Adcock, 2021). For these reasons, understanding the potential side effects of routine painful procedures like tail docking should be an utmost priority.

Tail docking causes acute pain, regardless of the method, and can cause chronic pain to at least a year of age (Larrondo et al., 2019). However, there are still large gaps in our knowledge regarding the procedure, which are essential to address when making science-based recommendations for best practices. My objective was to address some of these gaps by first detailing the healing process of tail docking wounds and its impact on the healing of ear tag wounds (Chapter 2). Secondly, I assessed how neonatal tail docking of ewe lambs impacted their social behavior later in life, specifically their interactions with potential mates (Chapter 3) and their own lambs (Chapter 4).

I found that, on average, tail docking wounds took six weeks to heal, and tails took almost three weeks to fall off (Chapter 2). In this study, I created a novel scoring system adapted from previous research on disbudding wounds in calves (Adcock and Tucker, 2018). I found that pus, a sign of infection, was present at least once in 89% of tail docking wounds and was associated with longer healing times, consistent with previous informal evaluations (Johnstone, 1944; Watts et al., 1979). However, slough, another sign of infection, did not prolong tail wound healing but was present at least once in 93% of lambs.

In addition to tail docking wounds, I assessed ear tag wounds, as these procedures are often performed concurrently. Surprisingly, I found that only 49% of the ear wounds were healed when the observations ended between 8 and 11 weeks. Further, ear tags with more occurrences of sanguineous exudate, or blood, were less likely to have healed by the end of the observation period. Lastly, I found that performing painful procedures concurrently, ear tagging and tail docking, compared to a single procedure, ear tagging, did not delay the healing time of ear tag wounds by weaning between 57-76 days of age.

I also assessed how neonatal tail docking of ewe lambs impacted their mating behavior at 7 to 8 months of age (Chapter 3), their maternal behavior at 13 months, and the behavior of their lambs (Chapter 4). Before discussing these results, it is worth noting that my experimental design does not allow for distinguishing between the potential physiological consequences of an early life painful procedure, such as altered neural pathways or chronic pain, and the effect of an altered appendage that may serve a communicative function. To disentangle these effects in the future, studies should at least include a tail-docked group that receives pain management. However, a pain-managed control should be used with caution since there is currently no validated approach to managing pain from tail docking, especially over the long term (reviewed

in Adcock, 2021). Ideally, studies could also include a treatment group of sheep genetically selected for shorter tails (James, 2006), which would remove the element of pain and allow us to assess the tail's role in intraspecific communication. Still, such genetic modifications may inadvertently affect other behavioral traits and should be interpreted with care.

To determine the influence of neonatal tail docking of ewe lambs on their behavior later in life, I first examined their mating behavior at 7 to 8 months of age by observing them in the first two hours of ram exposure (Chapter 3). A similar study found that rams preferred undocked ewes to docked ewes, but their ewes were restrained when exposed to the ram (Orihuela et al., 2018). In my study, each ram was exposed to four freely moving ewes (two twin pairs, two docked and two undocked) for 48 hours. In the first two hours I observed rates of rejected, accepted, and total mounts, headbutts, and the duration of perineal investigation. Additionally, I calculated a ram's latency to mount and investigate a ewe's perineal region. Finally, I used real-time location sensors to determine each ewe's distance traveled and her average distance to the ram. I observed no significant differences in any of these outcomes between docked and undocked ewes. However, I did observe two statistical tendencies that suggested neonatal tail docking may reduce a ewe's sexual attractiveness and receptivity. Specifically, I saw that rams tended to mount undocked ewes sooner, and undocked ewes had a higher probability of accepting a mount compared to docked ewes.

Three potentially overlapping mechanisms may explain the results: 1) undocked ewes can use their tails to visually and olfactorily stimulate rams during mating, 2) docked ewes are unable to withstand extended pressure on the anogenital region because of chronic pain at the docking site, or 3) early life stress experienced by tail docking disrupted docked ewe's behavioral or reproductive development. Although our results may reflect a combination of these mechanisms,

results from another study I have conducted support the chronic pain hypothesis. In collaboration with the University of Wisconsin-Madison Veterinary School, I harvested tail tissue for histological examination from a subset of 18 ewe lambs (8 docked, 10 undocked) from my original cohort that were slaughtered at 7 to 8 months of age (Pantel et al., In prep). Neuroma-like proliferations were found in all docked tails ranging from mild to severe classifications, indicating that docked ewes may experience chronic pain several months after the procedure.

In Chapter 4, I assessed whether tail docking ewe lambs as neonates impacted their maternal behavior and their offspring's behavior after lambing. At approximately 13 months of age, docked and undocked ewes gave birth to either singleton or twin lambs. Once born, ewes and their lamb(s) were moved to individual maternity pens, where I observed their behavior for two hours during the bonding period. Specifically, I analyzed the duration the ewe spent eating, pacing, lying, nursing, and grooming her lambs, as well as the duration the lambs spent tail wagging and lying. I did not find an effect of the dam's tail docking status on her behavior or her lamb(s) in the bonding period. However, lambs of undocked ewes tended to spend more time lying compared to lambs of docked ewes. Lying time is a context-dependent indicator of welfare, associated with both reduced or improved welfare across species. As such, this tendency may indicate that lambs of undocked ewes were more relaxed (better welfare) or had reduced vigor (worse welfare) than lambs of docked ewes, and further research is needed to clarify the welfare implications.

I also assessed docked and undocked ewes and their lambs' behavioral and physiological responses to lamb-dam separation 6 to 12 hours after lambing (Chapter 4). During the 15-minute separation, lambs were moved to a separate part of the barn. This restricted visual access between the ewes and their lambs and muted auditory cues but did not fully eliminate them. Ewe

and lamb eye temperatures were taken immediately before and after separation using infrared thermography. The same behaviors from the bonding period were coded in the 15 minutes before and after the separation. I did not find an effect of the dam's tail docking treatment on her behavior or her lamb(s) behavior before, during, or after the separation.

Regardless of tail status, ewes displayed behaviors indicative of distress in response to lamb-dam separation. Specifically, ewes ate less when they were separated and spent a quarter of the time pacing, a behavior they did not display in the rest of the study. Following reunion, ewe-lamb interactions increased relative to baseline, with ewe and lamb lying decreasing and grooming, nursing, and tail wagging increasing. However, no differences in ewe or lamb eye temperatures were found before or after separation. This aligns with some previous studies that also found no significant changes in eye temperature after a stressful procedure (e.g., Harris et al., 2021), while others have reported changes (e.g., Cannas et al., 2018). As such, further investigation into the optimal eye temperature metrics, including measurement timing and anatomical location, should be considered. Overall, my findings did not support the hypothesis that the lamb-dam bond is impaired by neonatal tail docking. However, the ewes found separation from their lambs stressful, emphasizing the importance of minimizing disruptions during the early postnatal period.

Considering the findings from these studies, the conclusions are nuanced. First and foremost, in Chapter 2, I presented the first formal evaluation of tail docking wounds, finding that, on average, they take almost two months to heal. This is particularly alarming as studies in disbudded calves have found that heightened pain responses persist throughout the healing process (Adcock and Tucker, 2018; Drwencke et al., 2023). Further, I found instances of infection in most lambs' tail wounds at least once over the study period, and the presence of pus

was found to delay healing. In my third chapter, I found some evidence that early life tail docking can impact mating behavior later in life, but I did not find similar effects on maternal behavior in my fourth chapter. This suggests that neonatal tail docking may impact some behaviors but not others later in life, although further work with larger sample sizes is needed to confirm these results. Given our limited understanding of the long-term impacts of tail docking sheep, and the inconclusive evidence supporting its rationale, we need more research to determine whether this practice is justified.

### **Ethical considerations and alternatives**

The research I have presented addresses significant knowledge gaps regarding tail docking in sheep, although our understanding of the procedure's short- and long-term implications is still limited. However, there is clear evidence that tail docking causes acute and chronic pain. Further, tail docking can result in infection, which may delay healing and lead to other morbidities. The question begs, should we continue inflicting tail docking pain on millions of sheep each year based on scarce and conflicting evidence that it may reduce fecal soiling and, in turn, may reduce blowfly strikes?

The adaptive function of the tail in sheep is poorly understood, but it may be important for intraspecific communication. Ungulates are known to use tail posture and movement to communicate emotional states (e.g., pigs: Camerlink and Ursinus, 2020; cattle: Schwartzkopf-Genswein et al., 1997). There has been very little research into how sheep use their tails to communicate, but there is some evidence that the tail might be used to portray strong emotional states (Kiley-Worthington, 1976; Reefmann et al., 2009). Further, evidence suggests that tail wagging in female ruminants may function as a proceptive behavior that increases attractiveness (Fierros-García et al., 2018; Haulenbeek and Katz, 2011). In addition to communication, there

may be other potential benefits of leaving the tail intact that deserve consideration. For instance, undocked ewes had fewer vaginal biota present during copulation when compared to docked ewes, suggesting that the tail may protect the vaginal environment (Orihuela et al., 2019). The tail may also aid in fly removal, as has been found in cows, but this has yet to be considered in sheep (reviewed in Sutherland and Tucker, 2011). As such, understanding how leaving sheep undocked impacts welfare and productivity warrants further exploration.

I focused on tail docking ewe lambs with rubber rings, but there are other docking options, such as hot iron cautery or removal with a knife or scalpel. The rubber ring method is widely assumed to be the most common method for tail docking in sheep, although recent empirical research has not confirmed this (Orihuela and Ungerfeld, 2019; Sutherland and Tucker, 2011). However, some research suggests that tail docking with a rubber ring may be more acutely painful, at least in comparison to hot iron cautery (Grant, 2004). Regardless, there is evidence that all methods of tail docking cause acute pain across species (reviewed in Sutherland and Tucker, 2011). As such, the use of pain relief, which is not currently required in the United States, needs to be considered for all methods of tail docking. The use of local anesthetic and analgesia has been found to alleviate the acute pain caused by docking, but some methods may be more effective than others (reviewed in Orihuela and Ungerfeld, 2019). Further, current pain management strategies relieve acute pain, but there is currently no solution to addressing chronic pain from tail docking (Adcock, 2021). If tail docking continues to be practiced, determining methods for relieving both acute and chronic pain must be a priority.

Amputating the tail is a permanent intervention to address a problem that occurs seasonally, as the greatest risk of flystrike is during spring and summer (reviewed in Lihou and Wall, 2019). Instead of focusing on a permanent approach, which may have negative welfare and

productivity consequences, we could pursue seasonal fly preventions that are already being used successfully. In flocks of docked sheep with no flystrike control, anywhere from 5% to 16% of the flock was affected (Broughan and Wall, 2007). However, when ewes were shorn mid-summer, there was a 95% reduction in the risk of flystrike in ewes (Broughan and Wall, 2007). Additionally, when the risk for flystrike is low, spot-treating affected animals, and when the risk is high, proactive flock-wide chemical treatments have been found to be sufficient solutions to reducing flystrike (reviewed in Lihou and Wall, 2019). Exploring flystrike rates in docked and undocked sheep under different fly control strategies would provide essential insight into potential alternatives to tail docking.

My research took place in Wisconsin, which has a humid continental climate with four distinct seasons. Given that the lifecycle of a blowfly follows four typical seasons and they do extremely well in humid environments (Lihou and Wall, 2019), Wisconsin provides an ideal climate for them to thrive. The Wisconsin climate shares some similarities with the temperate and subtropical climates found in areas where blowfly strikes are common, including the United Kingdom, New Zealand, and Australia. It is worth noting that none of the sheep in my research experienced flystrike, and there was no difference in fecal egg counts between docked or undocked ewe lambs while on pasture (Woods et al., In prep). However, my research took place during only one season and ewe lambs were only on pasture for 45 days between July and August. Blowfly strikes can vary considerably across seasons in the same location based on variability in pasture and weather conditions. Many risk factors for flystrike can be at play and need to be considered for future research, such as the age of sheep, season, rainfall, and pasture composition.

Further, breed characteristics may make sheep more susceptible to flystrike in humid climates. My study focused on Polpay sheep, which are primarily bred for meat production, but their wool is sometimes sold as a byproduct. Although blowfly strikes occur across breeds, there is clear evidence that sheep with breech wrinkles, like merino sheep, which are raised for wool, are more susceptible compared to non-wrinkled breeds like Polypays (James, 2006). Merino sheep are commonly equated with a wrinkly breech and are the most prevalent breed of sheep in Australia (Swan et al., 2017). Flystrike is a severe issue in Australia, costing the industry \$175 million annually (Kotze and James, 2022). However, in New Zealand, where flystrike is estimated to cost the industry \$50 million annually, the most common breed of sheep is the wool-producing Romney, which does not have breech wrinkles (Johnson et al., 2021).

If further research determines that tail docking reduces blowfly strike in certain environments or breeds, genetic selection for shorter tails and reduced wool on the breech could provide a potentially painless long-term alternative. Previous research has found that tail length can be highly heritable with rapid selection (reviewed in James, 2006). In an effort to find an alternative to tail docking, one study compared traits related to reproduction and productivity in ewes mated with rams with varying tail lengths (Hümmelchen et al., 2025). There was a tendency for ewes bred to short-tailed rams to have a lower pregnancy rate compared to those bred to long-tailed rams, but there were no differences in the other parameters measured, including twinning rate, average body length, or vitality of the lambs (Hümmelchen et al., 2025). However, possible negative side effects associated with the shortening of the spine in other species, such as urinary and fecal incontinence and restricted mobility of hind limbs, should be considered (Indrebø et al., 2008; Robinson, 1993). Genetic selection could be a viable, non-painful alternative to tail docking in sheep, but it requires further investigation.

### **Broader significance**

Given the limited research on the welfare implications of tail docking across species, my research results can inform recommendations for best practices and future research directions. In the past, using research findings to inform public policies has proven successful in reducing or eliminating common painful procedures in farm animals. For example, it used to be commonplace to tail dock cattle to improve cleanliness and, in turn, reduce mastitis-causing pathogens from infecting the udder, but there was no substantial evidence to support these claims (e.g., Schreiner and Ruegg, 2002). Further, tail docking reduced cows' ability to prevent flies from landing on their hind legs compared to cows with intact tails (Eicher and Dailey, 2002). These findings contributed to several countries and organizations discouraging or banning the procedure in dairy cattle, resulting in a steady decline in the practice worldwide (reviewed in Sutherland and Tucker, 2011). As research on the merits and pitfalls of tail docking sheep becomes available, it can be used to inform best practices through industry regulations and public policies.

Even as evidence mounts against the routine tail docking of sheep, subjective norms and industry traditions may still deter producers from ending the practice altogether. In addition to concerns about blowfly strikes, sheep are often tail docked at ultra-short lengths (e.g., less than 2 cm) for livestock shows, as it is believed that this length causes lambs to appear fuller and more square (Goodwin et al., 2007). However, as the length of the tail shortens, the risk of rectal prolapse has been found to increase in sheep (Thomas et al., 2003). To discourage producers from ultra-short docking, select states have enacted length recommendations, and the United States Animal Health Association and American Veterinary Medical Association have passed resolutions that lambs should not be accepted for exhibition if the tail is shorter than the distal

end of the caudal fold (Goodwin et al., 2007). However, establishing a reliable way to measure tail length has yet to be explored in sheep, making it difficult to maintain regulations (Goodwin et al., 2007; Woodruff et al., 2023).

Similarly, in dogs, breeders were (and still are) resistant to ending cosmetic tail docking because they believe docking maintains breed standards and tradition (Bennett and Perini, 2003). However, as public concern has increased surrounding the procedure's necessity, several countries have begun to outlaw cosmetic tail docking of dogs, including Australia, New Zealand, South Africa, and most of Europe (reviewed in Mellor, 2018). As such, tail docking in sheep and other species may follow a similar trajectory with enough public attention.

The research presented here can serve as a foundation for designing studies to assess the long-term impacts of tail docking in other species. For example, piglets are regularly tail docked early in life to prevent tail biting. Similar to sheep, tail docking piglets has been found to cause acute and chronic pain (reviewed in Adcock, 2021). However, the long-term welfare and behavior implications of neonatal tail docking in piglets are unknown. Twins, as used in my research, are ideal subjects for randomized controlled trials because each sibling experiences the same maternal environment, which may otherwise influence elements of the research (Sumathipala et al., 2018). Given that pigs produce larger litters compared to sheep, researchers are afforded the luxury of assigning individual piglets within the same litter to different treatments such as docked, undocked, and docked with pain management.

### **Conclusions**

These studies provide insight into the welfare and behavior implications of tail docking sheep. Previously, it was unknown when wounds resulting from tail docking with a rubber ring healed. We now know that they can take almost two months to heal, and signs of infection are

not only common but also delay wound healing. With this knowledge, further research can explore treatment strategies for these wounds and the healing and infection rates of wounds resulting from other tail docking methods. Further, alternatives to tail docking, such as leaving sheep undocked or genetically selecting for shorter tails, warrant consideration.

In examining the long-term behavioral impacts of neonatal tail docking, I found some evidence that it may influence a ewe's sexual attractiveness and receptivity. Specifically, rams tended to mount undocked ewes first, and undocked ewes had a higher probability of accepting a mount than docked ewes. This finding could result from altered behavioral responses due to early life stress, chronic pain, the lack of ability to communicate with the tail, or some combination of these mechanisms in docked ewes. However, we found no differences in the maternal behavior of ewes tail docked as neonates or in the behavior of their lambs, suggesting that tail docking may influence some behaviors more than others. These findings highlight the need for further research into the specific behavioral domains affected by tail docking to more fully understand its welfare implications and inform evidence-based decisions about the practice.

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