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Pre- and Postpartum Human-Animal Interactions reveal Consistent Individual Differences in Rangeland Breeding Ewes

By

KALEIAH SCHILLER

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Animal Biology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

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Maja Makagon

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Andrew Sih

Committee in Charge

2023

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ABSTRACT

Consistent individual behavioral differences (CIDs) are evident in breeding ewe populations and may carry fitness consequences that affect lamb outcome, especially in extensive settings that involve minimal human intervention. These CIDs are described as covarying behavioral and physiological response patterns to challenge, that may be elicited during human-animal interactions. The aims of this dissertation were to assess CIDs during human animal interactions (HAIs) in restrained and unrestrained contexts when the lamb was not present (Chapter 2); assess CIDs among ewes during and after lamb processing in relation to indicators of lamb outcome including birth weight, growth rate and weaning weights (Chapter 3); and evaluate the relationship between CIDs when the lamb is and is not present during pre- and postnatal HAIs with inclusion of indicators of lamb outcome (Chapter 4). Using practical, on-farm methods to evaluate CIDs among ewes, Chapter 1 revealed no evidence for a stable behavioral trait (two or more covarying behaviors) in the presence of a human, however, there was evidence for repeatability in singular behaviors including duration of 'head down' while ewes were being directly handled and frequency of 'environmental vigilance' when in the presence of a human. Previous research suggests a personality trait related to the presence of a human may be activated during lamb processing that is an unreliable indicator of adaptive maternal care. A unique behavioral response characterized by pacing, avoiding the human and restricted grazing was elicited during lamb processing and was unrelated to indicators of lamb outcome and adaptive maternal care (Chapter 3). Proximity maintained from the human during lamb processing (similar to maternal behavior scores, O'Connor et al. (1985) was inconsistent between years and seemingly a reflection of maternal investment which may be influenced by lamb weight. Finally, patterns of behavior were identified between periods when the lamb was

and was not present (Chapter 4). Duration of ‘head down’ when the ewe is being handled by the human was related to frequency of ‘open-mouth vocalizing’ after lamb processing, when the ewe and lamb were alone together and positively related to lamb birth weights. Few studies have explored the relevance of head postures in sheep, however, ‘head down’ behavior is thought to be indicative of stress and negative arousal and should be considered as a potentially, biologically important behavior in sheep, worth consideration into selective breeding programs.

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I would like to thank my advisor, Dr. Kristina Horback, from the bottom of my heart. I thank Dr. Horback for her patience, level-headedness, intelligence and perspective. I could not have asked for a better advisor. As Dr.Horback's first graduate student, she allowed me the freedom to envision and create my own project, with just the right amount of guidance and input when needed. Dr.Horback demonstrates dedication to her graduate students, evidenced in her willingness to adapt to our needs and individual selves. The hardest part of this dissertation was not the dissertation itself, but the personal and global events occurring simultaneously. Had I had a different advisor, I am not sure I would have had the space to endure these events and complete my graduate degree. I admire Dr.Horback's dedication to teaching and outreach most of all. Without education, communication and extension, I see little impact, even importance, of the research we put so much time into. Any graduate or undergraduate student lucky enough to share time with Dr.Horback and receive her knowledge and guidance is certainly on the right path.

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I have endless gratitude towards the hardworking shepherds, Dan Macon and Roger Ingram, who allowed me to conduct research on their breeding ewes in Auburn California. I met Dan at a sheep innovators conference while presenting research on a species I knew little about. From there, Dan and I formed a partnership, and friendship, with the goal of asking important questions that could benefit both small and large sheep operations. Dan and Roger allowed me to shadow them and learn from their incredible wealth of knowledge. I will always be in awe of the understanding shepherds carry of the land, animals, and environment. Dan Macon exposed me to the world of research and extension and cemented my own passion for education and outreach. With Dan, I was able to meet other equally impressive extensionists and collaborate on important projects/ workshops/ projects to better the sheep farming industry.

Undergraduate interns were the unpaid machine behind this dissertation. If I could have compensated every single one of them, I would have. They certainly deserve it. These interns spent hours and hours coding data for the purpose of their own interest and passion for research. All I can do now is dedicate some space for them here, and hope that each of them finds a career and life that is fulfilling and joyful. Here are some of the wonderful interns I worked with: Aleisha Burke, Stephanie Perez, Hannah Blackwell, Jaqueline Ayala-Hernandez, Erika Juarez, Ramya Pondicherry, Tania Vargas, Yerim Bae, Aishah Asif, Jessica Llamas, Valeria Prado-Castillo, Albert, Jamie Gallagher, Chloe Bolanos, Emrys, Beth Lyon, Katie Sontag, Con, Victoria Machuca, Hope Forsberg, Megan Porral-Hyunh, Susan Zhou, Erika Cromwell, Lauren Cromwell, Ryder Wu, Gouchun Li, Emily Chung, Victoria Alvarez, Sujata Shivakumar, Helen Racancoj, Linsey Sutton and Jennie Park.

Finally, to sheep #17. May you raise many lambs, receive much love and gentleness, and growl at any human who crosses your path.

DEDICATION

This dissertation is dedicated to shepherds Dan Macon and Roger Ingram who demonstrate limitless capacity for knowledge and compassion. This dissertation is also dedicated to the most loyal herding dogs, Mo and Mae, and reliable guardian dogs, Elko and Bodie.

Chapter 1

General Introduction

Sheep (*Ovis aries*) are a domesticated livestock species, bred for either their milk, meat (lamb and mutton) and/or wool. Sheep are an incredibly gregarious species and rely on their conspecifics in numbers (the flock) for antipredator protection and acquisition of resources. Due to their gregarious nature, sheep are best managed in flocks of at least 5, ideally more, to optimize grazing (Penning et al., 1993) and ease handling. The leading sheep production states in the U.S. include Texas, Wyoming and California (Jones, 2004) and like many California populations, the current study population was raised extensively on rangeland/ pasture. Small flocks (50 - 200 head) are the most common type of sheep operation in the Western U.S. These small flocks are common and convenient as sheep are able to utilize patchy land and different forage varieties more efficiently compared to other ruminants, such as cattle (Shapouri, 1991). The current study used adult female sheep (ewes) for data collection that were raised in Auburn, CA, and bred annually for raising lambs that will be slaughtered for meat at 5 months of age. Under these systems, breeding ewes undergo natural cover (mating) with rams (intact, adult male sheep) and will give birth to single or multiple lambs. Depending on the system and breed of sheep, farmers may choose to breed animals in the fall so they give birth in the spring, when vegetation is most plentiful.

Both adult sheep and their young can experience a level of vulnerability on rangeland due to weather, predation and/or poor shepherding. In general, animals on rangeland or within extensive conditions will face specific environmental challenges that intensively managed animals are not be exposed to (Dwyer, 2009). Lambs and ewes at the University of Hopland, Field Station, experienced predation at a rate of 1.5 – 10.4% and 2.7 - 3.8% over an 11 year period, mainly due to coyotes. Other types of predators in the west may include Bob cats, mountain lions and Golden eagles. Extreme and incremental weather events are another environmental issue leading to animal loss, especially in lambs, with losses reaching nearly 45% in cold and rainy conditions (Alexander, 1964). Shepherding or human management of sheep is necessarily to combat the impact of environmental challenges, however, even this aspect of the sheep's life can be an environmental stressor. Unfortunately, human-animal interactions between the shepherd and the flock within extensive operations are often stressful since the interactions are infrequent (Dywer, 2009). Within most sheep operations, sheep will experience some type of intensive handling procedure, be it shearing, foot trimming, drenching or even assistance at lambing. Shearing, in particular, can be immensely stressful for sheep (Hargreaves and Hutson, 1990) as this involves flipping the animal onto their rump. Less intense interactions, like drenching (vaccinating), that are quick and do not require lengthy handling have been observed to be less stressful compared to shearing or crutching (Hargreaves and Hutson, 1990). Reducing the level of stress on the animal during human-animal interactions is generally advised to reduce the risk of injury to the shepherd and animal and to make handling more efficient in the future. Flocking, following and vision are three descriptors that Hutson (2014) uses to encapsulate sheep, which are helpful to understand in order to reduce stress during handling. Hutson (1980c) advises that the best handling systems require a view of the exit route and where the animal is meant to move. For low stress handling, the 'Bud-Box' was developed to move sheep so they can see the exit and others ahead of them which will implicitly elicit movement from seeing movement of other conspecifics. Ultimately, using low stress handling methods that involve a familiar environment, minimal noise and physical contact with the sheep will make handling most efficient. Individual sheep may also be culled, or pulled out of the flock, if they are reactive to a point that will cause them to sustain injury during handling and human interventions.

Lambing season, when lambs are born, is the most intense and energy expending time of the year for shepherds and the flock. At this time, ewes may need assistance with the birthing process which would require the shepherd to be vigilant around the clock to identify parturition issues. Some operations may try to select sheep that are more likely to have twin lambs over singles lambs to increase profitability, however, this can and has led to lamb mortality issues when the ewe is unable to care for or identify both of her lambs (Purser and Young, 1964). After birthing, the quality of maternal care can be variable between ewes and this may or may not be detrimental to the lamb's health. Ewes that demonstrate poor receptivity of or selectivity towards the lamb may consequentially abandon or reject the lamb all together (Poindron et al., 2007). Some sheep operations will involve a type of selection program targeted at selecting ewes with the best maternal qualities based on perhaps their time at the birth site or quality of interactions with the lamb (Dwyer, 1998; Shillito-Walser, 1984). Adaptive maternal behaviors in sheep that could be selected for include frequent licking and grooming of the lamb, staying at the birth site for 4 – 6 hours after parturition (Nowak, 1996), positioning the body such that the lamb can more easily have access to the udder (Dwyer, 2008, Nowak, 1994) and frequent vocal communication with the lamb (Nowak, 1996). Ultimately, ensuring the survival of the lamb requires intensive oversight from the shepherd, good weather, low predation and quality maternal care.

Consistent individual differences (IDs) in behavior

Consistent individual differences (CIDs) have been widely reported in populations of domesticated sheep. These differences are expressed through temporally stable, behavioral and physiological responses to their environment with associated functional consequences in terms of fitness and survival of the species. Work investigating CIDs may have an applied framework, focused on understanding how animal CIDs relate to performance and management related parameters (e.g. growth rates, immune function, milk yield, offspring survival, etc.) or a theoretical framework targeted at uncovering the evolutionary development of CIDs and their current manifestation. CIDs in sheep have dimensionality that can be measured through specific behavioral and physiological traits by means of restrained (i.e., limit physical movement) and unrestrained (i.e., freedom of movement) testing. Restrained testing allows for on-farm testing and has been historically considered more reliable and predictive of growth and carcass traits in sheep. Due to the restricted nature of these tests, sheep have limited movement and therefore limited behavioral expression. Unrestrained tests, on the other hand, allow researchers to observe a wider range of behaviors for multifactor analysis to reveal latent traits and their relationship to other biological outcomes in sheep. Unfortunately, the coherency of aggregated behavioral correlates from unrestrained tests is variable, and their relationship to performance and biological outcomes are inconclusive. Previous work has explored the use of restrained and unrestrained tests to identify the relationship between CIDs in sheep and differences in maternal behavior, as these differences are vital for the likelihood of lamb survival in extensive systems where the ewe's behavioral expression can have detrimental consequences on the lambs' outcome (Porciuncula et al., 2022). Due to the inconsistencies present in restrained and unrestrained testing of ewes, the relationship of CIDs and maternal behavior is unknown. Additional longitudinal investigations are needed to understand the relationship between IDs of ewes and the relationship to maternal behavior.

For the purposes of this dissertation, the term animal CIDs will be used to refer to these unique response patterns. Within the definition, these response profiles may demonstrate differing levels of adaptivity and plasticity (Sih et al., 2004), assessed by introducing animals to a gradient of environmental challenges and collecting markers of fitness. Also within the framework of CIDs there is the concept that each CID has dimensionality (i.e., boldness, activity, aggressiveness, sociability, exploration) that may be revealed when testing animals using different eliciting stimuli and recording said, observable behavioral and physiological responses (Réale et al., 2007). From an evolutionary perspective, CIDs are established over time through natural selective processes, allowing populations of animals to have a distribution of interactions with their environment and social others; with the assumed goal of providing a more dynamic and beneficial response to challenging circumstances.

Assessing CIDs in sheep

Sheep CIDs, often referred to as temperament, are typically assessed with the goal of improving selective processes to enhance production. Heritability estimates of animal CIDS and the relationship of these CIDs to performance parameters are viewed as valuable pieces of information that can streamline the process for identifying animals that can not only behave, grow, and reproduce in a way that handlers prefer, but may also cope well with the pressures of management. To construct an index for selection, researchers may categorize the distribution of specific behavioral traits (two or more related behaviors) belonging to any dimension of an CID. Indices or scales describing behavioral traits range from 2 to 10 different criteria or levels, having varying degrees of efficacy and validity. By simplifying behavioral traits in this way, researchers hope to translate their findings into applicable tools for farmers. Within sheep literature, referring to sheep as either 'calm' or 'nervous' or dedicating a score from 1 to 5 based on the animal's reactivity to restrictive handling is a common way of scaling one or more dimensions of an animal's CID. This form of scaling is done to handle the qualitative aspect of behavioral traits in a way that can be compared to whatever physiological or biological parameter is of interest. For example, some authors have performed 30 second tests on weight scales to dedicate a value from 1 to 5 (1-calm, no movement; 2-calm with occasional movement; 3-moderate movement; 4-abrupt episodic movements; 5-continuous episodic movements) for each individual animal (Pajor et al., 2010, 2013; Gavojdian et al., 2015). The scores are sometimes further consolidated into calm/ more docile (1 and 2) or nervous/ less docile (3 - 5) groups for easier comprehension. Behaviors that may be collected during testing include open and closed mouth vocalizations, steps/ movement, postural changes and latency to settle. The weight scale is the most feasible method for scoring animal behavioral traits for farmers (Gavojdian et al., 2015; Dodd et al., 2012), however, other restrictive and non-restrictive methods can be used such as assessing flight speeds after restraint (faster time = more averse to handling), agitation in isolation, temperament assessments in a milking parlor, approach or avoidance levels of handler in a yard, laneway or during sensitive periods such as lambing season. Outside these practical approaches, more recent research has reached into human personality work to describe CIDs, using multivariate approaches to grasp at the greater complexities of sheep behavior in less restricted environments. Studies may employ arena or open field tests (OFTs) and labels from these assessments detour from the standard 'calm' or 'nervous' assignment to mention other dimensions of CIDs such as exploration, boldness, activity, response to novelty or sociability.

Assessing CIDs in sheep: restrained resting

Isolation box, flight speed and weight crate or scale tests are common types of restrained assessments used to measure the animal's level of agitation during confined isolation or in human proximity. Since the goal of these tests are to have on-farm practicality over theoretical substance, there are some issues related to the introduced subjectivity within scoring systems and the lack of specificity in behavioral measurements in terms of focusing on behavioral traits related to targeted dimensions of temperament or CIDs. In other words, these approaches may be measuring more than one property of animal CIDs and therefore it is not always appropriate to lump measurements of multiple tests into a single CID dimension or generalize the information from one test outside of its scope. Dodd et al. (2013) discussed this sentiment in a study assessing flight speed and agitation in lambs in relation to carcass quality. Though these two tests are often used to do a more objective assessment of CIDs in sheep, behavioral measurements from the two tests were not correlated, and were therefore assumed to be reflecting different aspects of behavioral reactivity, also in agreement with Brown et al. (2016). Furthermore, they appeared vulnerable to change by environmental effects between different flocks of sheep. Despite the potential for subjective ratings through inter-rater disagreement and non-specificity, relationships to measures collected in these restricted tests and growth parameters in lambs and physiological responses in adult sheep are somewhat repeatable up to 2.5 years (Murphy et al., 1994) and consistent across studies (Dodd et al., 2012) with only some contention (Amdi et al., 2010). Gavojdian et al.(2015) reported that calmer lambs tended to yield increased growth in the first three months and Pajor et al. (2013) found that lambs of calmer temperaments may have improved growth and reduced cortisol, non-esterified fatty acid, cholesterol, and glucose concentrations compared to more nervous lambs at the end of the fattening period. Furthermore, Horton and Miller (2011) reported calmer lambs to have heavier live weights than more nervous lambs in the weight crate. Among adult sheep, Pajor et al. (2010) found that calmer ewes in the weight crate had lower cortisol concentrations compared to their nervous counterparts, as well as offspring with improved weight gain. Studies have repeatedly observed that calmer adult ewes may also have improved milk quality and reduced risk of mastitis relative to their nervous conspecifics (Toth et al., 2017). With these harmonious findings, it is still important to recognize the appropriate scope to which these results may be assumed, as not all findings may extend to any aspect of performance or biological consequences. Zambra et al. (2014) cautions scientists to assess temperament repeatedly, over the animals' lifetime to understand how behavioral responses may change with handling experience.

Assessing CIDs in sheep: unrestrained testing

Abattoirs, stock yards, arenas and open field tests (OFT) are all areas in which unrestrained testing can be performed. Unrestrained tests may or may not involve the presence of a human stimulus (Dodd et al., 2012) or other stimuli that may be perceived as a predator, conspecific, or something ambiguous. Work in abattoirs and stockyards is almost entirely directed towards understanding the impact of handling procedures or ease of handling in sheep. Bonato et al. (2021) has explored sheep CIDs in more practical, unrestrained environments using applied methods for collection. Bonato et al. (2021) assigned a behavioral reactivity level based on ease of handling in a test pen, referring to this assay as a 'docility test'. Scores from the 'docility test' can then be related to practical performance parameters, such as reproductive potential (Bonato et al., 2021). MBS (maternal behavior score) is another practical assessment done in unrestrained environments; however, this score is solely used for understanding aspects of maternal behavior – something that has not been well framed under or associated with dimensions of sheep CIDs (Dwyer, 2008). Other authors have taken more theoretical approaches with unrestrained environments. Beausoleil et al. (2005) and others have performed extensive studies using the arena, which traditionally presents sheep with a motivational conflict to reinstate conspecific contact or avoid a human stimulus, to examine aversiveness of various stimuli, divergent selection for temperament traits (Beausoleil et al., 2008; 2012), stability of temperament (McBride and Wolf, 2007) and variation in behavioral and physiological traits (Yu et al., 2021) in sheep. Though clarity on dimensions of CIDs in sheep is needed, there is not necessarily consistent inter-experimental terminology used or evidence presented that confirms the arena test is measuring the same dimensions of temperament or sheep CIDs every time. In general, this may be due to the structure of the test itself, intentionally eliciting different motivations. Furthermore, Yu et al. (2021) reported behavioral and physiological trait instability over the course of ontogeny in sheep and McBride and Wolf (2007) reported essentially an effect of duration of the test on manifestation of behavioral traits. Further, Wolf et al. (2008) reported evidence of behavioral plasticity between repeated arena tests in certain behaviors, something which is often overlooked. The most prevalent behavioral trait across studies using the arena procedure is a trait or factor related to activity level, typically comprised of vigilance and locomotive behaviors. ‘Activity level may either accompany other traits that manifested in the arena test, or be the sole focus (i.e., more versus less active individuals).

From a practical perspective, behavioral assessments in unrestrained environments have shown less promise with regard to refining selective processes to improve growth and carcass performance, however, some authors have reported valuable relationships between sheep CIDs and immune response and HPA-axis activation (Beausoleil et al., 2012), fertility and lamb survival (Kilgour and Szantar-Coddington, 1995), maternal behavior and rearing ability (Kilgour, 1998) and aspects of handling (Bonato et al., 2021) using the arena test. Behaviors collected within the arena test, such as vocalizations and locomotion, show moderate to high repeatability (Wolf et al., 2008; Dodd et al., 2012) and worth consideration. Within this group of unrestrained testing, it seems that for the number of results supporting relationships with CIDs and various biological consequences, there is a comparable amount of evidence muddying or conflicting these results. Similar issues with restrained testing transcend to unrestrained testing in terms of lack of specificity in measurement collection, with a need to target a limited set of behavioral traits. Studies using the OFT method, in particular, seem to find weak evidence for relationships between behavioral traits and functional consequences such as rearing ability (Kilgour and Szantar-Coddington, 1995) and maternal selectivity in ewes (Bickell et al., 2009). For this reason, it is perhaps inappropriate to generalize results across all forms of unrestrained testing.

Human stimulus in ID assessments

Humans have been used as stimuli to provoke behavioral responses from sheep in both restrained and unrestrained testing environments, either performing a passive or active role (Dodd et al., 2012). For this reason, a satisfactory amount of information can be complied to understand the impact of human presence and proximity on sheep, perception of the human stimulus relative to other stimuli, and behavioral responses to the human stimuli in relation to important management considerations (e.g., handling ease and maternal care). Though it is convenient to apply a human stimulus for eliciting behavioral responses in sheep, interpreting the response comes with a level of complexity above other, more ambiguous stimuli (e.g., box) or biologically relevant (e.g., herding dog) cues. Previous work has referred to the human as a potential predator, however this notion is not supported by later reports of differential responses to dogs compared to humans (Beausoleil et al., 2005; Hemsworth et al., 2011). Humans have a more contextually diverse relationship to this species in that humans may be agents to 'force', 'handle' (Hutson, 2000) or even gentle. Sheep experience a variety of care and interventions under human management, all of which may be perceived as aversive, neutral or positive (Hargreaves and Hutson, 1990). It is generally understood that common handling procedures are associated with differing levels of fear or stress in sheep (Hemsworth et al., 2011), depending on the amount of handling required, wait time and noise levels (Schiller et al., 2020) and amount and speed of movement (Dwyer, 2009) of the handlers. In contrast, certain experiences such as feeding or gentling could be perceived as neutral or positive, especially if it is by an affiliative human handler (Coulon et al., 2015). Given this complex relationship that humans can have with sheep, the actual motivation underlying responses to the human stimuli may be unclear. Despite there being a level of uncertainty to how each population of animals may perceive a familiar or unfamiliar human, there is also a substantial amount of practicality in employing the human stimulus.

From what has been observed in restrained tests discussed earlier in this chapter, it is evident that the experience of restraint in a chute or weight crate by a human is capable of eliciting repeatable, biologically relevant responses in sheep. Unrestrained tests are vulnerable to producing greater variability in behavioral responses since they can be performed in a wider variety of contexts, however specific behaviors such as vocalizations, locomotion and 'human-related' fear behaviors (approach or avoidance) appear to be indicative of CIDs in these assessments (Dodd et al., 2012). Interestingly, there is consistency in the literature which insinuates that individuals who were more active and likely to approach a human in the arena test were those who had lower plasma CORT concentrations (Beausoleil et al., 2008) and were considered bolder or less fearful than their less active conspecifics (Beausoleil et al., 2012). This finding is contrary to previous assumptions that more active individuals are those experienced a greater level of fear or distress in experimental setting. Though habituation is possible through repeated testing, there is evidence that the response to a human stimulus in arena tests can elicit behavioral traits that are stable between tests (McBride and Wolf, 2007), which is an integral assumption of CIDs. Vocalizations in the presence of a human also rank similarly within-subjects across repeated arena tests (Wolf et al., 2008) and should be considered as indicators of IDs in sheep. There is also consistent evidence of behavioral measurements related to movement and travel in the arena being related to lamb rearing ability (Kilgour, 1998) and lamb survival (Kilgour and Szantar-Coddington, 1997). It should be noted that both Beausoleil et al. (2012), and Kilgour et al. (1998), were working with sheep selected for differential activity levels in the arena test and maternal abilities, so the results may be considered circular or expected.

The human stimulus, CIDs and maternal behavior

The relationship between CIDs and maternal behavior is sparse in sheep literature. Evidence of a consistent relationship between the response to human and maternal care is demonstrated through the MBS (maternal behavior score; 1=ewe stays close to lamb during tagging, 5= ewe runs away and does not return during tagging) index developed by O'Connor et al. (1985). This index does have some promise as a tool for selecting ewes that can perform adaptive maternal care (i.e., rear lambs to weaning), yet this scoring system does not measure traditionally explored dimensions of CIDs in sheep. There have been some studies comparing behaviors from restrained and unrestrained tests to maternal behaviors or aspects of maternal performance (i.e., rearing capability), with inconclusive findings. The presumed purpose for investigating the relationship between CIDs in ewes and maternal performance is to predict the ewe's likelihood of success at raising a lamb to weaning. The range of lamb mortality rates across farms is any were from 15 to 50% mortality (Dwyer, 2008), which is astronomical compared to more intensive farmed animal systems. Sources of lamb mortality usually stem from starvation or hypothermia, which may be a consequence of a poor ewe-lamb bond. Strength of attachment of the ewe-lamb bond is variably dependent of the mother's maternal behavior, reflected through maternal responsiveness and selectivity (Poindron and Keller, 2007), time spent at the birth site (Nowak, 1996), and essential behaviors such as licking, grooming and low-pitched bleating. It is evident that there are inter-individual behavioral differences in how ewes express maternal care with their offspring, however, the relationship to maternal differences and individual differences outside of the lambing season need more attention. Kilgour and Szantar-Coddington (1997) have found that it is possible to predict rearing success in ewes by observing behavioral measurement in the arena test with a human stimulus with ewes that have been selectively bred for that exact performance outcome. Other studies that have performed tests on selected or unselected ewes have produced minimal evidence supporting a relationship between adaptive maternal performance in ewes and CIDs observed outside of lambing season. Behavioral measurements from arena and box tests were minimally related to maternal behaviors such as time spent licking the lamb, time at birth site, and distance the ewe retreated from the lamb during observation periods (Murphy et al., 1994), unlike late reports that demonstrated the opposite relationship (Murphy, 1999). Bickell et al. (2010) found maternal behavior of extensively raised ewes to also be moderately related to dimensions of ewe CIDs. CIDs in this dissertation were determined by reactivity to humans in the arena test and scores generated from movement and vocalizations in the isolation box test. Authors found that calms ewes tended to stay on the birth site and lick their lambs for longer durations compared to nervous ewes, however, there was no difference in lamb mortality reported between the two lines. Bickell et al.

(2009) reported no difference in bond establishment between ewes of a calm or nervous temperament. Alternatively, Peeva et al. (2009) found that calmer ewes expressed more aggressiveness towards alien lambs and allowed their own lamb to have greater durations of udder access compared to nervous ewes. Brown et al. (2016) also observed adaptive maternal traits associated with ewes categorized in the calm group. They reported that ewes with slower flight times had a significantly negative genetic relationship maternal behavior scores (reactivity to a handler at tagging; 1= good, 5=poor). The authors concluded that maternal behavior and temperament could be improved by targeted selection (Brown et al., 2016). The inconsistencies in this area of research reveal the need for more standardized comparisons between ewe CIDs and maternal behavior.

Dissertation Objectives

The overall objective of this dissertation is to assess the quality and consistency of sheep response to a human stimulus during pre- and post-natal experiments and evaluate the relationship of such responses to indicators of lamb outcome. I will assess the quality and consistency of unrestrained responses to a human stimulus when the lamb is not present (Chapter 2); the quality and consistency of unrestrained responses to a human stimulus during and after lamb processing in relation to indicators of lamb outcome including birth weight, growth rate and weaning weights (Chapter 3); and the relationship between responses towards a human stimulus when the lamb is and is not present in addition to their relationship to indicators of lamb outcome (Chapter 4).

CHAPTER 2

Varying degrees of human-animal interactions elicit weak evidence of a temporally stable behavioral trait in rangeland breeding ewes

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Abstract

Coherent patterns of inter-related behaviors that occur across time and contexts, also known as personality or consistent individual differences (CIDs), are important to identify among livestock as they carry valuable information for selective breeding purposes. Repeated testing is necessary to reveal behavioral patterns, yet temporal stability of behavioral traits is often assumed. In this study, three human-animal interaction (HAI) tests were employed within a flock of sheep maintained in the Sierra-Nevada foothills to assess intra-individual consistency and inter-relatedness of behavioral responses. Ten groups of five ewes (n= 38 - 51) were placed in three HAI tests (restrained Human Contact, unrestrained Human Presence and unrestrained Human Approach) at the time of post-breeding, gestation and weaning (3x per year; over 2 years). Composite traits were created using principal component analysis (PCA) of behaviors from the unrestrained Human Presence test and were compared to the behaviors from the two other HAI tests to evaluate cross-contextual relationships. Frequency of 'environmental vigilance' (EV), 'investigating fence' (IF) and duration of 'head down' (HD) were the only variables to achieve a moderate repeatability estimate [EV: 0.32 ± 0.09, [CI (0.21, 0.48])]; IF: 0.29 ± 0.13, [CI (0.14, 0.48)]; HD: 0.44 ± 0.15, [CI (0.22, 0.68)]. PCA revealed a single component in all Human Presence tests that was characterized by loadings (>0.45) for duration of 'grazing' and 'environmental vigilance'. Spearman's rank order analysis revealed no significant correlations between PC1 of each replicate. There was also no significant inter-relatedness identified between behaviors of each HAI test. Taken together, it is suggested that the rangeland ewes of this study exhibited consistency of some individual behaviors within the Human Contact and Human Presence test, however, did not demonstrate a temporally stable trait that can be generalized across multiple contexts. Future work should evaluate the influence of extensive versus intensive management styles on the development and maintenance of human-oriented behavioral traits.

Keywords: Sheep, temperament, human-animal interactions, consistent individual differences

1. Introduction

The existence of correlated behavioral and physiological responses during human-animal interactions (HAIs) that reflect a certain consistent, inherent quality in lambs and ewes has been a thoroughly explored topic in the last 10 years. A common goal of gathering potentially inter-related behavioral and physiological measures among livestock is to describe a trait (two or more related behaviors/ biological markers) that may be associated with health (Toth et al., 2017), performance (Dodd et al., 2014; Sart et al., 2014) and the animal's ability to cope with aversive stimuli (Finkemeier et al., 2018). Theoretically, expression of these responses are stable or may be up- or down- regulated depending on environmental conditions (Dingemanse and Wolf, 2013), across time. This framing process involves the use of an umbrella personality paradigm (i.e., sociability, aggressiveness, docility, exploration, novelty) yet may also branch into coping styles (proactive vs. reactive) or something related to a different aspect of personality, like temperament (e.g., docility) or behavioral syndromes (i.e., relationships between behavioral traits). Research involving livestock typically frames the individual differences when exposed to a human under experimental (arena, IBT) and applied (milking parlor, scale, yard) conditions as that of their ‘temperament’, ‘docility’ score, or ‘coping style’. Recent authors such as Cakmakci et al. (2022), Atkinson et al. (2022) , Beausoleil et al. (2012 and 2015), Yu et al. (2021), and others, have explored the existence of individual differences in ‘temperament’ and ‘coping styles’ in the presence of a human stimulus using unrestrained (arena, open field) and restrained (isolation box, scale) tests, finding varying degrees of between- and within-measure relationships. Based on the results, each animal is assigned a trait category that varies on a spectrum, ranging anywhere from a ‘nervous’ to ‘calm’ behavioral type, or in a dichotomy as having a ‘proactive’ or ‘reactive’ coping strategy. The behavioral categories are inferred to gauge the animal’s ability to cope with or manage intensive or infrequent interactions with a human handler and be used for selective purposes.

Respective to sheep literature, ‘proactive’ or ‘more active’ individuals are those that display a greater magnitude of behavioral responses such as vocalizations and locomotion (Aydogdu and Karaca, 2021), explore more and have a greater propensity to take risks or approach a human stimulus, while ‘reactive’ or ‘less active’ individuals tend to take fewer risks, and exhibit reduced vocalization, exploratory and locomotory behaviors. These behavioral types seem to be observed more distinctly in open environments where risk taking, exploration, and boldness can be assessed (Aydogdu and Karaca et al., 2021; Cakmakci et al., 2022; Yu et al., 2021). In juxtaposition, sheep labeled with a ‘calm’ temperament may be those that perform reduced movement and vocalizations when restrained by a human or isolation, compared to ‘nervous’ individuals (Pajor et al., 2010; 2013). While there is some consistency in the literature that ‘calm’ ewes in restrained tests display preferred maternal care (Aydogdu and Karaca., 2021; Brown et al., 2016; Peeva, 2009) such as, a longer duration of licking and grooming of their lamb and time spent on the birth site, and have greater success with raising and weaning lambs (Pajor et al., 2008), the evidence of maternal performance being related to behaviors performed in an unrestrained testing environment is not as coherent (Aydogdu and Karaca., 2021; Dodd et al., 2012). Moreover, some authors have observed the expected relationship with ‘coping styles’ and HPA axis-activation, with ‘reactive’ individuals having greater activation measured by serum cortisol concentrations compared to ‘proactive’ individuals (Aydogdu and Karaca., 2021; Yu et al., 2021) after human exposure, however, a number of authors have reported no differences in cortisol concentrations between these individual types in sheep (Beausoleil et al., 2012; Cakmakci et al, 2022) when assessed in an unrestrained environment. Unrestrained and restrained environments have also been used to observe the relationship between inter-individual variability and immune competence in ewes (Toth et al., 2017) and lambs (Zhang et al., 2021), however, Schiller et al. (2023) found there to be no association between response in restraint and open environments to cell and antibody mediated responses. Considering these discrepancies, revealing patterns of consistent individual differences in both contexts of open and restrained environments is essential if we aim to detect associations to the stress response or biological outcomes. The current lack of evidence of salient relationships between behaviors exhibited by sheep in open and restrained environments when exposed to a human have implications for welfare on farm settings where the ability of sheep to cope with multiple daily stressors (e.g. shearing, foot trimming, vet care, loading, moving and sorting, etc.) is inherently linked to the individual's response those challenges. Moreover, patterns of response that overlay HPA-axis activation can indicate individuals that may be more or less suitable under human management.

The objective of the current study was to use various degrees of human-animal stimuli (contact, presence, and approach) under different contexts (restraint versus non-restraint) to characterize the existence of one or more behavioral traits by exploring 1.) repeatability of singular behaviors from three human-animal interactions that may contribute to a behavioral trait and 2.) inter-relatedness of behaviors from the three human-animal interactions to characterize a behavioral trait. The authors predict that unique patterns of individual behavioral responses will arise across the various testing environments such that ewes will exhibit greater reactivity (determined by clusters on inter-related behaviors) in one context (i.e., restraint) will consistently perform the same magnitude of response in a separate condition (i.e., non-restraint). This hypothesis is informed by findings from Beausoleil et al. (2012) reporting domain general responses in ewes subjected to varying stimuli over time. The authors also predict that responses between coinciding periods of the year will be temporally stable (e.g., between weaning events, pre-breeding events, and post-breeding events), informed by the understanding that ewes go through significant neuroendocrine and morphological modification during gestation and after lactation, that is unique to that time of year, and can have consequences on expression of consistent individual differences (Biro and Stamps, 2010).

2. Methods

2.1 *Animals*

The current study was approved by the University of California, Davis Institute of Animal Care and Use Committee (protocol# 20926) and conducted at two distinct sheep handling sites (Blue Oak Ranch [site A] and Belmantro Station [site B]) located within the Sierra

Nevada foothills in Auburn, California, USA. The study flock consisted of terminal Shropshire (n=20; terminal line), Blue-faced Leicester x White faced crossbred ewes (n=20; replacement line), and Blue face Leicester x Mule crossbred ewes (n=20, terminal line) kept as a subset of a greater flock of 120 individuals. Prior to the data collection, study ewes were selected based on their breeding group. Due to the longitudinal structure of the study, a mixture of primiparous and multiparous Shorpshire and Blue-faced-Leicester x White breeding ewes were selected between the years 2018-2019 as this set of breeding ewes were likely to be maintained for at least two consecutive years. Between the years 2019-2020 an additional (n=20) primiparous terminal Mule ewe from the terminal line were entered into the study to supplement for ewes that were culled due to either age, reproductive or other health related issues.

Study ewes were maintained together on range as a single breeding group with *ad libitum* access to forage on pasture and restricted access to protein supplements prior to the breeding season (August – September 2019, 2020) for flushing. For the first year of the study, ewes were fed alfalfa on irrigated pasture for flushing, and in the second year given a mixture of dry cob, corn oats, barley, and chia seed (1.25lbs/ head/ day) for flushing. All ewes were exposed to approximately 190 acres of total rangeland throughout the entire year, with rotation occurring every 5 days within and outside of the lambing season. During the lambing season (February – March) ewes were rotated across 9 different paddocks, ranging from 4 to 15 acres of available space each. Outside of lambing season (June – February) ewes were moved across 62 separate paddocks. Each year of the study ewes were introduced to rams (30:1 ratio) for natural cover in the beginning of October and the rams were removed in November. To reduce predation, ewes were kept with two guardian dogs and protected by electric fencing. During moving and husbandry events, shepherds used low-stress handling techniques that included guiding the flock with guardian dogs to be rotated in combination with applying pressure-release strategies with herding dogs to facilitate movement. To avoid introducing unnecessary stress, shepherds refrained from the use of loud vocal cues or livestock prods while moving and handling animals. Outside of husbandry events, ewes were turned out on rangeland with a shepherd visiting the paddocks daily to feed the guardian dogs.

Six human-animal interaction (HAI) replicates were conducted across roughly 2.5 consecutive years starting in January of 2019 and ending in June of 2021. Study ewes were tested at one of two handling sites, determined by stage of production cycle, with each handling site consisting of a holding pen surrounded by wire fencing that contained a wooden Bud-Box developed for low-stress moving in sheep and cattle. All ewes were previously familiarized with both handling sites during common husbandry procedures that included drenching, foot-trimming, and body condition scoring. Initial dates of HAI replicates within the study were determined by the shepherd's management schedule to allow researchers to emulate the flocks standard level of exposure to humans. The current study consisted of three replicates per year conducted over two successive years for a total of 6 replicates per animal. The first replicate of each year was conducted during the post-breeding phase when rams were pulled from the ewes (November), the second replicate of each year took place during gestation when ewes received perinatal booster shots (January), and the third replicate was conducted at weaning time, when lambs were pulled from the flock (June) (Figure 1). The post-breeding and gestation replicates were performed at site A (Blue Oak Ranch) and the weaning replicates were performed at site B (Beltramo station). Temperatures during the post-breeding trials ranged between 12.8 °C and 18.3 °C at 0700 – 1400 hrs. At weaning time, temperatures ranged from 16.7 – 32.3°C at 0700 – 1400 hrs. Additional (n=20) ewes were entered into the study during the first year's weaning trail to supplement for any animals that needed to be pulled from the study flock. Unfortunately, data from the first year's Human Contact test at gestation was lost and all data from the second year's weaning event was not able to be used due to extreme heat (>32.2°C) affecting the ewes behavior. During this trial sheep began to perform open-mouth panting, and there was a collective decision to stop the trial. Upon completion of the study twenty-eight ewes were present for all replicates (excluding the sixth replicate) and had complete data sets.

2.2 *Experimental setup*

All ewes in the study were subjected to three tests, which were conducted in the same order during each replicate: (1) Human Contact test: ewe receives human contact within a 0.5m x 8m raceway followed by a (2) Human Presence test: ewe is subject to a stationary human stimulus within a 10.5m x 10.5m modified open field test (mOFT) and finally an (3) unrestrained Human Approach test that also took place in the mOFT. For each trial, three unfamiliar humans (A, B and C) were assigned to be one of the human stimuli for either Human Contact, Human Presence or Human Approach test. A new group of researchers would be on-boarded for each replicate to limit the potentiality of ewes responding based on familiarity as opposed to general human contact, presence, or approach.

Before each HAI replicate, the entire flock, made of study and non-study ewes (120 animals total), were gently pressured from a holding pen 1 (9.1 x 9.1) into the Bud-Box pen (3.3 x 2.7m) by two herding dogs with 15 - 20 sheep entering the Bud-Box at a time (Figure 2). From the Bud-Box, the ewes were ushered into groups of five in single file into the raceway portion of the handling system. Within the raceway, ewes were confined by (~ 1m tall) wooden vertical boards that allowed them 270-° vision, including into holding pen 2 where sheep were still being held. Due to the raceway being oriented towards an open and familiar pasture, ewes typically moved freely into the raceway with minimal encouragement. At this time pre-selected study ewes were marked with livestock spray distal to the shoulder and proximal to the tail end with their assigned visual identification numbers to be seen on camera for behavioral analysis. Individuals that had an even electronic identification number (EID) on their ear were marked with a large number using blue spray paint, and individuals that had an odd electronic identification number were marked with a large number using red paint. After all study ewes were marked, they were released into holding pen 2 while non-study ewes were released out of the exit gate at the end of the raceway and moved into a separate paddock.

2.2.1 *Restrained Human Contact test.*

The study ewes (those that had been moved into the holding pen 2) were moved once more into the Bud-Box (15-20 animals at a time) prior to the start of the Human Contact test (Figure 2). At the start of the Human Contact test, study ewes were moved for the second time into the raceway. Human A was positioned approximately 8 m perpendicular to the last sheep in the raceway before approaching this last individual animal and applying light pressure under the muzzle with one hand and light pressure on the top of the rump with the other hand for 10 seconds. Human A was given prior practice using non-study ewes on the adequate amount of pressure to apply for this treatment and to release the ewe if she took > 2 steps forward or backward, or if she crouched in the raceway making it hard to sustain physical contact. If the ewe broke free during the contact treatment, Human A was tasked with attempting to reinstate contact, within the 10 second time period. If Human A could not reinstate contact, the restraint test was not extended. Human A would then move to the next ewe (in front of the ewe that had just received the contact treatment) until all five individuals in the group had received the contact treatment. During the "Human Contact" test, study ewes that had not received the 10 sec handling treatment yet were able to hear vocalizations or movement from the ewes behind them that were receiving handling.

Each Human Contact test was recorded on camera by a researcher positioned behind (1m) Human A, and when all ewes in the raceway had received the treatment, they would be let out of an exit gate at the end of the raceway to be entered directly into the mOFT. The shepherd would once more fill the raceway with five ewes, single file, after the previous set of ewes were done with the five-minute (mOFT). For each trial Human A wore dark jeans and a dark blue or black long sleeve shirt to minimize the incidence of ewes responding to color compared to the human stimulus. Video data (Sony Handycam DCR SX85; Sony Corporation of America, New York, NY, USA) was analyzed for frequency of turning around, kneeling, stepping with right front foot (for standardization), vocalizing, head position changes (above, at or below shoulder line) throughout the entire restrained test (Table 1).

For the Human Contact tests, interobserver reliability was established amongst 3 observers prior to behavioral annotation of video data using The Observer XT v. 11 (Noldus Information Technology, Wageningen, Netherlands). Observers were trained on ~5min worth of video from two Human Contact tests. To assess reliability, each observer was tasked with coding two different videos they had not yet seen, each containing five animals being tested in the raceway. Using data the observers had coded, interobserver agreement was established (Cohen's kappa = 0.80) on durations of 'head up', 'head down' and frequency of 'stepping'. Duration of 'kneeling' and frequency of 'vocalizing' and 'turning around' were not used as they rarely occurred.

2.2.2 *Unrestrained Human Presence test (mOFT)*

After the last ewe of each group passed the raceway exit gate and entered the mOFT, the Human Presence test would begin. The mOFT was constructed prior to each trial using eight wire fence panels (1m x 5.3m) secured together with black paracord and positioned upright with T-posts at each junction (Figure 3). Four camcorders were positioned in each corner and secured approximately 2m from ground level using tripods and recorded footage continuously for the duration of each test. The mOFT ground was mowed and visually partitioned using red spray paint into 25 boxes (2.1m x 2.1m), each associated with either zone 3 (box with human B), zone 2 (intermediate boxes touching zone 1) or zone 1 (boxes peripheral to zone 2, touching fence line). The mOFT was partitioned in this way to assess distance traveled during the test, inter-individual proximity, and proximity to the Human B. After the Human Contact test, Human B stationed themselves in the mOFT before the sheep entered and was tasked with standing motionless in the center, facing the raceway exit gate, for the duration of each 5-minute Human Presence test. Video data were annotated for duration of vigilance (directed at human or environment), 'walking', 'grazing', 'lying down', 'investigating human', 'investigating fence' and frequency of 'head-butting', displacement (initiator or receiver), 'stomping', and 'vocalizing' (Table 1).

For the Human Contact tests, interobserver reliability was established amongst 12 observers prior to behavioral annotation of video data using The Observer XT v. 11 (Noldus Information Technology, Wageningen, Netherlands). Observers were trained on thirty minutes worth of video using a single Human Presence test clip from one trial. For training purposes, each observer was tasked with coding behavior on each of the five ewes within the test they were assigned. To assess reliability of data from the Human Presence test, each observer was tasked with coding for two different ewes, which typically took another thirty minutes per animal.

Using data the observers had coded, interobserver agreement was established (Cohen's kappa = 0.80) on all behaviors listed in the ethogram with exception of frequency of 'head butting', 'displacement' and 'vocalizing' as these behaviors rarely occurred.

2.2.3 *Unrestrained Human Approach test*

At the conclusion of the unrestrained Human Presence test, Human C was signaled to approach the testing area at a steady pace (~ 2 steps/sec) and untie the exit gate for sheep to be released into a familiar home paddock. Human C then entered and circled the mOFT in a counterclockwise direction to encourage the sheep to exit the testing area. Human C circled the arena until all sheep exited (typically taking 10 – 45 seconds). This human was directed not to contact the sheep, make vocalizations or clap while in the mOFT. Video data were annotated for latency to 'look at human' (Human C), 'step', and 'leave the arena' (Table 1). Within-observer reliability was assessed using a single observer for the Human Approach test, simply due to time constraints. The single observer coding for the Human Approach test worked with practice video from two separate tests, each taking about thirty minutes to analyze. Intra-rater reliability (Cohen's kappa = 0.80) was assessed on latency to 'step', latency to 'look at human' and latency to 'leave arena'.

2.3 *Statistical Analysis*

Using R Statistical Software Version 4.2.1 (R Core Team 2018), repeatability of singular behaviors was explored using the *rptR* package (Stoffel et al., 2017; 2019). Due to animal drop-out and addition (at weaning time), the sample size for each trial was varied (Year 1: post-breeding (R1: n = 39), gestation (R2: n = 38), and weaning (R3: n = 51); Year 2: post-breeding (R4: n = 46), gestation (R5: n=44)). For assessing repeatability in the Human Contact test, the models included a term for 'Replicate' that consisted of data from the first year's post-breeding

(R1) and weaning event (R3), and the second years post-breeding (R4) and gestation event (R5) for a total of four replicates. As stated earlier, data was lost and dropped from the first year's gestation trial and the second year's weaning trial. To assess repeatability of singular behavioral measures from the Human Presence and Human Approach tests, the models included data from the first year's post-breeding, gestation, and weaning trial and the second year's post-breeding and gestation trial for a total of five repeated trials. The *rptR* package is useful when exploring repeatability with non-gaussian (Poisson) distributions (Stoffel et al., 2017). Most variables showed a negative binomial or Poisson distribution. For repeatability assessments, durations and frequencies of behaviors were fit as the response, with 'Replicate' as a fixed effect and individual (ID) and group membership as a random effect. Data were also fit with a fixed effect of breed, pregnancy status and weaning status, and a covariate term for age when required. Models were also either fit with a log or sqrt link function and the nonparametric bootstrap method was used to assess confidence intervals.

Example model: Y = Replicate + Breed + Age + … + (1|ID) + (1|Group)

After assessments of repeatability, authors wanted to explore for co-variation amongst behavioral variables to see if two or more behaviors changed across time together (i.e., behavioral trait). Data that were found to be over dispersed due to zero-inflation were not included in repeatability estimates since there is currently no acceptable method to assess repeatability and accurate confidence intervals of a zero-inflated random effect using linear mixed effects models. Multivariate hierarchical linear mixed effects modeling for repeated measures using the *MCMCglmm* (Houslay and Wilson, 2017) or brms packages in R software were considered to assess covariation of behavioral variables. Due to zero-inflation, multivariate approaches using the *MCMCglmm* and *brms* package were deemed inappropriate. Though data in this study had a log or sqrt transformed Poisson error or negative binomial distribution, most multivariate mixed modeling approaches are equipped to handle gaussian error distributions with a limited number of zeros. Therefore, authors used the *glmmTMB* (Brooks et al., 2017) package in R software that can handle Poisson or negative binomial distributions in the response variable and multiple random effects with restricted effects maximum likelihood (REML) to gather residual data, controlled for the fixed effect of trial, pregnancy status, weaning status, breed and the random effect of group membership. This package can also allow for an autoregressive component of repeated random effects of individual sheep (Replicate | ID). Residual data from the *glmmTMB* (Brooks et al., 2017) was then used for principal component analysis, similar to what was done in Diess et al. (2012).

Following residual data collection, principal component analysis (PCA) was used to describe clusters of related behaviors within trials for the unrestrained Human Presence test, and Spearman's rank order correlations were used to explore intra-individual consistency of components and relatedness of behaviors from the other two human-animal interaction tests. Behavioral variables including durations and frequencies to 'vocalize', 'headbutt', 'displace', 'investigate human' and 'stomp' in the unrestrained Human Presence test, and frequency to 'turn around' and 'kneel' in the restrained Human Contact tests were rarely performed and therefore excluded from analysis.

2.3.2 *Principal Component Analysis*

Data from the restrained Human Contact and unrestrained Human Approach test were not entered into PCA since variables were not considered entirely mutually exclusive from one another, nor did they appear to have patterns of inter-relatedness within individuals according to preliminary assessment using spearman's rank order correlations. Data was ascertained to be adequate for clustering using the Bartlett's test of sphericity ($P<0.05$) (Tobias and Carson, 1969) and the KMO (Kaiser-Meyer-Olkin) index, as a measure of sampling adequacy (MSA), which must be equal to or exceed (0.6) to be considered acceptable. Biplots were displayed to observe the contribution of each behavioral variable to the principal components. Principal components with eigenvalues exceeding 1 and identified to be at the 'break point' on the scree plot were retained for interpretation.

To investigate manifestation of behavioral traits, loadings of specific behavioral variables to the principal components were observed and considered to be associated with the factor if their loading was greater than 0.4 and had the highest amount of association relative to other components (Table 3). Ewes received a score for each principal component using the least squares regression approach. Regression factor scores predict the location of each individual on the component. This standardized method produces scores similar to a Z-metric, where values range from approximately -3.0 to 3.0. Relationships between principal components of the unrestrained Human Presence test and behaviors from the restrained Human Contact and unrestrained Human Approach test were assessed using Spearman's rank order correlations with alpha set to 0.05.

3. Results

3.1 *Repeatability of individual behaviors from the three Human-Animal Interaction tests*

Repeatability estimates were performed on data demonstrating a gaussian, Poisson or sqrt/log transformed Poisson distribution, including duration of 'head down' and 'hands on' in the Human Contact test and duration/frequency of 'environmental vigilance', 'walking', and ‘investigating fence’ in the Human Presence test (Table 2). Frequencies of ‘investigating fence’ (0.29 SE ± 0.13; CI [0.14, 0.48]), ‘vigilance in the environment’ (0.32 SE ± 0.09; CI [0.21, 0.48]) and ‘head down’ in the raceway (0.44 SE ± 0.15, CI [0.22, 0.68]) were the only variables to achieve a moderate repeatability estimate (>0.3, Turner et al., 1969). Other variables such as duration ‘grazing’, ‘environmental vigilance’, and duration of ‘head up’ had a lower repeatability range between R= 0.10, SE ± 0.06 to R= 0.21, SE ± 11. Behaviors such as duration of ‘walking’, ‘looking at the human’ and ‘hands on’ had even lower repeatability estimates 0.01 SE ± 0.03 - 0.04 SE ± 0.05 (Tables 2) .

3.2 *Principal component analysis from the unrestrained Human Presence test*

Residual data from the linear mixed effects models with a fixed effect for replicate, random effect for ID and group membership and an autocorrelation structure (when required) were used to explore inter-relatedness of variables within the Human Presence test. Preliminary analysis using Spearman’s rank order correlations revealed a potential pattern of inter-relatedness between behaviors within the unrestrained Human Presence test that was not identified amongst behaviors from the other two human-animal interaction tests, therefore, authors used mutually exclusive behavioral variables from this test to explore for a latent trait using five behaviors (duration of ‘grazing’, ‘vigilance at human’, ‘vigilance in the environment’, ‘walking’ and ‘investigating fence’). The second-year weaning trial (R6) is excluded from analysis due to extremely warm conditions interrupting normal behavioral performance. For all replicates, Bartlett’s Test of Sphericity reached statistical significance ($p<0.05$). For replicates 2 - 5, meaning the first year’s gestation trial (R2), first years weaning trial (R3), second years post-breeding trial (R4) and second years gestation trail (R5), KMO = 0.60. The first years post-breeding trial (R1) did not achieve an appropriate KMO value, hence results from this replicate were omitted. The first component that exceeded an eigenvalue > 1 for replicates 2 – 5 explained 37.6 – 59.3 % of the variance of the five behavioral variables used. The PC that manifested for replicates 2 - 5 is generally characterized by having a loading greater than ±0.45 for duration of 'grazing' and 'environmental vigilance'. The composite trait manifesting from gestation in the first year (R2) can be described as having a negative loading for 'environmental vigilance' (-0.52) and a positive loading for grazing (0.50) while weaning time in the first year (R3) can be described as having a positive loading for 'environmental vigilance' (0.47) and 'vigilance at human' (0.53) and a negative loading for grazing (-0.56). Post-breeding and gestation events in the second year (R: 4 and 5) also had a negative loading for grazing (>-0.45) and positive loadings for 'environmental vigilance (>0.55). The second-year post-breeding event also had a positive loading for 'vigilance at human' (0.53) and R5 also had a positive loading for 'walking' (0.50) (Table 3). After correcting for multiple correlations there were no significant relationships identified between replicates for the 'alertness' response.

3.3 Inter-relatedness of behavioral variables from the three Human-Animal Interaction tests

Spearman's rank order correlations were performed to investigate relationships between PC 1 (of replicates 2 – 5) from the unrestrained Human Presence test and behaviors from the restrained Human Contact and unrestrained Human Approach test. After correcting for multiple correlations, not significant relationships were found between behaviors of different tests. Though insignificant, there was a positive trend between latency to 'look at human' was and PC 1 from the first-year post-breeding phase (R2, r_s = -0.39, P=0.06) and within the second-year pre-breeding phase (r_s = -0.28, P=0.07) (Figure 5) .

4. Discussion

Categorizing consistent individual differences (CIDs) in animals is helpful for identifying individuals that may incur fitness or biological consequences that could be detrimental or beneficial to their own health (e.g., chronic stress causing inflammation) or welfare (e.g., stress or anxiety during handling events leading to animal injury) under human management and within farming operations. Before being able to firmly ascertain the existence of a biologically relevant trait (two or more covarying responses) in sheep, more understanding is needed on the expression of and relationship between behavioral variables across varying contexts. For example, some studies have reported that individuals that move and vocalize more frequently during restraint are those that are more 'nervous' or 'anxious' sheep (Gavojdian et al., 2015; Pajor et al., 2008; Doyle et al., 2015) and have increased cortisol concentrations relative to less active individuals (Pajor et al., 2013), while others have reported that less movement in restraint and isolation is associated with more stress indicated through higher serum cortisol levels (Schiller et al., 2023) or no relationship at all (Rietema et al., 2015). The underlying arousal state of individuals that move frequently during an open arena test is also up for debate. Beausoleil et al. (2012) found serum cortisol levels to be similar between more active and less active sheep in an arena test while Aydogdu and Karaca (2021) and Beausoleil et al. (2008) found that sheep that moved and vocalized less had higher cortisol concentrations. Though less movement and vocalizations could be related to increased propensity to become stressed during a challenging event, Zhang et al., (2021) found individuals who move less have a decreased likelihood to experience oxidative stress and inflammation, contradicting Aydogdu and Karaca (2021) and Beausoleil et al. (2008) since greater levels of circulating cortisol is assumed to lead to oxidative stress in animals. To further confuse the story, Murphy et al. (1994) found that response in restraint (movement) and response in an open arena (movement and vocalizations) are unrelated, meaning these tests could be eliciting different CID traits in sheep. To approach these areas of inquiry, the current study aimed to (1) evaluate within-subject repeatability of behavioral responses to a varying gradient of human-animal stimuli (contact, presence, and approach) under different contexts in order to help characterize the existence of one or more temporally consistent and cross contextual (restraint versus non-restraint) behavioral traits.

Several recent studies have investigated behavioral traits among sheep in unrestrained arena tests and restrained isolation box tests (IBT), reporting that single behaviors, such as vocalizations and locomotion in the arena (Cakmakci et al., 2022; Yu et al., 2021; McBride and Wolf, 2007) and agitation in the IBT, are repeatable (Atkinson et al., 2022; Cakmakci et al., 2022) and that inter-relatedness of behaviors from open field tests (i.e., zones crossed, vocalizations), risk-taking measures from an arena (i.e., movement) and also markers of arousal after testing (cardiac activity and serum cortisol concentrations) can change across time (Yu et al., 2021). CIDs that are expressed despite a changing stimuli and time have only been identified by Beausoleil et al. (2008; 2012) using an arena test. Beausoleil et al. (2008, 2012) selectively bred sheep for locomotor and vocal activity based on the outcome of arena tests and found that offspring display similar levels of reactivity (zones crossed, vocalizations) as their parents over subsequent rounds of testing, and that these responses were consistent even when the eliciting stimulus was changed. Though Zhang et al. (2021) did not look at the relationship of responses across different stimuli or context, they did find that frequency of zones crossed, and vocalizations were positively correlated in the arena, perhaps reflecting consistent individual differences (CIDs). This co-variation in locomotor and vocal activity in the arena is the only obvious evidence for a domain-general and possibly biologically relevant trait in sheep occurring within an open environment, to the authors' knowledge. Beausoleil et al. (2012) termed this behavioral consistency in the arena a general temperament trait characterized by 'high' activity, and present across differing stimuli. Outside of the findings of Beausoleil et al. (2008; 2012), the existence of domain general traits is unknown.

Characterizing consistency and up- or down-regulation of inter-related behaviors across multiple contexts would indicate an inherent quality of the ewe that can be observed under one condition and may hold information about the same individuals under another condition, possibly reflective of a CID and/or biological correlate. This is an important consideration under livestock production settings where being able to target sheep for certain biological or performance outcomes based on behavioral performance can be used as a management and selection tool. Though the current study found evidence for within-subject repeatability of individual behaviors (similar to Atkinson et al., 2022 and Cakmakci et al., 2022) for frequency of 'investigating fence', 'environmental vigilance' in the Human Presence test and duration of 'head down' behavior in the Human Contact test, there is weak evidence for the existence of a trait occurring in response to all three types of human stimuli. With this said, more investigation is needed to understand if the singular repeatable behaviors that do indicate individual differences in sheep (frequency of 'environmental vigilance', 'investigating fence' and duration of 'head down') may be related to biological outcomes such as immune performance, arousal state (i.e., cardiac and catecholamine activity) or maternal care and offspring outcome. Schiller et al. (2020) found that responses in restraint (frequency of steps, vocalizations) are repeatable, significantly correlated and related to arousal thresholds (heart rate, ocular temperature) in ewes, suggesting that response in restraint can indicate a CID and is related to propensity to become excited during human contact. If in fact frequency of 'environmental vigilance', 'investigating fence' and duration of 'head down' are related to biological and arousal correlates, they should be included in management and selection decisions to potentially improve herd performance and potentially welfare.

4.1 *Repeatability of HAI responses*

Frequencies of 'environmental vigilance' and 'investigating fence' in the Human Presence test, and duration of 'head down' in the Human Contact test were considered moderately repeatable according to their repeatability estimates. Repeatability estimates represent variation in the behavioral measure explained by the within-subject differences relative to variation explained due to the environment and between-subject differences (Wolak et al., 2013). Repeatability estimates are useful for understanding how the animal will behave across varying environments and can also be used to assess heritability (Boake et al., 1989). After summarizing the data from the unrestrained Human Presence test using PCA, results indicated weak evidence for a transient behavioral response that was related to attention paid towards environmental stimuli in an open setting in two out of the five trials. Of the five behavioral variables entered, duration of 'grazing', 'environmental vigilance' and 'vigilance at human' had a considerable loading to each PC 1 from replicates 2 – 5. Authors of the current study consider PC 1, consisting of 'environmental vigilance', 'grazing' and 'vigilance at human' to be potential evidence of an 'alertness' response in an unrestrained environment, however, greater repeatability estimates for these behaviors are needed to indicate a temporally stable trait. Evolutionarily speaking, sheep are a prey species, reliant on predator protection strategies including the "many eyes" (Lima, 1990; 1995) and "dilution" (Delm, 1990) effect. As a group-living animal, they perform these mechanisms with conspecifics to be able to graze and reduce the likelihood of being predated. The 'alertness' response could be explained within one or both of these evolutionary adaptive mechanisms, exceedingly so when the sheep are tested in groups as compared to isolation when these mechanisms are impractical.

Expression of CIDs in animals may demonstrate plasticity across time and in terms of this study and it is possible that there was a lack of repeatability in durations of ‘grazing’ and vigilance behaviors due to allostatic changes, including going into gestation and preparing for parturition. Biro and Stamps (2010) state that CIDs are, amongst other factors, regulated by energy acquisition and metabolism suggesting that this is in part a state dependent response due to the ewes gestating and preparing for pregnancy. For example, at times of greater energy acquisition (ingesting more food) such as in the winter/spring for this population of sheep, it is expected that they would perform greater durations of ‘grazing’ behavior since they need more fuel for the metabolic system to function, support a growing fetus and store energy for lactation. Interestingly, Vierne and Bouissou (2001) reported that fear reactions during pregnancy were reduced in response to isolation, however, not in response to a human stimulus (glances at stimulus, vocalizations, locomotory activity, willingness to feed).

Lack of repeatability in vigilance behavior could also be a result of the ewes orienting their attention on the separation process from their lambs than on feeding. Abrupt weaning can be stressful for ewes and lambs, so this response could be related to an increase in cortisol levels post-separation compared to a slower weaning process. In the current study, durations of ‘vigilance at human’, ‘grazing’ and ‘environmental vigilance’ did load onto PC 1 from each replicate, however, these variables had rather low repeatability estimates, calling into question their contribution towards a stable trait in ewes. Another explanation for variance in response to humans could be individual differences in perception of the human. The current study’s population of sheep, like many, have varying degrees of interactions with humans that may range from positive (e.g., feeding) to negative (e.g., shearing), making response towards or near the human stimulus in an open environment hard to interpret. Sheep that received rewarding stimulation from a human (brushing) compared to neutral stimulation (presence) showed differences in ear and head postures (Tomioso et al., 2018), and animals that received chronic (6 weeks) aversive treatment (predator cues, rough handling) approached a stationary human less and vocalized more compared to control animals (Destrez et al., 2013). Regardless of the origins of the response in sheep, exposure to humans elicits temperament traits more effectively than having no human present (Goddard et al., 2000; Kilgour and Szantar-Coddington, 1995) and observing responses during human-animal interactions is the most streamlined method to assess CIDs in farming environments since it would require no additional equipment or change of protocol.

Frequencies of 'environmental vigilance' were moderately repeatable within ewes and considered indicative of individual differences in the unrestrained Human Presence test. Sheep that were given an anxiolytic treatment showed heightened vigilance behavior in an attention bias test (Monk et al., 2019; Lee et al., 2016) and less motivation to eat in a familiar home pen with close visual and auditory contact to conspecifics (Doyle et al., 2015). Though willingness to eat was not considered repeatable, the current study did provide sheep with a familiar and unrestrained home environment for testing similar to Doyle et al. (2015). Given findings from Monk et al. (2019), Lee et al. (2016) and Doyle et al. (2015) it could be that individuals performing more vigilance behaviors and less grazing were experiencing a greater level of anxiety compared to individuals who had longer durations of grazing. Other studies have found certain stimuli (i.e., dogs) to trigger more heightened and focused attention from sheep when tested alone and together (Beausoleil et al., 2005; Hemsworth et al., 2011). Stimuli, such as herding dogs used for moving, are known to elicit anxiety-induced vigilance behavior (Lee et al., 2016) possibly because they are a more salient representation of a predator compared to humans (Beausoleil et al., 2008) who work with sheep under multiple contexts (moving, drenching, shearing, etc.). Sheep subjected to a multitude of blind and carnivore stimuli (blind stimuli: human, trolley, ball and trolley; carnivore stimuli: stuffed wolverine on trolley, stuffed lynx on trolley, stuffed bear on trolley, and man with leashed dog) showed greater flight distances to the carnivore stimuli compared to the blind stimuli (Hansen et al., 2001).

Duration of ‘head down’ had the highest repeatability estimate of all behavioral variables tested. Hemsworth et al. (2011) found that increased head down behavior in ewes is associated with increased postmortem cortisol concentrations, so it is possible that ewes who demonstrated this behavior more while being handled were experiencing a greater level of stress relative to the other ewes, however, this needs more validation. Little research has been done on the meaning of head postures in sheep during restraint. Given ‘head down’ in the raceway was repeatable even in a group testing format it may be worth investigating as an indicator of other biological outcomes in sheep (e.g., maternal behavior, lambing success, immunology). Other responses in restraint (movement) are repeatable across time (Schiller et al., 2020) and related to weight parameters in sheep (Pajor et al., 2010; Gavojdian et al., 2015), so head postures may also be worth investigating for their repeatability and biological relevance.

4.2 *Inter-relatedness of responses from all three Human-Animal Interactions tests*

Behaviors between the restrained Human Contact test and PC 1 of each replicate from the unrestrained Human Presence test showed little intra-individual correlation to each other. This was similar for behaviors between the restrained Human Contact test and unrestrained Human Approach test. These findings are likely a result of limited individual expression due to testing methods (crowding in the back of the raceway) in the Human Contact test, and perhaps an expected outcome based on previous literature. Murphy et al. (1994) found measures taken from a restrained (isolation box test; agitation score based on movement and vocalizations) and unrestrained (arena; movement and vocalizations) environments to be unrelated. Atkinson et al. (2022) found that behaviors from different tests that displayed intra-individual consistency did not show much inter-relatedness. The only behavioral response that seems to be ubiquitously domain-general across studies is vocalization (Atkinson et al., 2022). The current study measured vocalizations in the restrained Human Contact and unrestrained Human Presence test, however, due to individuals being group tested, vocalizations were rarely performed across all trials. Dodd et al. (2012) states that arena behaviors in the presence of a human are indicative of responses to novelty, social isolation and surprise while tests involving direct handling by a human are more related to arousal or stress of simply being handled or in close contact with the human. Likewise, Hargreaves and Hutson (1990) suggests that procedures involving direct handling have a greater impact on sheep and cause a greater increase in cortisol concentrations compared to other procedures that involve no handling. Hemsworth et al. (2018) also found higher post-slaughter cortisol and lactate concentrations in lambs that received lifting and pulling into the forcing pen relative to lambs that did not receive direct handling.

Bickell et al. (2010) and Aydogdu and Karaca (2021) clustered animals based on reactivity during restrained and unrestrained testing, however, caution should be placed on this method of categorizing individuals as output from these assessments could be producing individual types based primarily on one of the contexts. Though behaviors from the restrained Human Contact test were weakly associated with behaviors from the other two HAI tests, restraint testing can be an effective way of assessing intra-individual consistency and inter-individual variability in the arousal/stress response in sheep towards a human stimulus (Murphy et al., 1994; Schiller et al., 2020) and is related to biological correlates (Toth et al., 2017; Gavojdian et al., 2015; Pajor et al., 2010). In sum, responses from the Human Contact and Human Presence tests may not be generalized to responses in other contexts. Dodd et al. (2013) cautions that response in restraint may not be reflecting the same inherent quality within individuals as responses in an open testing environment.

A positive trend was identified between latency to ‘look at human’ and PC 1 of the first year gestation phase (R2) and a negative trend between PC 1 of the second year's post-breeding phase (R4). This indicates that sheep that were quicker to look at the approaching human at the conclusion of the unrestrained Human Presence test were those that had shorter durations of grazing behavior and longer durations of vigilance behaviors. These results are intuitive since individuals who maintained greater durations of vigilance behaviors in the unrestrained Human Presence test would be quicker to spot another approaching human in their environment. Goddard et al. (2000) indicates that the presence of a stationary or moving human is more stressful than no human at all, and level of stress increases as the human becomes more mobile. Monk et al. (2019) found that vigilance may be indicative of anxiety states in sheep, and it is possible that sheep that were already vigilant in the unrestrained Human Presence test experienced elevated levels of stress or anxiety once they were aware of another human approaching within their environment. This may be useful when considering the consequences of frequent or unpredictable movement around sheep, which is already considered a stress-inducing experience. Alternatively, it is possible that sheep that had a shorter latency to look at the approaching human had learned from previous tests that a human would be arriving to release them from the mOFT. Though these results may suggest the presentation of a ‘domain-general’ trait in an unrestrained environment across a stationary and moving human stimulus, authors are hesitant to draw this conclusion since the Human Presence and Human Approach tests were conducted in quick succession and not entirely exclusive of one another.

The purpose of this study was to explore repeatability and inter-relatedness of behaviors that may be reflective of a trait performed under a variety of human-animal interactions. Results indicated that there may be a context-specific ‘alertness’ response in the presence of a human stimulus in an unrestrained environment, and this response is unrelated to responses during the closer, more restrained human interaction. The current study suggests that responses in the unrestrained environment cannot be generalized across multiple types of human-animal interactions. In the very least, more investigation is needed to understand if the ‘alertness’ response is associated with other types of behavioral variables found under close human contact. According to previous literature there is a trend that suggests unrestrained environments are more useful for assessing emotional states (e.g., anxiety and depression) and personality paradigms (e.g., boldness, exploration, novelty, aggressiveness) in sheep while restrained environments are better equipped to gauge HPA-axis activation (Pajor et al., 2010), and perhaps, performance-related parameters such as maternal behavior (Aydogdu and Karaca., 2021; Brown et al., 2016; Peeva, 2009), net feed intake (Amdi et al, 2010) and growing traits at the end of the fattening period in lambs (Pajor et al., 2008; Pajor et al., 2013).

Stress experienced on farms may be triggered by any aversive environmental stimuli, or those perceived as aversive, and result in short- or long-term consequences on the animals’ biological functioning. Short term stress responses are considered normal and even adaptive, however, long term consequences of chronic HPA-axis activation leading to increased levels of systemic cortisol concentrations may cause oxidative stress, hypertension, worsened immune function, reproductive disorder, stereotypical behaviors, and other complications. Ability to identify coherent behavioral patterns associated with stress and biological outcomes is complicated, as each type is likely to experience tradeoffs. For example, sheep that are willing to explore or approach novel items in unrestrained environments may be those that experience a reduced HPA-axis response (Goddard et al., 2000; Yu et al., 2021) and anxiety (Doyle et al., 2021) relative to sheep that are less willing to explore or approach novel items, however, they may also have greater risk of predation (Stamps, 2007) or injury. To complicate the issue further, only a few studies have thoroughly assessed the consistency of inter-relatedness between behavioral and/or physiological responses across time and multiple contexts. Many authors have found specific behaviors to be repeatable, such as vocalizations (Yu et al., 2021) and single arena behaviors (Dodd et al., 2012), or consistent despite the stimulus used (Beausoleil et al., 2012), however, the stability of the association between behaviors is not well characterized. Yu et al. (2021), found that relationships between physiological and behavioral variables (i.e., cortisol concentrations, heart rate, response to novelty, vocalizations and exploration) changed over the course of ontogeny in sheep and individual responses to specific stimuli are subject to diminish or increase. In contrast, Schiller et al. (2020) reported consistent individual responses to physical restraint; such that movement, vocalizations, heart rate and eye temperature measures were moderately inter-related within individuals across a five-week study period and individuals performed similar magnitudes of behavior relative to their conspecifics, indicating a level of within-subject consistency. This behavioral characterization is important when desiring to select individuals that may have, for example, reduced arousal responses in restraint that result in easier handling and lowered risk of injury or harm for the animal and handler.

4.3 *Considerations for group testing*

Certain elements of the unrestrained Human Presence test environment did elicit individual differences amongst the ewes; exactly what the eliciting stimulus was is uncertain. Though it is possible that the stationary human was not the eliciting stimulus in this testing environment, it seems that the presence of a human tends to bring out inter-individual differences better than having no human present (Kilgour and Szantar-Coddington,1995). Although other activity-related responses such as locomotion and vocalizations are replicable and reflective of individual behavioral types in an arena with a human stimulus (Dodd et al., 2012), sheep in the current study were group-tested with direct conspecific contact, and demonstrated less activity (locomotion, vocalizations) compared to sheep tested alone in the arena. Kilgour (1997) reported that single tested sheep had greater levels of movement and vocalizations compared to group (n=4) tested animals, who seemed to stay close together and remained immobile for the duration of the arena test. The traditionally used arena test elicits conflicting motivations to reinstate social contact with flock mates or avoid the human stimulus, so it is logical that authors find indicators of sociability (i.e., vocalizations) and activity to be the most reflective of individual behavioral types. It is possible that the effects of testing sheep in groups diminished their arousal responses that would otherwise be observed when isolated with a human stimulus. Salvin et al. (2022) reported that social buffering may encourage individuals to initiate eating in an unfamiliar testing environment, however, may not be effective at reducing startle responses after the stimulus has been applied. In the current study there were group effects detected in the Human Presence test, however, there was still spread in observed grazing behavior despite group membership (Figure 4). It is possible that social contact during testing can still be a suitable method for assessing individual differences in frequency of environmental vigilance, however, this response may not be as strongly expressed compared to when sheep are subjected to novel, risky or challenging situations in isolation (Reale et al., 2007). Villalba et al. (2009) reported sheep that were more agitated during social isolation were also less reluctant to accept novel food items. Individual differences in grazing and vigilance behavior among social groups of ungulates has been observed, and it may be that the level of sociability in sheep drives their propensity to graze or remain vigilant in an open environment (Villalba et al., 2009). Finally, group tested sheep from extensive and semi-intensive management systems showed a greater increase in heart rate when a human was stationary or moving compared to when they were tested with a group of three other conspecifics and no human present (Goddard et al., 2000). This indicates that a stationary and moving human stimulus may still induce the stress response when sheep are group tested. Though Goddard et al. (2000) did not investigate for evidence of individual differences in the stationary and moving human tests, authors state that based on distribution of behavioral response, there appeared to be the presence of ‘proactive’ and ‘reactive’ behavioral types.

5. Limitations

Though the random effects of group membership were controlled for in residual data used for non-parametric analysis, it is still possible the effects of group diminished expression of a potential behavioral trait related to the arousal responses when isolated with a human. All three human-animal interaction tests were done consecutively in the same order for each trial, making it impossible to test for the effects of test order or interpret the tests results as entirely separate from one another. The restrained Human Contact test was brief and the ewes experienced crowding in the back of the raceway which limited their ability to move and adjust posture. While this study controlled for sheep entry numbers in the raceway, the effects of crowding could not be completely nullified. Having as many replicates as possible is always desirable when wanting to understand consistency or repeatability of behaviors, however, all data from trial 6 was not presented as this date achieved extreme heat conditions that appeared to interfere with the animals' normal behavioral performance. Authors understand that the output from PCA can be very vulnerable to transformation based on the behavioral variables entered. This should be considered with conducting a PCA to describe clusters of related behaviors.

6. Conclusion

Results of the current study provide weak evidence of a temporally stable 'alertness' trait manifesting in the unrestrained Human Presence test, generally characterized by individual differences in durations of 'grazing' and 'environmental vigilance'. Due to the unrestrained Human Presence and Human Approach tests being conducted in immediate succession the authors are not certain that responses from either test are entirely separate. The most convincing evidence for consistent individuality in this study was indicated through performance of the frequency to become environmentally vigilant in the unrestrained Human Presence test and duration of 'head down' behavior in the Human Contact test. Frequency of 'environmental vigilance' behavior may be interpreted as the animal's propensity for awareness in their surroundings during human-animal interactions and may be reflective of individual emotional states (i.e., anxiety or fear). According to this study, behavioral responses within tests were not able to be generalized across contexts (i.e., restraint vs non-restraint). Though habituation can be an issue when subjecting sheep to repeated, human-animal interactions (Cakmakci et al., 2022; Erhard et al., 2006) assessing sheep in this context may still allow for observation of intra-individual variability in frequency of vigilance. Behaviors in an unrestrained environment (i.e., arena) in the presence of a human should be considered for temperament and personality assessments as they have been found to be both repeatable despite age (Cakmakci et al., 2022) and management differences (Goddard et al., 2000).

7. Figure captions

Figure 1. Eight to ten groups of five ewes (n= 40 - 50) were placed in six HAI replicates over two years at the time of post-breeding when the rams were pulled from the ewes in November, gestation when ewes were brought in for perinatal booster shots and deworming in late January and at weaning in June of each year. Each star represents the period when a trial was conducted.

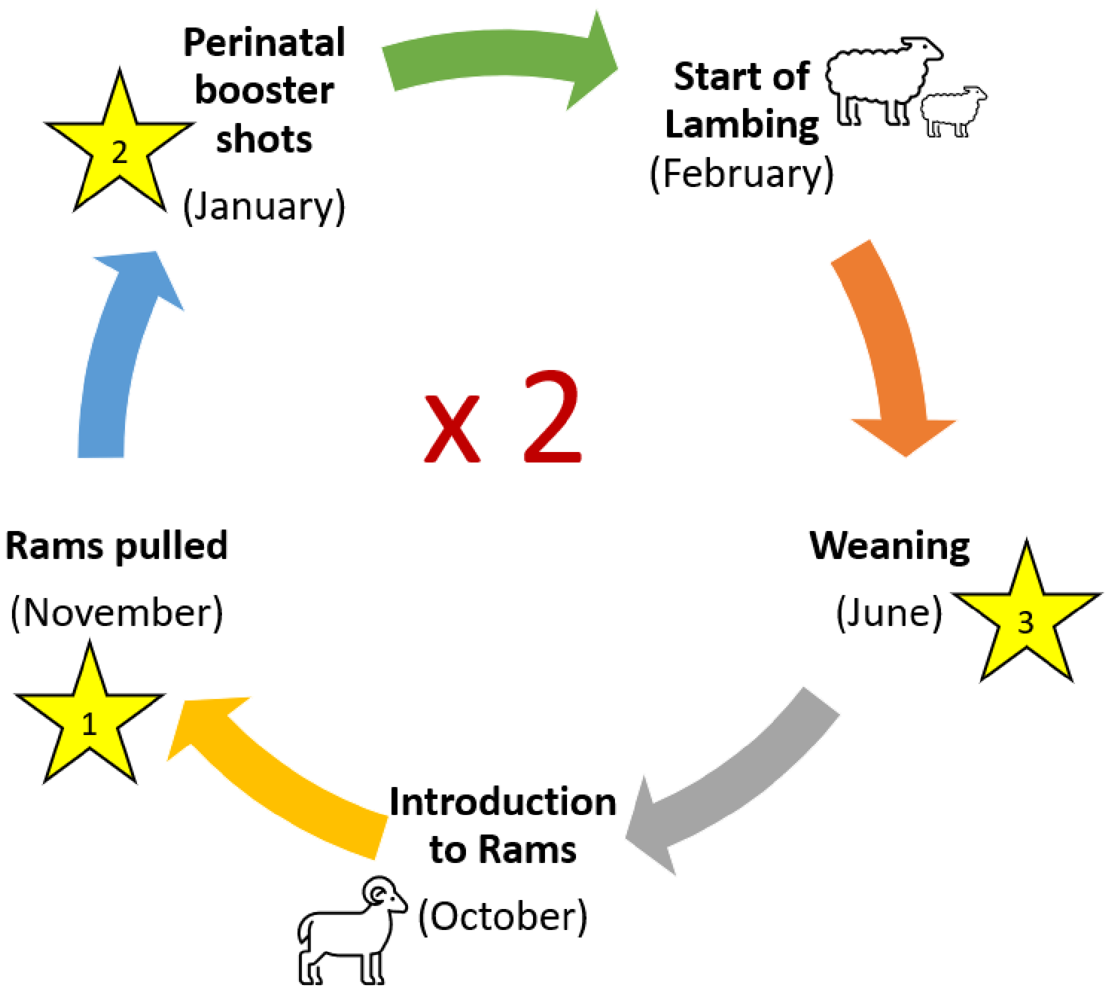


Figure 2. Bud-Box and raceway set up. Ewes (n=5) were subjected to a contact treatment for 10-seconds within the raceway. Human A was positioned approximately 8 m perpendicular to the last sheep in the raceway and would approach each individual animal, apply light pressure with one hand under the muzzle and with the other hand on the top of the rump of each sheep for 10 seconds. Each approach and contact treatment were video recorded, and when all ewes in the raceway had received the treatment, they would be let out of the exit gate at the end of the raceway to be entered in the modified open field test. Another group of five ewes were ushered single file into the raceway after the 5-minute modified open field test was completed.

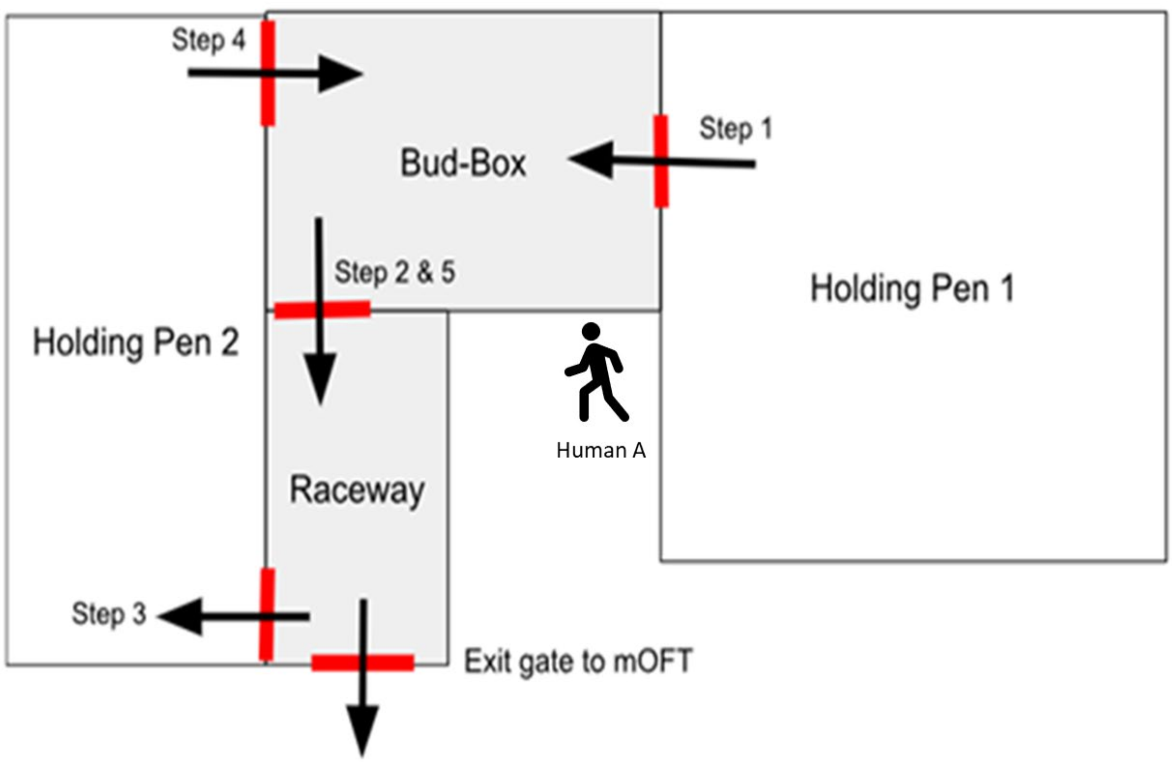


Figure 3. Modified open field test (mOFT) set up. The mOFT (10.5x 10.5m) was visually partitioned using red spray paint into 25 boxes (2.1m x 2.1m), each associated with either zone 1 (box with Human B), zone 2 (intermediate boxes touching zone 1) or zone 3 (boxes peripheral to zone 2, touching fence line). After the last ewe of each group (n=5) passed an exit gate (figure 1) and entered the mOFT, the Human Presence test would initiate. After the 5-minute duration of the test, Human C would approach the exit gate and walk counterclockwise at a steady pace to encourage sheep to exit the arena. Zone '1' is illustrated as the darkest gray shade and zone '3' is the white zone in the center of the mOFT..

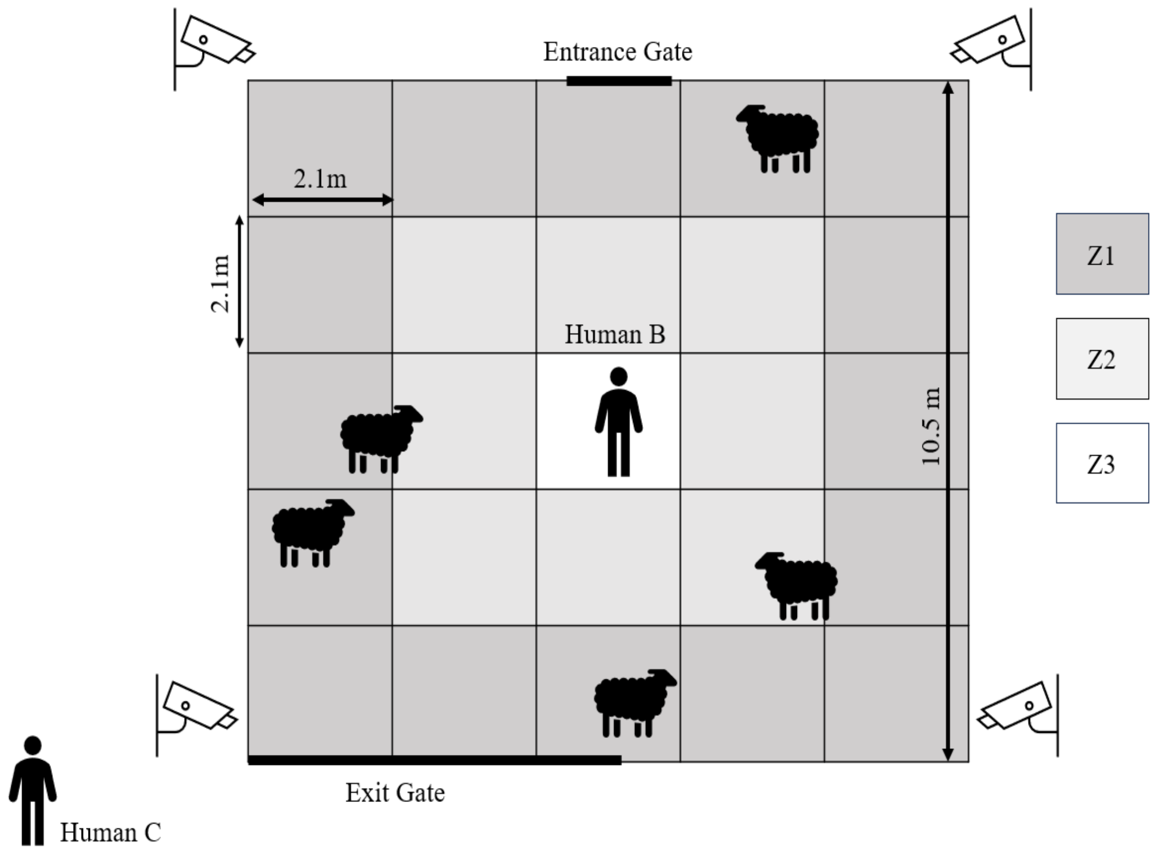


Figure 4. Distribution of raw grazing data per replicate of the Human Presence test, colored by group number (n=5).

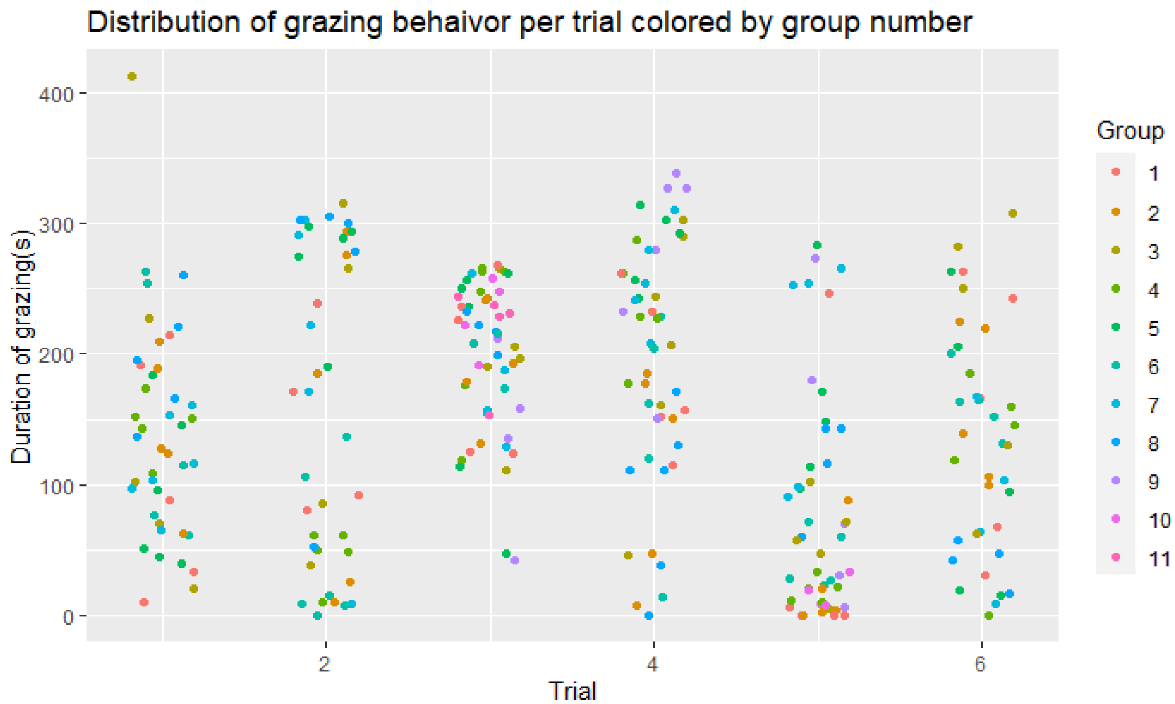
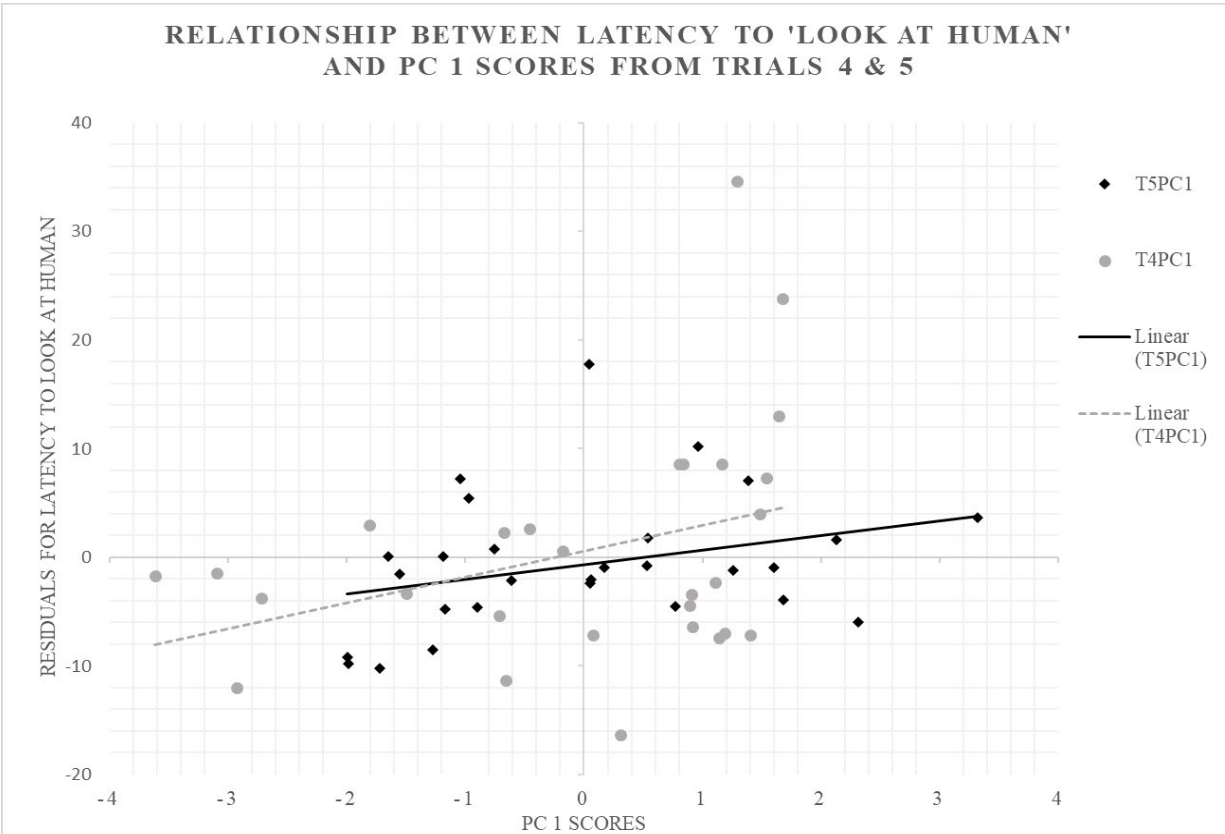


Figure 5. PC 1 of the Human Presence test from the second year's post-breeding (R4) and gestation event (R5) demonstrated a positive trend to latency to 'look at human' from the Human Approach test within replicate 2 (P=0.06) and replicate 4 (P=0.07).



8. Tables

Table 1. Ethogram including behaviors from the Human Presence, Human Contact and Human Approach tests with associated operational definitions

Behavior	Operational definition
	Human Presence test
Displacement (initiator/receiver)	**Initiator**: Focal sheep moves non-focal sheep to a different position either with contact (top of head/ shoulder) or with close proximity (within a sheep's distance); Focal sheep may replace with position of non-focal sheep **Receiver**: Focal sheep is moved by non-focal sheep to a different position either with contact (top of head/shoulder) or with close proximity (within sheep's distance); Focal sheep may be replaced by non-focal in position
Head-Butt (initiator/receiver)	Head-butting: Focal sheep's poll is in contact with non-focal sheep's poll
Vigilance (environmental)	Focal sheep has head positioned at or above shoulder line; nose it directed away from the human stimulus; ears are erect and perpendicular to head (inner ear is facing forward) OR directly backward from position of muzzle and body/legs are still (motionless); ewe may or may not be chewing feed simultaneously; ears are in aligned position
Vigilance (human)	Focal sheep has head positioned at or above shoulder line; nose is directed towards the human stimulus; ears are erect and perpendicular to head (inner ear is facing forward) OR directly backward from position of muzzle and body/legs are still (motionless); ewe may or may not be chewing feed simultaneously; ears are in aligned position
Investigate (fence)	Focal sheep is making direct contact with fence; can be making contact with fence using feet, snout, head or side of body; ewe is sniffing fence with snout or licking fence with tongue; may be moving
Investigate (human)	Focal sheep is making direct contact with human using feet, snout, head or side of body; sheep is sniffing/chewing human with snout or licking human with tongue
Graze	Focal sheep has head below shoulders; and is sniffing or manipulating grass with nose/mouth or ingesting vegetation with mouth; snout is contacting or close to ground; ewe may or may not be motionless; may fluctuate between walking and grazing
Walk	Focal sheep takes at least two consecutive steps with right foot; head may be positioned above, at or below shoulder line; >/2 steps per second

Stomp	Focal individual has ceased pacing or walking and remain still; focal individual lifts one foot off the ground and placed foot down in same position; wool or flank may shake; focal individual makes no forward or backward movement
Vocalize	Individual makes audible, open mouth vocalization
Human Contact test	
Kneel	Focal sheep has right, left or both front knees contacting the ground
Vocalize	Individual makes audible, open mouth vocalization
Turn Around	Focal sheep points nose in direction towards rump in direction of entrance gate; focal sheep may step one or both front feet in direction of entrance gate
Head Up	Top of the focal sheep's head (poll) is above shoulder line
Head at Shoulder	Top of focal sheep's head (poll) is in line with the shoulder
Head Down	Top of focal sheep's head (poll) is below the shoulder line
Step	Focal sheep lifts and places front feet on ground making forward or backward movement
Human Approach test	
Latency to Leave the Arena	Amount of time for focal sheep's rump to pass the exit gate after the human has touched the gate
Latency to Look at Human	Amount of time for focal sheep's muzzle to be pointed at the human stimulus after the signal to approach as been initiated
Latency to Step	Amount of time for focal sheep to take a step forward or backward after the human has touched the exit gate

Table 2. Repeatability estimates, SE and CIs for behaviors within the Human Contact and Human Presence test.

Duration 'grazing'	R	SE	2.5%	97.5%
org	0.052	0.0358	0.0143	0.121
link	0.10	0.0604	0.0280	0.210
Duration of 'vigilance at human'	R	SE	2.5%	97.5%
org	0.0437	0.0492	8.46e-15	0.131
link	0.0435	0.0484	8.40e-15	0.128
Duration of 'environmental vigilance'	R	SE	2.5%	97.5%
org	0.109	0.048	0.0277	0.165
link	0.197	0.0737	0.0495	0.261
Duration of 'walking'	R	SE	2.5%	97.5%
org	0.032	0.0391	3.44e-10	0.102
link	0.0397	0.0472	4.19e-10	0.122
Duration of 'investigating fence'	R	SE	2.5%	97.5%
org	NA	NA	NA	NA
link	0.21	0.06	0.003	0.17
Frequency of 'investigating fence'	R	SE	2.5%	97.5%
org	0.09	0.8	0.02	0.25
link	0.29	0.13	0.14	0.48
Frequency of 'environmental vigilance'	R	SE	2.5%	97.5%

org	0.10	0.10	0.05	0.18
link	0.32	0.09	0.20	0.48
Duration of ‘head down’				
org	-	-	-	-
link	0.44	0.15	0.22	0.68

Table 3. Composite traits from replicates 2 – 5 in the Human Presence test are presented. Each replicate is composed of a trait that is generally characterized by considerable loadings for grazing (>0.40) and environmental vigilance. Replicate 2 can be characterized as having a positive loading for grazing (>0.50) and a negative loading for vigilance in the environment (>-0.50), with inverse presentation for trial 3 – 5.

Replicate	Y1: Post-Breeding Behaviors	R2 PC 1 (59.3%)
2	Grazing	**0.50**
	Investigating fence	-0.42
	Walking	-0.38
	Vigilance at human	-0.40
	Vigilance in environment	**-0.52**
Replicate	Y1: Weaning Behaviors	R3 PC 1 (45.3%)
3	Grazing	**-0.56**
	Investigating fence	0.44
	Walking	0.03
	Vigilance at human	**0.53**
	Vigilance in environment	**0.47**
Replicate	Y2: Pre-Breeding Behaviors	R4 PC 1 (48.1%)
4	Grazing	**-0.47**
	Investigating fence	0.39
	Walking	0.18
	Vigilance at human	**0.53**
	Vigilance in environment	**0.56**
Replicate	Y2: Post-Breeding Behaviors	R5 PC 1 (37.6%)
5	Grazing	**-0.45**

	Investigating fence	0.03
	Walking	**0.50**
	Vigilance at human	0.44
	Vigilance in environment	**0.60**

CHAPTER 3

Postpartum human-animal interactions reveal a potential 'pacing/ avoidance of human' response in extensively managed ewes

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Abstract

Lamb welfare, performance and survival on rangeland is partially determined by the quality of maternal care received (i.e., high durations of licking/grooming, allowing udder access, and staying close to the lamb). Previous studies have explored maternal behavior scores (MBSs), as a tool to select ewes based on their proximity to the lamb and the shepherd, finding variable evidence towards sensitivity and repeatability in scores in ability to gauge lamb outcome (weight gain and weaning weight), survival, and maternal care throughout the lambing season. Though this scoring system is convenient, previous research indicates another behavioral attribute related to fear of humans may be elicited during lamb tagging, caused by presence of the human. This longitudinal study investigated for the presence of a trait related to fear, anxiety or stress caused by the human handler that may be operating separately from measures of proximity or separation behavior in the ewe. Across both years of the study, twenty-two extensively managed breeding ewes were placed in a Lamb Handling test during lamb processing with a human close (<1m) and a Lamb Tie Down test with a human far (>30m) during ewe and lamb interactions. The Lamb Handling and Lamb Tie Down tests occurred over two consecutive years to test for the presence of a temporally consistent trait that may be related to indicators of lamb outcome (birth, growth and weaning weights). Using principal component analysis, a single ‘pacing/ avoidance of human’ response manifested during the Lamb Handling test, characterized by ‘pacing’ ‘investigating the human’, ‘grazing’ and ‘environmental vigilance’ in the first year and ‘grazing’ and ‘investigating the human’ in the second year ($P<0.001$). This ‘pacing/ avoidance of human’ response was unrelated to indicators of lamb outcome and maternal behaviors and may be context-specific to human-animal interactions in the lambing season. The most similar measure to ewe MBS (duration of ‘close proximity’ to the lamb) was not consistent between years 1 and 2 ($P = 0.30$) of the study and highly affected by birth weight of the lamb ($P<0.001$). It is likely that proximity to the shepherd and lamb during processing is modulated by maternal investment. Researchers or farmers desiring to gauge maternal attachment and lamb performance or outcome in the early postpartum season may consider observing adaptive maternal behaviors without the human handler present. Future research could focus on identifying if the ‘pacing/ human avoidance’ trait is context specific to the lambing season, or generally performed in response to a human handler.

Keywords: ewe-lamb, human-animal, maternal behavior, sheep, temperament

1. Introduction

In the first few days postpartum, lamb survivability, health and growth on extensive rangeland settings depends heavily on the juvenile environment (i.e., weather, protection from predators, supplemental nutrition) and maternal behavior. Lamb mortality on rangeland can be exceedingly high (15 – 50% lamb loss, Dwyer et al., 2008), incurring costs to the farmer, and drawing concern to the conditions which lead to the lamb's death. These conditions may include lack of shelter provision, other aspects of inappropriate management, and, aggression, neglect and lack of selectivity from the ewe. In conditions with adequate shelter and space allowance, "good" maternal behavior is an additional benefit to the lamb and its ability to survive the first few days of life and maintain proper health and growth though weaning. Without specific displays of maternal behavior from the ewe, the ewe-lamb pair may fail to form a strong bond and the lamb may experience significant detriments to its own welfare (Nowak, 1996) including starvation and hypothermia, or even physical aggression from the ewe herself. In the case of high neonate loss, and the factors contributing to poor welfare prior to the lamb's death, this may reflect a greater issue relative to insufficient nutrition or management (Dwyer et al., 2008). To form a strong ewe-lamb bond in the first 6 hours postpartum, the ewe must engage in a coordinated response of behaviors including low-pitched bleats towards the lamb (Dwyer, 1998, Shillito-Walser, 1984), frequent durations of licking/grooming (Alexander, 1988) and sniffing (Morgan et al., 1975), udder allowance for sucking (Nowak, 1994), and must stay near or on the birth site for that duration of time (Nowak, 1996). After the first 24 hours, it is considered important for the ewe to stay in close proximity to her lamb(s) (Alexander et al., 1977), have frequent vocal communication (Nowak, 1996) and display consistent udder allowance (Dwyer, 2005; 2008) so mutual preference between the ewe and lamb can be reinforced (Nowak et al., 1997). Given that the proper endocrinological activity is initiated (i.e., activity of oestradiol and progesterone) (Dwyer et al., 2008; 2014; Poindron et al., 2007), with minimal stress (Coulon et al., 2014) and proper labor management (Alexander et al., 1977), it is typically expected that the ewe can sustain her lamb(s) in extensive environments with little assistance until reaching weaning time.

Between 6 – 36 hours after birth, the shepherd will collect the lamb(s), and walk away from the birth site to a different location to process the lamb while the ewe is allowed freedom of movement. Lamb processing includes ear tagging for individual identification, castrating ram lambs, tail docking ewe and ram lambs, and spraying the umbilical cord area with iodine treatment. Scoring the ewe during processing is done based on her proximity to the human and lamb, and is a traditional method to assess the ewe's potential attachment to her offspring(s) (O'Connor et al., 1985; Everett-Hinks, 2005). It is a relatively easy measure for shepherds to record since it requires little deviation from the regular management protocols. The assumption behind this mechanism of scoring the ewe is that differences in score values will be associated with differences in lamb performance/outcome (birth, growth, and weaning weights) and survivability. O'Connor et al. (1985) was the first to develop this scoring tool which includes five points. Low scores are designated to ewes that flee (>30m) while the shepherd is working with the lamb and high score are designated to individuals who remain near the shepherd and the lamb (< 1 m). Maternal behavior scores have yet to receive vigorous validation, so distinction in behavior and proximity between scores is ultimately subjective. Additionally, scores are often reduced to binary or tertiary categories either because they lack variation or lack the sensitivity to pick up on differences in lamb outcome (growth and weaning weights) and other maternal behaviors (i.e., allowing udder access, low-pitched bleating, sniffing/licking). Even with the lack of validation, this score is assumed to be reflective of an underlying, inherent quality in the ewe that will be consistent across lambing seasons.

Previous work suggests that the expected relationship between the scoring system and lamb outcome is that as the scores increase, the lambs will have a greater chance of surviving until weaning, heavier weaning weights, and greater weight gain post-weaning. There is a general notion that selection based on this score will also lead to greater birth weights in subsequent lambing seasons. It is presumed that higher score values will be associated with "better" maternal behaviors throughout the lambing season. Some authors have found this relationship to be true observing that ewe score values above the lowest score (proximities < 30m) were associated with better chances of the lamb surviving from birth to weaning and having significantly higher weaning weights compared to ewes with a score of 1 (O'Connor et al., 1985; 1996). Other authors have reported mixed findings when looking at the role of MBSs in response to human presence, maternal behavior, and lamb outcome. Lamb et al. (2001) found that there were no differences in lamb weight gain between MBS categories and Everett-Hinks (2005) concludes that given a sufficient environment and good shepherding, MBS is likely to have negligible effects on lamb outcome. It is possible that scoring proximity of the ewe during processing, in the presence of the human, is eliciting a temperament trait related to fear of the human or fear of the lamb's well-being, that may or may not be related to maternal behavior and lamb outcome. Further, the processing event itself could be eliciting a temperament trait that is also observable outside of the lambing season and related to fear or anxiety in the midst of a challenging circumstance. Results from other studies have indicated that testing for consistent individual differences (CIDs) in the dry period, with or without a human present (i.e., isolation box test, open field tests) may have a unique, and separate relationship to behavior at lambing time, such that open field tests are related to ewe rearing performance from birth to weaning (Kilgour and Szantar-Coddington 1995; Kilgour 1998) and isolation box tests are be related to maternal behavior scores (Plush et al., 2011).

Currently, proximity maintained from the lamb and shepherd at processing is used to gauge lamb outcome *and* maternal care throughout the lambing season. Evidence so far demonstrates variability in sensitivity and robustness of proximity to the shepherd as an indicator of lamb outcome and maternal behavior. Expanding on the ewes' behavioral repertoire, with inclusion of measures of proximity, may expose if there are separate behavioral responses activated during this time that are consistently related to maternal behavior and lamb outcome. Revealing a separate trait during processing may help explain why some studies have identified an association between ewe CIDs during the dry period and behavioral reactivity at processing (Bickell et al., 2009, 2011; Aydogdu et al., 2021). Further investigation into measures of proximity may also elucidate the viability of this response, as previous studies have shown that the ewe's proximity to the shepherd and lamb pair during processing (i.e., MBS) has poor to moderate repeatability (0.06 – 0.32) and may be highly affected by temporary environmental effects (Everett-Hinks, 2005; Lambe et al., 2001; O'Connor et al., 1985) calling into question the stability of proximity measures between the ewe and human. The first aim of this study was inspired by a sentiment from Dwyer and Lawrence (2005), stating that the ewes' response during lamb processing may reflect an underlying CID, as much as it measures their maternal behaviors. The first study aim is to test for the existence of a postpartum behavioral trait among ewes during lamb processing (Human < 1m from lamb; ewe has freedom of movement) test and evaluate the trait's relationship to indicators of lamb outcome (i.e., birth weights, weaning weights and growth weights), adaptive maternal care (i.e., durations of 'nosing/licking/sniffing', 'closed mouth bleating' and 'udder allowance') and proximity to the shepherd/lamb pair based on the ewes body length (within body length = 1m; outside body length = >1m). The second aim of this study is to summarize non-maternal behavioral responses (e.g., grazing, pacing, open-mouth bleating) among ewes during ewe-lamb interactions after the lamb has been processed, and evaluate their relationship to lamb outcome, performance, adaptive ewe maternal care, and proximity to the lamb and shepherd at processing. Summarizing the data in this way will help identify if there is another potential stress or CID related response persisting in the site of processing even after the human in absent. The third aim of this study was to evaluate the relationship between indicators of lamb outcome/performance and adaptive maternal behaviors.

Lambe et al. (2001) states that recording separate ewe-lamb interactions may help identify maternal responses more associated with indicators of lamb performance and outcome, however, this can be difficult in extensive settings. In terms of the first study aim, we predict that the presence of a human handler during processing will elicit a unique behavioral trait that is unrelated to indicators of lamb outcome or adaptive maternal care and proximity measures. This trait may be reflected through behaviors that do not have a direct or salient connection with maternal care and are more associated with stress or fear/anxiety responses in sheep, such as high durations of ‘pacing’ and ‘open mouth bleating’ and low durations of ‘grazing’, ‘investigating the human’ and ‘vigilance’. In terms of the second study aim, we predict that there will be a temperament trait during ewe-lamb interactions after processing (human > 30m away from lamb), when the ewe and lamb are alone together, that will be related to adaptive maternal behaviors and lamb outcome and characterized by higher durations of ‘grazing’, and lower durations of ‘pacing’ and ‘open-mouth bleating’. Finally, in accordance to the third study aim, we predict that behaviors reflective of adaptive maternal care (allowing udder access, closed-mouth bleating sniffing/nosing/grooming) will have a positive relationship to indicators of lamb outcome and performance.

2. Methods

2.1 Animals

The current study was approved by the University of California, Davis Institute of Animal Care and Use Committee (protocol# 20926) and conducted at two distinct sheep handling sites (Blue Oak Ranch [site A] and Belmantro Station [site B]) located within the Sierra Nevada foothills in Auburn, California, USA. The study flock consisted of terminal Shropshire (Shrop) (Year 1: n= 11 ; Year 2: n= 15) ewes and Blue-faced Leicester x White-faced (BLW) crossbred ewes (Year 1: n=19; Year 2: n= 19) . Due to some animals not becoming pregnant or not being physically present to be observed on camera, twenty-two individuals (Shrop: n= 10 ; BLW: n=11) were consistently video recorded between years 1 and 2. All study subjects were kept as a subset with a greater flock of 120 individuals and allowed access to approximately 190 acres of total rangeland throughout the entire year and rotated every 5 days to a different paddock. During the dry period, ewes were moved across 62 separate paddocks. Study ewes were maintained together as a single breeding group with *ad libitum* access to forage on pasture and restricted access to protein supplements prior to breeding throughout August and September. Ewes had sufficient forage each year of the study (2020 and 2021), however, 2021 was affected by the drought and had less forage available than the year before. Ewes were flushed (increased nutrients in diet to prepare for pregnancy) on alfalfa hay on irrigated pasture the first year and a mixture of dry cob, corn oats, barley and chia seed (1.25lbs/ head/day) the second year. For predator abatement, ewes were maintained with electric fencing and two guardian dogs socialized to the flock as pups.

Ewes were introduced to rams for natural cover or natural breeding (30 F:1 M) in November through October and lambed from late February to early April. Lamb mortality was not recorded as it was generally low among the study ewes (1 – 5%), likely due to 2020 and 2021 being relatively dry years. Harsh, wet winters are more likely to cause high rates of lamb mortality (Alexander et al., 1977). During the lambing season, ewes were rotated across 9 different paddocks, ranging anywhere from 4 to 15 acres. The shepherds of the study flock have been using the EZ Care system developed to score ewes based on lambing ease, mothering ability and lamb vigor for the past 10+ years. During the study period (years 2020 – 2021), numbers of ewes needing extra lambing assistance during labor was minimal (1 in 20). Shepherds were able to scan lambing data (birth weights, mothers EID, sex) using ear tags and an electronic reader for easier record keeping. Shepherds maintaining this flock used low-stress handling techniques (Hutson and Grandin, 2014) when moving animals or during any procedures that involved direct human contact to avoid inducing unnecessary stress upon the animals.

2.2 *Experimental Design*

Researchers were called to the lambing site between 6 – 36 hours after each lamb was born, according to the shepherd's records. Data collection started in the early morning, between 0600 – 0800 hours, temperature range (7.2 – 12.8 C). Data were collected after the 6 hours postpartum period when forming the ewe-lamb bond is crucial for establishing mutual selectivity between the ewe and the lamb (Nowak, 1996). The observation period (6 - 36 hours postpartum) seemed like the optimal range for studying ewe behavior since lambs can readily identify their mothers at close range (Nowak and Lindsay, 1990), reducing the potential of this ewe-lamb bonding aspect to affect results of the current study. Prior to data collection, the shepherd would state which ewes were to be recorded that day, and observers would set up cameras placed on tripods approximately ~10 m away from the ewe lamb pair, near where the shepherd placed lamb processing equipment. Eight observers were trained using an operational ethogram (Table 1) and scored three practice videos (each ~15 mins) including the Lamb Handling and Lamb Tie Down test. Interobserver reliability was established amongst the eight observers using two ~15 min reliability videos (Cohen's kappa = 0.80) the observers had never seen before, (Cohen's kappa = 0.80) prior to behavioral annotation of video data using The Observer XT v. 11 (Noldus Information Technology, Wageningen, Netherlands).

2.2.1 *Lamb Handling test (Processing)*

Two video cameras (Sony Handycam DCR SX85; Sony Corporation of America, New York, NY, USA), stabilized on tripods, were placed approximately 25 – 30 m away from the shepherd's toolbox that was placed in the pasture. The shepherd would then approach the ewe and lamb pair, collect the lamb(s) using a shepherd's hook and walk the lamb away from the ewe to where the toolbox was placed. The toolbox was placed approximately 50 – 100m away from the birth site. Recording started when the shepherd approached the lamb for collection and continued through lamb processing (i.e., tail docking, castrating ram lambs, ear tagging for individual identification, iodine treatment of umbilical cord, and marking spray to flanks). Processing would typically take 5 minutes per lamb. During this time, the shepherd would announce the ID of the ewe (indicated after scanning their ear EID using the electronic scanner), the sex of the lamb(s) and the lamb(s) weight. Video was analyzed for durations of 'close proximity', 'nosing/sniffing/licking' the lamb(s), 'environmental vigilance', 'investigating the human', 'grazing', 'pacing', 'walking', and 'pacing'. Video was also analyzed for frequencies of ‘closed mouth’ and ‘open mouth’ bleating. Video recording still took place if the ewe was not immediately visible near the lamb and shepherd at the time of processing.

2.2.2 *Lamb Tie Down test (ewe-lamb interactions)*

After lamb processing, the lamb was tied to the ground with twine and a camping stake by one of their back legs at the same location that processing had occurred. The lamb(s) was tied in this way to keep them from moving out of the view of the camera, and to be able to have interactions with the ewe, without the human present At this time, observers and the shepherd walked at least 30m away from the pair to record another ewe-lamb set. Videos recorded the ewe lamb pair for approximately 10 mins per set, or longer if the ewe had been interrupted by another ewe or a camera was knocked over. The same behaviors were assessed from video as those observed in the Lamb Handling test, with addition to frequencies of the lamb vocalizing and durations of ‘sucking’, ‘standing active’, ‘standing inactive’, ‘lying active’ and ‘lying inactive’. Video recording still took place if the ewe was not immediately visible in order to code lamb behaviors that may affect the ewe from a distance (i.e., vocalizations).

2.3 *Statistical Methods*

Raw data were transformed into proportions and rates based on the duration the ewe was seen on camera. Using R Statistical Software Version 4.2.1 (R Core Team 2018), non-parametric Principal component analysis (n=29) was conducted in order to summarize the multitude of behavioral variables collected from the Lamb Handling and Lamb Tie Down tests into composite traits. Spearman’s rank order correlations were then used to investigate for within-subject consistency on composite traits for ewes that became pregnant and could be seen on camera in years 1 and 2 (n = 22). Using linear mixed effects models with restricted effects maximum likelihood, residual data controlled for environmental and endogenous effects was used in principal component analysis and Spearman's rank order correlations. . For residual data collection, all behavioral variables from each test (Lamb Handling, Lamb Tie Down) were entered as the response in a general linear model with various factors fit as covariates (i.e., age, day, lamb activity) or fixed effects (i.e., breed, parity, litter size, day of birth) with a quasibinomial distribution to accommodate for the proportional nature of the data. Models from the Lamb Tie Down test also included lamb behaviors such as duration of inactive standing, active standing, inactive lying down, active lying down and vocal activity (Table 4). For ewes that had multiples, each lamb was scored, and their behavioral data was combined into a single variable. For example, if a ewe had two lambs, their raw durations of 'lying inactive' were combined into a single variable and divided by the amount of time one lamb was observed on video (~10 min). Combining the data in this way allowed for a single behavioral variable to encompass each lamb's activity, which was typically double what the ewes with singles were experiencing.

To assess the relationship between behavioral variables and lamb weights, birth and weaning weights were adjusted for day born and sex. Unfortunately, the majority of data on the hour of birth was missing and could not be included in the models (section 5). What data was available on hour of birth was included as a covariate in models assessing the ewes behavior. Hour of birth can certainly be an important factor in terms of the ewes interactions with the lamb and should normally be included in the models. Raw measures of birth and weaning weights were used in analysis to look at the relationship between behavioral performance and lamb outcome variables for single bearing ewes. Growth rates in singles were established by subtracting birth weight from weaning weight. For multiple bearing ewes, birth and weaning weights were combined into a single variable (CombWGT). Growth rates in multiples were established by taking the sum of the two weaning weights minus the sum of the two birth weights.

Data from the Lamb Handling and Lamb Tie Down tests were entered into PCA representing the 29 individuals that were present either year to explore for latent behavioral traits and to summarize the number of variables into more manageable components. Twenty-two ~~two~~ individuals consistently lambed or were present on camera for observation each year. Data were subject to Bartlett's test of sphericity ($P<0.05$) (Comrey and Lee, 1992) and the KMO (Kaiser-Meyer-Olkin) index ($\geq$0.60) as an indication of sampling adequacy (MSA). Principal components with eigenvalues ≥ 1 was retained for interpretation. Loadings of each variable were considered to be associated with the component if they were equal to or exceeded 0.4 (Table 2). Using the least squares regression approach, ewes received a score for each principal component that had a satisfactory eigenvalue, and these scores were used to indicate the location that ewe assumed on the composite trait. This approach is standardized and produces scores like a Z-metric, with values ranging from -0.30 to 3.0. Within subject consistency using Spearman's rank order correlations, with alpha set to 0.05, were used on the twenty-two individuals that were consistent between years 1 and 2 to explore if ewes had similar locations on the composite traits between years 1 and 2.

Linear effects models for repeated measures and *REML* using the glmmTMB package were performed to assess if composite traits were significant predictors of performance and outcome variables in the lambs (birth weights, weaning weights, growth rates). Outcome variables were fit as the response with day of birth as a covariate, lamb sex as a fixed effect, subject as a random effect and composite traits as a linear predictor.

2.4 *Post hoc analysis*

Lamb sex was not a significant term in the Lamb Handling test, and so was initially not included as a fixed term in the Lamb Tie Down models. Post hoc analysis revealed that inclusion of a male lamb was a significant term for behaviors that went into the PCA for the Lamb Tie Down test (Table 5).

3. Results

3.1 Lamb Handling

Principal component analysis revealed two composite traits within each year from the five behavioral variables entered (pacing, environmental vigilance, grazing, open mouth bleating and investigating the human) of the Lamb Handling test. Within the first year, the two composite traits that manifested explained (60.5%) of the variance amongst data. PC 1 can be characterized as having a positive loading for pacing (>0.40) and negative loadings for environmental vigilance, grazing and investigating the human (< -0.40). PC 2 from the first year can be described as having negative loadings (> -0.40) for pacing, investigating the human and grazing. With the same variables entered into PCA the second year, two composite traits manifested that explained (62.3%) of the variance in the data. The first PC explained 38.5% of the variance in the data and can be characterized as having negative loadings (< -0.40) for open mouth bleating and pacing. The second PC in year 2 explained 23.9% of the variance and can be characterized as having a positive loading for investigating the human and a high positive loading (>0.75) for grazing (Table 2).

Spearman's rank order correlations revealed a significantly negative correlation between the first PC from year 1 and the second PC from year 2 ($r_s = -0.70$, $P < 0.001$). Given the behaviors that determine this trait, it is referred to as the 'pacing/ avoidance of human' response

(Figure 1). In lieu of maternal behavior scores from previous research, consistency of duration of ‘close proximity’ to the human and lamb(s) was explored to assess viability of this measure as an indication of individual differences in ewes during lamb processing. Duration of ‘close proximity’ was not consistent between years ($r_s = 0.26$, $P = 0.30$).

3.2 *Lamb Handling responses and relationship to lamb outcome*

Results from the linear mixed effects models with the fixed effect of lamb sex and day of birth were used to explore the relationship between behaviors from the Lamb Handling test and principal components. Inclusion of PC 1 (‘pacing/avoidance of human’ trait) from year 1 did not improve fit in the models over the null for birth, growth and weaning weights. According to Spearman’s rank order correlations adjusted for multiple tests, there were no significant relationships identified between component 1 of year 1 (‘pacing/ avoidance of human’ response) and maternal behaviors including ‘udder allowance’, ‘sniffing/nosing/licking’, ‘closed proximity’ or ‘closed mouth bleating’. Duration in ‘close proximity’ to the lamb and shepherd was a significant predictor of weaning weights in multiples ($z = 2.18$, $P=0.03$). Specifically, greater durations of ‘close proximity’ lead to greater weaning weights for multiples and lower weaning weights for singles. Duration in ‘close proximity’ to the lamb and shepherd was also a significant predictor of lamb birth weights for multiples ($z= 4.4$, $P < 0.0001$). As duration of ‘close proximity’ increased, lamb birth weights also increased for multiples. Duration of ‘close proximity’ was not a significant predictor of growth for single and twin lambs across both years of the study. In the second-year duration of ‘close proximity’ was again a significant predictor of birth weights for multiples ($z = 5.46$, $P <0.0001$).

3.3 *Lamb Tie Down*

Principal component analysis revealed two composite traits within each year from the four behavioral variables entered (pacing, environmental vigilance, grazing, open mouth bleating and pawing) of the Lamb Tie Down test. Within the first year, the two composite traits that manifested explained 68.7% of the variance amongst the data. The first PC from year 1 explained 44.2% of the variance and can be characterized as having strong negative loadings (<-0.40) for pacing and open mouth bleating and positive loadings (>0.40) for pawing (Table 3). The second PC from year 1 explained 24.5% of the variance amongst the data and can be characterized as having a high positive loading for grazing (>0.80) and a negative loading for pawing. Within the second year, two composite traits explained 64.2 % of total variation in the data. The first PC of year 2 explained 39.1% of the variance and can be characterized as having strong negative loadings (<-0.40) for 'open mouth bleating' and 'pacing', and a positive loading for 'grazing' (Table 3). The second PC of year 2 explained 25.1% of the variance and had a high negative loading for pawing (< -0.90). Spearman's rank order correlations revealed that the second PC from year 1 and year 2 had a significant positive relationship ($r_s = 0.55$, $P = 0.02$). This trait is subjectively entitled the 'probing' trait (Figure 2). As discussed in section 3.4, sex of the lamb was a significant predictor of the 'probing' response in year 1 (F =0.05; F/M < 0.01; M/M = 0.04). Sex of the lamb was not initially accounted for when assessing residual data for PCA.

3.4 *Lamb Tie Down responses and lamb outcome indicators*

Linear mixed effects models including the fixed effects of lamb sex and day of birth were used to evaluate the relationship between behaviors from the Lamb Tie Down test, and, lamb performance and outcome variables and maternal behaviors. The second principal component from both years of the 'probing' trait did not improve fit over the null model for any of the lamb performance or outcome variables. According to spearman's rank order correlations, corrected for multiple tests, there were no associations found between the 'probing' trait and durations of 'udder allowance', 'sniffing/nosing/licking', 'closed mouth bleating' or 'close proximity'. The second principal component of year 2 ('probing' trait) was related to duration of 'sniffing/nosing/licking' the lamb (r_s= 0.45, p=0.02) , however, this became insignificant after adjusting for multiple correlations.

3.5 *Relationship between adaptive maternal behaviors and lamb outcome*

Spearman's rank order correlations adjusted for multiple tests was used to explore for relationships between adaptive maternal behaviors. In the first year, duration in 'close proximity' to the lamb and 'sniffing/nosing/licking' the lamb were significantly correlated (r_s= 0.52, P=0.03). There were no significant relationships identified between adaptive maternal behaviors in the second year of testing. Linear mixed effects models with a fixed effect for lamb sex and day of birth were used to evaluate the relationship between maternal behaviors ('udder allowance', 'sniffing/nosing/licking', 'close proximity') and lamb outcome variables. In the first-year adaptive maternal behaviors were not significant predictors of birth weights for single and multiple bearing mothers. Duration of 'closed mouth bleating' (z = -2.86, P=0.004) and 'udder allowance' (z = -2.37, P=0.02) were significant linear predictors in the weaning weight models for multiple bearing mothers. Duration of 'udder allowance' (z = -2.47, p= 0.01) was not a significant predictor of lamb growth rates for single lambs, as was duration of 'close proximity' to the lamb (z = -1.68, P = 0.09). In the second year, duration of 'closed mouth bleating' was again a significant linear predictor of weaning weights for multiple bearing mothers (z = -2.03 , P=0.04), growth from birth to weaning for single bearing mothers (z = 3.33, P < 0.001) and a nearly significant predictor of growth in multiples ($z = -1.67$, $P=0.09$). Duration of ‘udder allowance’ was also a significant linear predictor of growth in single lambs ($z= -3.48$, $P <0.001$).

3.5.1 *Effect of lamb sex on ewe behavior*

Post hoc analysis revealed that sex of the lamb had significant effects on behaviors during the Lamb Tie Down test that went into the PCA, particularly if the ewe had a male lamb. The presence of a male lamb had significant effects of durations of ‘pawing’ (Y1 & Y2: $P=0.05$) , ‘close proximity’ (Y1: $P=0.01$) and ‘grazing’ (Y1: $P<0.001$). After controlling for the effect of day of birth and birth weight, sex of the lamb was a significant predictor in the ‘probing’ response models in year 1 in that there was a significant effect of having a single female lamb ($p<0.001$), a single male lamb ($P<0.01$), twin female lambs ($P=0.001$), twin male lambs ($P<0.001$) and a male and female lamb ($P<0.0001$) (figure 5). Unfortunately, the sample size was too small to look at differences between sex categories, however, visually it appeared that having twin males was associated with high scores on the ‘probing’ trait in the first year. Sex of the lamb was not a significant factor in the ‘probing’ trait model for year 2. Since the second year ‘probing’ trait was mostly represented by negative loadings for ‘pawing’, which was effected by lamb sex, it is possible that the correlation between PC 2 of year 1 and PC 1 of year 2 is driven by duration of ‘pawing’.

4. Discussion

The present study evaluated the consistency of behavioral traits among extensively managed ewes during two contexts: at lamb processing with the human present and during lamb restraint with no human present. While ewe behavior at lambing time, namely proximity to the shepherd, is often used as a predictor for ‘maternal quality’ (O’Connor et al., 1985), this response to human presence may not actually predict lamb growth or weaning weights (Yilmaz et al., 2011; Moraes et al., 2016). Yilmaz et al. (2011) reported very poor repeatability with ewe maternal behavior scores (MBS) collected while the shepherd was tagging the lambs. Other studies have reported that shepherds may benefit from selecting ewes based on MBS (Brown et al., 2016) and scores >3 may generate better lamb survival rates due to a reduced risk of starvation (Everett-Hincks and Dodd, 2008). The current study did not look at subjective MBS explicitly, and instead evaluated the ewe's proximity to the lamb and shepherd based on the ewe's body length; allowing for a proximity measure 'within' or 'outside' of her body length to establish categorical differences in the distance the ewe maintained from the shepherd and the lamb. For the current study, propping flags to indicate distance from the shepherd may have startled the ewes and so this was avoided. Additionally, MBS is often reduced to binary or tertiary categories either due to low variance in scores within a flock or negligible statistical differences between scores in lamb outcome and other maternal behaviors. In the current study, duration within 'close proximity' to the shepherd and lamb pair during processing was not consistent between years, aligned with previous studies finding low repeatability in MBS. This measure was also not consistently related to lamb growth (birth to weaning) and weaning weights. Duration of 'close proximity' to the lamb(s) and shepherd was however related to birth weights for multiples in both years of the study. As birth weights increased, so did duration of 'close proximity' despite effects of sex and day of birth. In a study with captive Bongos, birth weight of the offspring influenced maternal investment such that heavier offspring received more care (Forthman et al., 1993). Maternal care in sheep is generally more variable when the offspring are multiples (Silva et al., 2020), and it could be that the ewe may invest more in multiples of heavier weights. Silva et al. (2020) also found that ewes obstructed suckling bouts less with singles compared to twins in lambs that were 10 – 20 days of age. Duration of 'close proximity' to the lamb during the Lamb Handling test was not consistent between years nor was it consistently related to other indicators of lamb outcome, so it is likely that this is a response influenced by lamb weights and reflective of maternal investment.

Results of the current study indicate the presence of a 'pacing/avoidance of human' response characterized by positive loadings for 'pacing' and negative loadings for 'investigating human' and 'grazing' in the first year and a secondary response (explaining less variance) in the second-year that had a negative relationship to PC 1 in the first year, manifesting with positive loadings for 'investigating human' and 'grazing'. This 'pacing/avoidance of human' response was unrelated to indicators of lamb outcome and is related to the ewe's willingness to approach or avoid a human or levels of fear/anxiety due to the lamb being handled. Inherent differences in ewe behavior at processing are present despite breed, age and parity differences, and could be representative of an underlying CID (Alexander et al., 1983). Though it would be convenient to be able to observe the ewe's behavioral activity during and after lamb processing, behavioral expression at that time was unrelated to maternal care and lamb outcome in the current study. Even behaviors during the Lamb Tie Down test that formed a 'probing' trait were unrelated to lamb outcome measures after controlling for lamb sex. In terms of the current study, it is possible that the stress induced due to the processing event is enough to obscure normal displays of maternal behavior that may otherwise be observed during later periods in the lambing season. When identifying ewes that perform adaptive maternal care and can raise lambs to weaning, it would be best to observe individuals when they are alone with the lamb, before processing or days after. Shepherds should consider observing displays such as licking and grooming, low-pitched bleating and durations of close contact with the lamb (Dwyer and Lawrence, 2005).

4.1 *Relationship between the Lamb Handling test and indicators of lamb outcome*

Due to the seemingly unique presentation of the 'pacing/ avoidance of human' response during the Lamb Handling test, during processing, it does seem that the human stimulus or act of handling the lamb may have activated a separate response related to the ewe's CID, level of fear/anxiety, or perception of the human. From previous studies outside of the lambing season, the ewe's response to a human stimulus can be referred to as their propensity for risk-taking or level of 'activity' (Beausoleil et al., 2008; Yu et al., 2021) and is repeatable when assessed in the arena test, involving a stationary human between the ewe and their flock mates (Murphy et al., 1994). Further, Dodd et al. (2012) states that responses towards a human in the arena such as vocalizations, locomotion and fear or 'boldness' related behaviors are replicable and can be a true measure of CIDs in the ewe, corroborated by Cakmakci et al. (2022). To date, few studies have investigated for consistent CIDs between the dry and lambing season that observe behaviors other than flight or retreat distance from the human. Aydogdu and Karaca (2021) did report that ewes selected for increased or decreased behavioral reactivity based on arena and scale tests (response towards humans in restraint) showed little difference in maternal care and maternal behavior scores. Responses within isolation and to a human stimulus in the arena was not associated with maternal recognition or preference in ewes during a choice test (Bickell et al., 2009). Gavojdian et al. (2015) reported no significant relationships between behavioral reactivity on the scale and litter size, daily gain, or growth rates of unweaned lambs, concluding that selection for CIDs in this context will not result in satisfactory improvement in performance of the lamb. Though the current study does not report results from outside of the lambing season it is possible that the behavioral trait(s) previous studies refer to (Aydogdu and Karaca, 2001; Bickel et al., 2009; Gavojdian et al., 2015) are related to the single 'pacing/ avoidance of human' response observed in the Lamb Handling test at lambing. This trait could also be connected with the ‘shy’ versus ‘bold’ or docility spectrum given that willingness to graze in the presence of, or even investigate (i.e., sniff), a potential threat (i.e., human) may be considered a risky choice. Though it is possible the ‘pacing/ avoidance of human’ response is related to levels of boldness, previous studies have found that increased locomotion in the presence of a human is reflective of greater ‘boldness’ (Beausoleil et al., 2012) , which seems contradictory to results of the current study.

The ‘pacing/ avoidance of human’ response may also be related to levels of anxiety experienced by sheep, as Doyle et al. (2015) reported that sheep administered an anxiolytic showed lower feed motivation in an open pen with auditory and visual contact to conspecifics. Feeding behavior varies between individuals in domesticated ruminants and individuals that show greater levels of fear or reactivity towards a human, in the presence of a human, will restrict feeding more often compared to less fearful or reactive individuals (Neave et al., 2018). Sheep that scored high on the ‘pacing/ avoidance of human’ response, with lower durations of grazing, may have also been more social compared to conspecifics with longer durations of grazing. Levels of sociability and fearfulness tend to covary together in sheep, and individuals considered highly social were less likely to trade-off the opportunity to graze if it meant being separated from conspecifics at large distances (Sibbald and Hooper, 2003). Though differences in sociability is another potential explanation, ewes almost always choose to isolate themselves from the flock in the early postpartum period, making the ‘sociability’ paradigm hard to explore during this stage. Shillito-Walser et al. (1983) reported that ewes challenged with a T-maze test given the choice to join flock mates or rejoin close contact with the lamb largely made the choice to be near the lamb. Alternatively, it is possible that variation in this response could be due to differences in the perception of the human stimulus, as sheep that have had previous aversive experiences with handlers are more likely to avoid humans compared to sheep that have received gentling (Destrez et al., 2013). Ewes subjected to postpartum stressors including isolation from conspecifics and the lamb, presence of herding dogs, and presence of unfamiliar humans in a pen performed less nursing, low-pitched grunts and nuzzling/licking of the lamb compared to control ewes (Leedy and Alexander, 2007).

Within the Lamb Handling test, lamb birth weights in multiples were positively related to durations in 'close proximity' to the shepherd and lambs for both years of the study. Tradeoffs between degree of maternal investment and lamb size have an adaptive evolutionary basis in most mammalian species (Keller and Chasiotis, 2007). With sheep, the traditional belief was that ewes will put more investment into singles compared to twins (Forthman et al., 1993), however, Wilson et al. (2009) found that bearing twins will lead to greater fitness (survivability and reproductive success) benefits across a variety of environmental conditions but especially those that promote offspring survival (mild weather). Perhaps ewes that spent longer durations in 'close proximity' to the lamb and shepherd at processing gave birth to optimally sized twin lambs that would be more likely to withstand the extensive rangeland environment. Ewes that gave birth to lighter lambs may have not gotten in close proximity to the human due to the costs of approaching the human being higher than the cost of abandoning the lamb. These findings could also explain why 'close proximity' was not an inherent or consistent quality of the ewe. Everett-Hinks (2005) concluded that proximity to the shepherd (i.e., MBS) is vulnerable to temporary environmental effects and likely to change between seasons. Yilmaz et al. (2001) also found that heritability and repeatability of MBS is low (0.09) and shepherds desiring to increase litter survival may focus more on management strategies over scoring the ewe at processing.

Survivability of the lamb is highly dependent on lamb birth weights, so it seems reasonable to posit that breeding simply for increased lamb weights could harness better flock performance *and* survivability if this is coupled with good shepherding at the beginning of the postpartum period.

4.2 *Relationship between the Lamb Tie Down test and adaptive maternal behaviors*

Behaviors from the single 'probing' trait in the Lamb Tie Down test were influenced by sex of the lamb in the first year, especially if the ewe had twin male lambs. This trait was not related to indicators of lamb outcome (growth and weaning weights). In the first year the 'probing' trait during the Lamb Tie Down test possibly manifested due to the lamb's experience of pain. Though the objectives of the current study were to explore for individual traits moderated by the ewe herself, the influence of lamb pain on maternal behavior during processing is often not a consideration on sheep operations. Futro et al. (2015) found that the experience of castration increased pain related behaviors of the lamb, especially in those that received tight rubber rings for castration. Male lambs in the current study were rubber band castrated and tail docked and more than likely experienced high levels of pain during the Lamb Tie Down test relative to female lambs who only received tail docking with the rubber ring. Futro et al. (2015) found that ewes licked/sniffed lambs more after lambs received procedures inducing more pain related behaviors. Hild et al. (2011) subjected 3- and 4-day old lambs to pain and stress treatments including social isolation (stress) and tail docking (pain) whilst recording the ewes' behavior. According to Hild et al. (2011), the duration of time the ewe spent investigating the lamb and number of glances at the lamb was related to the lamb's active pain avoidance and postural behavior. There were no differences observed in the ewes' behavior between stress and control lambs. The current study controlled for covariation of lamb behavior on ewe behavior, however, a more detailed behavioral ethogram including pain-related behaviors (rolling, site directed licking) in lambs is needed. All lambs (male and female) in the current study were assumed to be under some level of pain due to tail-docking, however, future studies should consider the impact of the ewes' perception of pain in their offspring as it relates to individual differences in maternal behavior.

Sex of the lamb influenced ‘pawing’ behavior in both years, yet no other behaviors in the ‘probing’ trait. In the second year, the ‘probing’ trait was most represented through negative loadings for ‘pawing’, which would explain the positive relationship between year 1 and 2 despite the lack of sex effects in the year 2 ‘probing’ trait. In other words, it is likely that behaviors that went into the year 2 ‘probing’ trait masked the effect of sex that would otherwise been observed when looking at just the ‘pawing’ behavior.

4.3 *Relationship between adaptive maternal behaviors and lamb outcome*

Findings between maternal behaviors and lamb outcome variables in the current study were unusual. Generally, as adaptive maternal behaviors increased (closed mouth bleating, udder allowance) weaning weights and growth from birth to weaning decreased. The expected direction between adaptive maternal behaviors and lamb outcome would be that as one increases, so does the other. Maternal behaviors including licking/grooming, low-pitched bleating and udder allowance are known to be adaptive and beneficial for lamb survival (Dwyer et al., 2005). Previous studies have identified that the closer the ewe stays near the lambs during the early postpartum period, the more nutritive sucking the lambs may perform (Nowak, 1994; 1996) and the less likely the lamb is to die due to starvation from separation (Stevens et al., 1982), especially in twins. Duration of sucking is also highly important for the ewe and lamb pair to establish a strong bond based on preference (Nowak et al., 1997). It is possible that the methodological approach in the Lamb Tie Down test affected maternal behaviors, however, the exact explanation is unknown. Even so, the authors expected that there would be no relationship found between maternal behaviors and indicators of lamb outcome if maternal expression was obstructed by methods of the study. The only explanations that seem plausible is that ewes that regularly performed more adaptive maternal care throughout the lambing season did not display these behaviors during the Lamb Tie Down test if they were under stress or perceived their lambs to be in danger. Additionally, there may have been an environmental covariate unaccounted for during the Lamb Tie Down test. The lambs were tied down and had restricted movement which was likely stressful for both parties. There is not much literature that explores the effect of acute lamb stress on maternal behavior and so this is an area worth future investigation.

5. Limitations

Due to small flock size and animal drop out, this study did have a smaller sample size than previous studies assessing maternal behavior in ewes, which may have affected results. Other indicators of lamb outcome and survival such as ewe selectivity, receptivity, recognition, length of labor and precise weather conditions between years were not included in the study. Further studies that include lamb survival from birth to weaning should consider these environmental factors in analysis. PCA is a non-parametric technique to identify clusters of interrelated behaviors, and it is susceptible to outliers or individuals that perform outside of the mean duration or frequency of responses. While video recording data, ewes were occasionally interrupted by yearling ewes interested in the lamb. This data was not included in the study; however, it is possible that the behavior of the yearling ewes influenced maternal ewe behavior . In the current study, all lambs were tail docked and assumed to be experiencing some level of pain, however, only males were castrated. Due to methods of the Lamb Tie Down test, it is possible that the ewe and lambs were under stress and that regular maternal behavior was inhibited. Hour of birth was included in the models with ewe behavior as a response, however, most of this data was missing in year 2 and so it was hard to assess if the number of hours between birth and observation was a significant covariate. Ewe-lamb interactions were observed during the Lamb Tie Down test, however, this was likely too stressful of an event to evaluate adaptive maternal care. Ideally, ewe-lamb interactions should have been observed directly before or a couple days – weeks after processing when the lamb is not tied down or in pain.

6. Conclusion

Scoring maternal behavior at the time of lamb processing is a procedure used to potentially infer the ewe's attachment to the lamb(s) and possibly the lamb's survival likelihood or physical outcome (i.e., birth weight, weaning weight, weight gain). Findings from the current study suggest that the presence of a human during processing could elicit a trait related to fearfulness, anxiety, or differential perception of the human handler that may be moderated by separate biological processes relative to those moderating maternal behavior. Durations of 'close proximity' to the lamb(s) during processing (similar to the Maternal Behavior Scoring System; O'Connor et al., 1985) were related to indicators of lamb birth weights, however, this measure itself was not consistent across years and was driven by lamb weights. It is likely that this measure is mainly driven by maternal investment, in that ewes with larger lambs had more to lose compared to ewes with smaller lambs and so stayed near the shepherd/lamb pair during processing. Maternal behaviors after processing, while the ewe and lamb were alone, were related to indicators of lamb physical outcome (i.e., durations of licking/sniffing, sucking and weaning weights), however, not in the expected direction. Though this is not supported by the current study, behavioral selection based on expression during and after the processing period may not lead to better lamb outcome over simply good human management, nutrition (Dwyer, 2008) and shelter provision. It would be best to consider observing the ewes before processing or days – weeks after processing when the lamb is not in pain.

7. Figure Captions

Figure 1. Scatterplot of the PC 1 from the first year and PC 2 from the second year of the Lamb Handling test. PC 1 of the first year and PC 2 from the second year had a significant negative relationship (r_s = -0.70, P <0.001).

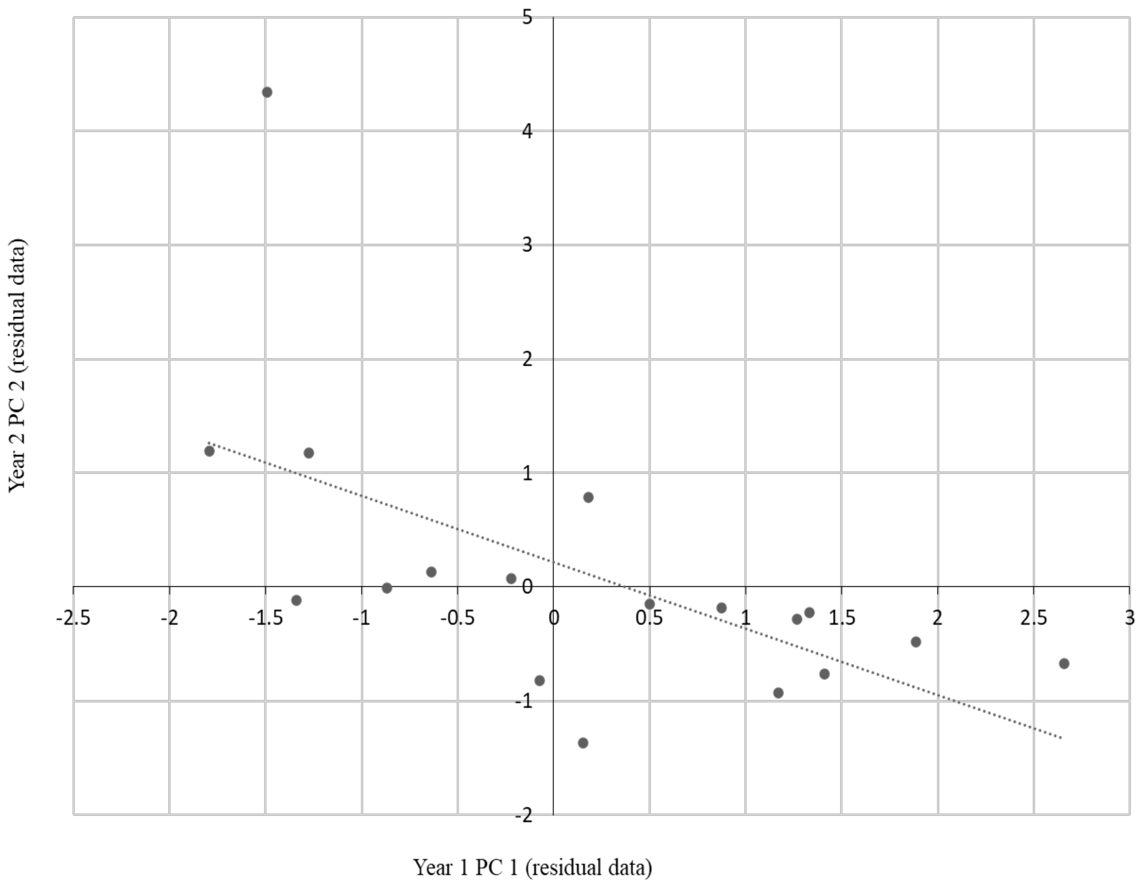


Figure 2. Scatterplot of PC 2 from the first and second year within the Lamb Tie Down test. PC 2 from year 1 and Year 2 were significantly and positively correlated (r_s=0.55, P=0.02).

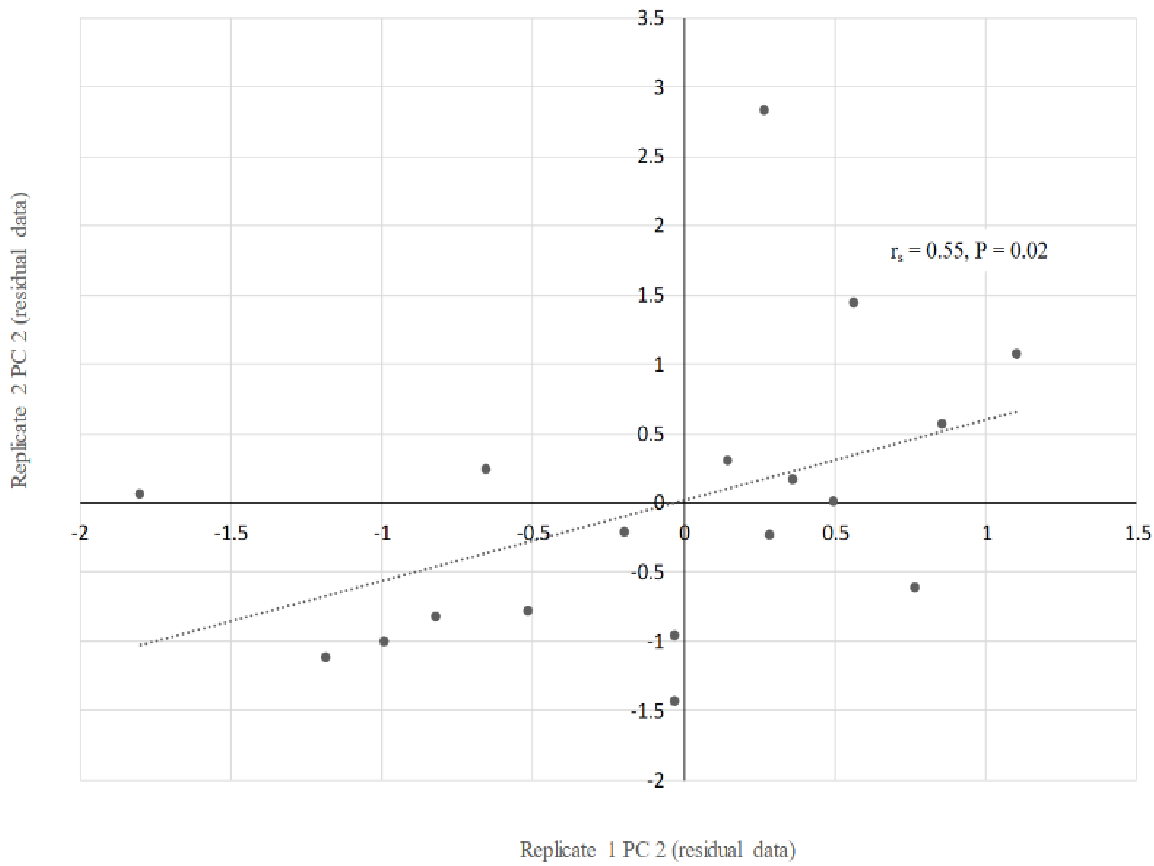


Figure 3. Differences in ‘probing’ between single male and female lamb, two male and female twin lambs, and male and female twins lambs. There was a significant effect of having a single female (P<0.001), male (P<0.01), a male and a female as twins (P<0.0001), twin female lambs (P=0.001) and twin males lambs (P < 0.001).

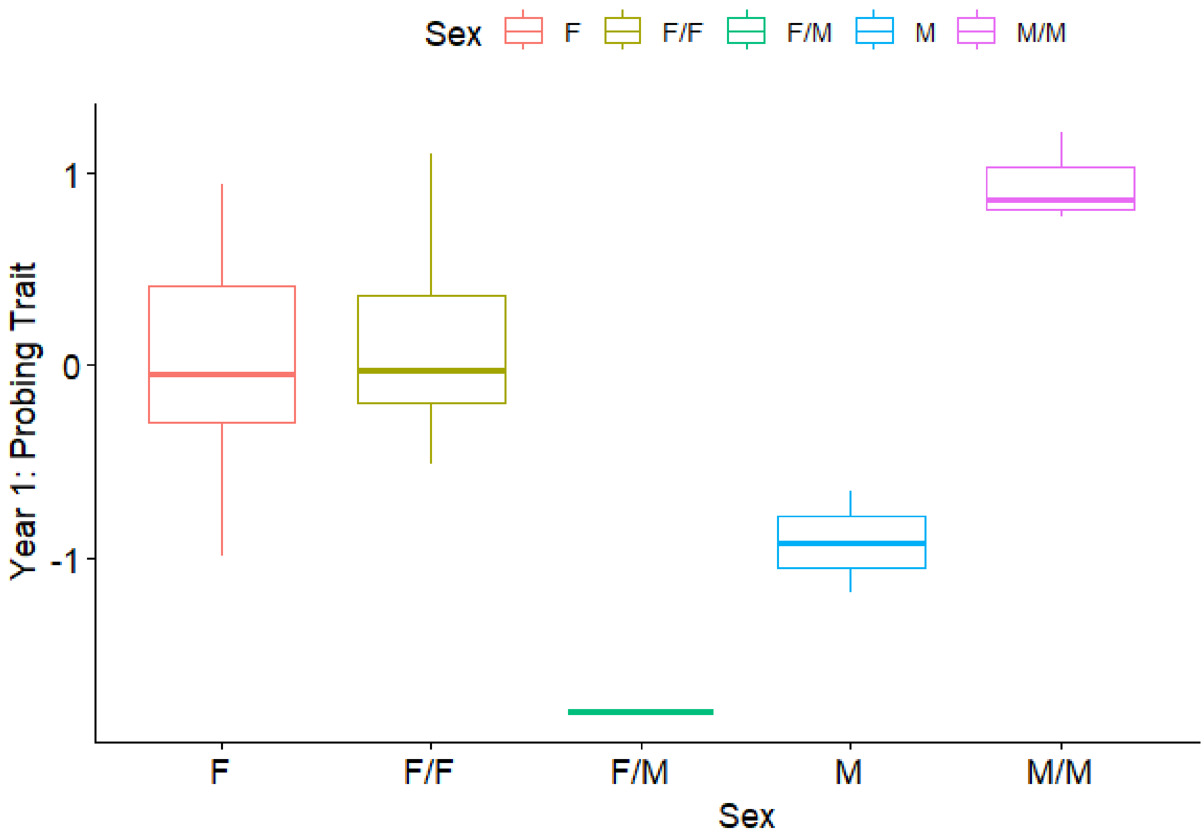
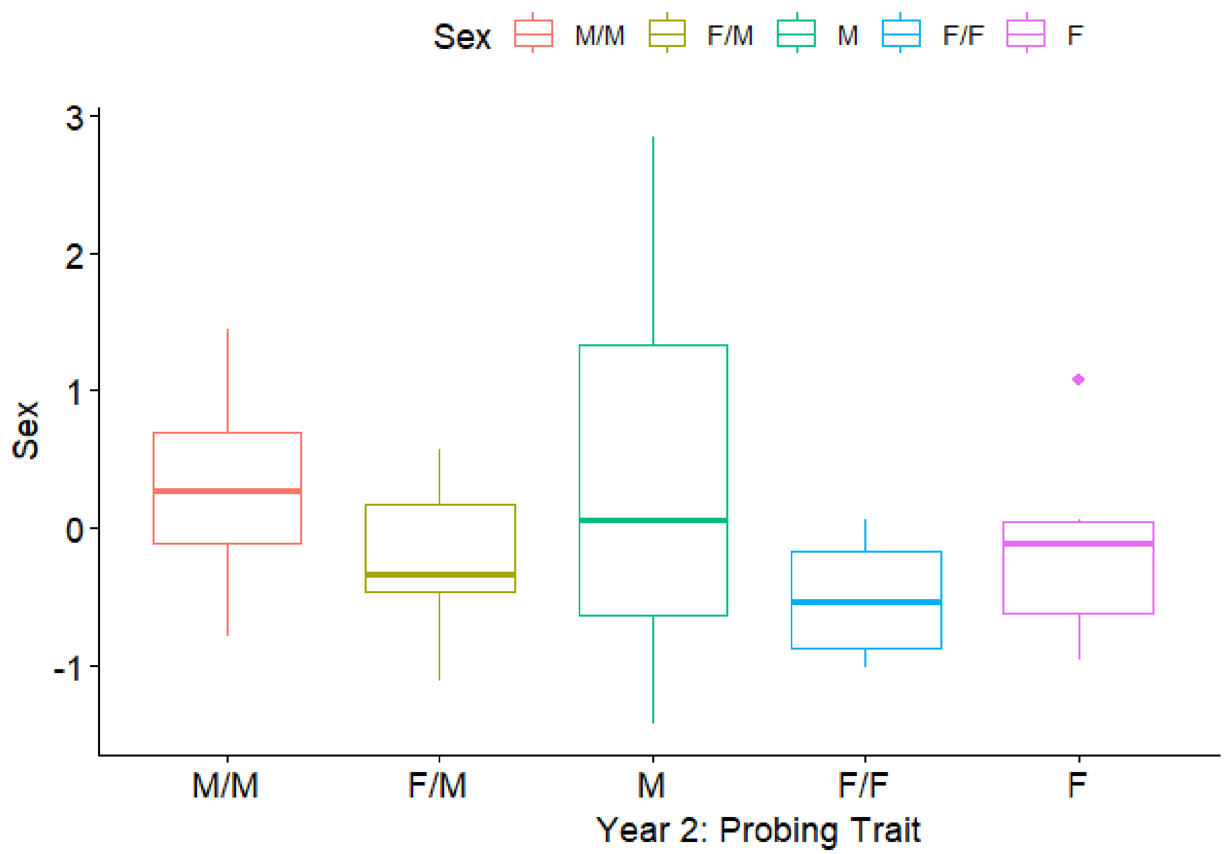


Figure 4. Similarities in 'probing' across lamb sex categories including single male and female lambs, twin male and female lambs, male twins and female twins. There was no effect of sex on the 'probing' trait across all lamb sex categories.



8. Tables

Table 1. Operational definitions of ewe behaviors recorded during the 'Human Presence' and 'Post Human Presence' tests.

Behavior	Operational definition
Vigilance (environmental)	Focal sheep has head positioned at or above shoulder line; nose is pointed away from the human and lamb; ears are erect and perpendicular to head (inner ear is facing forward) OR directly backward from position of muzzle and body/legs are still (motionless); ewe may or may not be chewing feed simultaneously; ears are in aligned position
Vigilance (human/lamb)	Focal sheep has head positioned at or above shoulder line; nose is pointed in the direction of the human and lamb; ears are erect and perpendicular to head (inner ear is facing forward) OR directly backward from position of muzzle and body/legs are still (motionless); ewe may or may not be chewing feed simultaneously; ears are in aligned position
Sniff/Lick/Nose (lamb)	Focal sheep is making direct contact with lamb(s); can be contacting the lamb(s) with nose or any part of the head including tongue
Investigate (human)	Focal sheep is making direct contact with human using feet, snout, head or side of body; sheep is sniffing/chewing human with snout or licking human with tongue
Graze	Focal sheep has head below shoulders; and is sniffing or manipulating grass with nose/mouth or ingesting vegetation with mouth; snout is contacting or close to ground; ewe may or may not be motionless; may fluctuate between walking and grazing
Walk	Focal sheep takes at least two consecutive steps with right foot; head may be positioned above, at or below shoulder line; ≤3 steps per second
Pace	Focal sheep takes at least two consecutive steps with right foot; head may be positioned above or below shoulder line; >3 steps/ second

Stomp	Focal individual has ceased pacing or walking and remain still; focal individual lifts one foot off the ground and placed foot down in same position; wool or flank may shake; focal individual makes no forward or backward movement
Open mouth bleating	Individual makes audible, open mouth vocalization
Closed mouth bleating	Individual makes audible, closed mouth vocalization
Close proximity	Ewe is within her body's length of the lamb and shepherd

Table 2. Composite traits from year 1 and 2 of the Lamb Handling test. The Lamb Handling test manifested one consistent behavioral trait between years consisting of PC 1 in the first year and PC 2 in the second year. These PCs were significantly, negatively correlated (r_s= - 0.70, P<0.001)

Lamb Handling test		
Year 1	PC 1 (40%)	PC 2 (20.6%)
Pacing	**0.52**	**-0.48**
Environmental Vigilance	**-0.53**	0.39
Investigating Human	**-0.40**	**-0.63**
Open-mouth bleating	0.31	0.02
Grazing	**-0.43**	**-0.47**
Year 2	PC 1 (38.4%)	PC 2 (23.9%)
Pacing	**-0.61**	0.07
Environmental Vigilance	-0.35	0.23
Investigating Human	**0.48**	**0.55**

Open-mouth bleating	**-0.60**	0.21
Grazing	0.05	**0.77**

Table 3. Composite traits from year 1 and 2 of the Lamb Tie Down test. The Lamb Tie Down test manifested one consistent behavioral trait between years consisting of PC 2 in both year 1 and 2. These PCs were significantly, positively correlated (r_s= 0.55, P=0.02)

Lamb Tie Down test		
Year 1	**PC 1 (44.2%)**	**PC 2 (24.5%)**
Pacing	**-0.62**	0.21
Grazing	0.27	**0.88**
Open-mouth bleating	**-0.56**	-0.15
Pawing	**0.47**	**-0.41**
Year 2	**PC 1 (39.1%)**	**PC 2 (25.5%)**
Pacing	**-0.57**	0.03
Grazing	**0.49**	0.06
Open-mouth Bleating	**-0.66**	0.10
Pawing	-0.05	**-0.99**

Table 4. Effect of lamb behavior on ewe behavior. Lamb activity in the Lamb Tie Down test. including ‘active standing’, ‘inactive standing’, ‘active lying’, ‘inactive lying’ and ‘vocalizations’ was a significant term in a variety of the models. A/S = active standing; I/S= inactive standing; A/L = active lying; I/L = inactive lying; vocalizations = voc.

Behavior	Estimate	Std. Error	t-value	P-value
Pacing Y1 (I/L)	0.1772	1.1863	0.149	0.882
Pacing Y2 (I/L)	-3.8330	2.0016	-1.915	0.0661 .
Grazing Y1 (I/L)	-0.2442	0.7750	-0.315	0.75527
Grazing Y2 (I/L)	1.2467	0.5835	2.137	0.0418 *
Invest. Lamb Y1 (I/S)	0.8107	0.4849	1.672	0.10701
Invest. Lamb Y2 (I/L)	-0.84035	0.27757	-3.027	0.00537 **
Envo. Vig. Y1 (I/L)	0.9694	0.5283	1.835	0.0785 .
Envo. Vig. Y2 (A/S)	-1.0683	0.4631	-2.307	0.02898 *
Vig. Lamb Y1 (A/S)	0.8273	0.4368	1.894	0.0699 .
Vig. Lamb Y2 (I/L)	0.1243	0.5107	0.243	0.809
Open mouth voc. Y1 (voc)	-0.002506	0.004565	-0.549	0.588
Open mouth voc. Y2 (voc)	23.1192	7.8402	2.949	0.00651 **
Closed mouth voc Y1 (voc)	-0.01092	0.14089	-0.078	0.939
Closed mouth voc Y2 (voc)	7.1191	9.5157	0.748	0.461

Table 5. Effect of lamb sex on behaviors from the Lamb Tie Down test. Sex was a significant term for duration of 'pawing' in years 1 and 2, duration of 'close proximity' in year 1 and duration of 'grazing' in year 1 (Year 1 data = Y1; Year 2 data = Y2).

Behavior	Estimate	Std. Error	t-value	P-value
Pawing Y1	-4.4418	2.1656	-2.051	0.0505 *
Pawing Y2	-5.1249	1.8562	-2.761	0.0109 *
Close Proximity Y1	2.3475	0.8941	2.625	0.01483 *
Close Proximity Y2	-1.831	2.744	-0.668	0.510
Grazing Y1	-2.2081	0.5056	-4.367	0.000208 ***
Grazing Y2	0.4628	1.4761	0.314	0.7564

CHAPTER 4

Consistent behavioral patterns during pre-and postnatal human animal interactions in rangeland breeding ewes

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Abstract

Consistent individual behavioral differences (CIDs) among livestock are known to be inherent qualities of the animal that are repeatable over time, across contexts, and can be related to production. Shepherds rely on qualities of the ewe to promote lamb health, survival and performance, and selecting ewes based on desirable phenotypes may be one way to benefit lamb outcome. Previous research indicates that traits observed among breeding ewes in restrained contexts during human-animal interactions (HAIs) may have a greater association with maternal care and lamb outcome compared to responses in an open testing environment. The current study investigated the relationship among various behaviors in multiparous ewes (n = 29) in two distinct contexts: human-animal interactions during post-breeding, gestation and weaning (no lamb present) and when the lamb was present 6 – 36 hours after parturition. Ewe responses to human contact while in the raceway during a Human Contact test and responses to human presence in a modified open field testing (mOFT) during a Human Presence and Human Approach test were recorded. Ewe responses were also recorded after parturition during a Lamb

Handling test (at processing) and after processing during a Lamb Tie Down test. Loadings from multiple factor analysis (MFA) were used to assess the association of each behavioral variable to a dimension and were considered significant if they were >0.40. MFA revealed negative patterns of association between rate of 'open mouth bleating' (>-0.60) during the Lamb Tie Down test and duration of 'head down' behavior during the Human Contact test at post-breeding (0.58) and weaning in year 1 (0.71), and, at post-breeding (0.64) in year 2. For multiple-bearing mothers, lamb birth weights were also associated with duration of 'head down' behavior during human contact at weaning in year 1 (P=0.05) and at post-breeding in year 2 (R4: P<0.001). Duration of 'close proximity' to the lamb during the Lamb Tie Down test was associated with duration in the 'zone with human' during the Human Presence test within year 1 at post-breeding and gestation and within year 2 at post-breeding. Behaviors from the restrained raceway test and unrestrained mOFT can be used to gauge aspects of maternal care and lamb outcome in sheep. Previous research indicates unrestrained responses towards a human stimulus are not associated with aspects of maternal care or lamb outcome, however, the specific mOFT set up in the current study is able to elicit biologically relevant responses from the ewe in this respect. With the increasing interest in precision breeding of commercial flocks for behavioral and physiological traits, the results of this study suggest that routine assessment of ewe response to human proximity could be used as a management tool to select ewes with desirable phenotypic traits.

1. Introduction

Extensively farmed sheep may be subject to numerous challenges throughout their lifetime including exposure to harsh weather conditions (Dwyer and Lawrence, 2005; Nowak and Poindron, 2006), infrequent and stress-inducing interactions with human handlers (Porciuncula et al., 2022), potential predation risk, and minimal assistance with raising lambs on range. Lambing specifically is a time of intense resource and financial acquisition for the farmer and a period of high vulnerability for the ewe and lamb-pair (Dwyer, 2008). Lamb mortality could be as high as 44% (Alexander et al., 1974) when weather conditions are poor and management input is minimal. Another issue that can exacerbate lamb loss is poor bond establishment between the mother and lamb (Nowak, 1996, Nowak and Poindron, 2006). Behavioral interactions between the ewe and lamb, including suckling bouts and frequency of vocalizations (Nowak, 1996) and duration of sniffing (Alexander and Shilllito, 1985), are crucial for bond development between the pair. Without the proper facilities in place nor adaptive maternal behaviors performed (i.e., low-pitched bleating, allowing udder access) the lamb may suffer starvation or hypothermia and consequently poor welfare (Dwyer, 2008) resulting in death.

Amongst low stress handling practices and allocating proper shelter and care, shepherds may select animals based on observable behavioral responses that are more likely to be accommodated to their farming environment and able to raise lambs until weaning. Behavioral responses towards a human, explicitly, are the most convenient to observe and record and may be incorporated into the current program under most farming systems during common interventions (e.g., deworming, weighing, transportation). According to previous literature, response towards a human while ewes are restrained and not with offspring, may be indicative of adaptive maternal behaviors, even over responses towards a human in the early post-partum period when the lamb is present (Yilmaz et al., 2011; Everett-Hinks, 2005). Maternal behavior scores, developed by O'Connor et al. (1985), showed promise as a tool for assessing ewes based on retreat distance from the shepherd during lamb processing, however, more current research indicates that this response will have little impact on lamb outcome (Lambe et al., 2001; Aydogdu et al., 2021).

Previous literature suggests that ewe behavior, when not with offspring, in unrestrained experiments involving human proximity or approach (e.g., arena test, open field test, yard test) are negligibly related to response towards a human in the early post-partum period or maternal behavior and are negligibly related to indicators of lamb outcome (Aydogdu and Karaca, 2012; Peeva, 2009). On the other hand, ewe behavior towards a human during physical restraint tests (e.g., scale, squeeze chute, raceway) may be more indicative of maternal behavior and lamb growth and performance (Dodd et al., 2012; Plush et al., 2011), such as live weight and post-weaning weight gain (Pajor et al., 2010; Gavojdian et al., 2015). The current study used multiple contexts (restrained and unrestrained) to assess the relationship between responses towards the human when the lamb is not present (June – January) to responses when the lamb is present in the postpartum period. To assess restrained responses of the ewe towards human contact, grouped ewes received a brief handling treatment using a common raceway setup. Ewes were then entered into a modified open field test (mOFT) for the Human Presence test to assess unrestrained responses towards a stationary human. Finally, response towards an approaching human were assessed when ewes were released from the Human Presence test. Behaviors performed during a close Human Contact test in the raceway (e.g., 'stepping', 'head up', 'head down'), are expected to be related to adaptive maternal behaviors (i.e., udder access, closed mouth bleating, sniffing/licking/nosing) and lamb outcome (birth, growth and weaning weights). This assumption is based on previous literature indicating a connection between lamb outcome and ewe behavior in environments involving restriction of movement (Dodd et al., 2012). Authors of the current study also expect a weak to no association between behavior in the

Human Presence test (e.g., duration in ‘zone with human’, ‘alertness’ response) and behaviors during the lambing season as previous research has indicated that unrestrained responses outside of lambing are not unrelated to maternal behaviors and lamb outcome (Dodd et al., 2012).

2. Methods

2.1 *Animals*

See chapters 2 and 3 (section 3.1) for full description of animals and husbandry. In brief, the current study was approved by the University of California, Davis Institute of Animal Care and Use Committee (protocol# 20926). Without the lamb present (post-breeding, gestation, and weaning) assays were conducted at two handling sites (Blue Oak Ranch [site A] and Belmantro Station [Site B]) located in the Sierra foothills of Auburn, California, USA. The study flock consisted of terminal Shropshire (n=20; terminal line), Blue-faced Leicester x White face crossbred ewes (n= 20; replacement line) and Blue-face Leicester x Mule crossbred ewes (n= 20, terminal line) kept as a subset of 120 animals. Observations at lambing season were conducted on the (n= 29) individuals in year 1 and 2 who lambed. Repeated human-animal interaction trials (HAIs) were performed at three coinciding times of the year across the 2 years of the study (2019 – 2021) for a total of six trials. Data collection occurred when the lamb was not ‘on the ground’, during post-breeding body condition and health checks when rams were pulled from the ewes (November), gestation when ewes received prenatal vaccinations (late January) and weaning when lambs were pulled from the ewes (mid-June) (Figure 1). The sixth replicate was dropped due to extremely high temperatures affecting ewe behavioral performance. Temperatures from June - October ranged from 12.8 °C and 18.3 °C at 0700 – 1400 hrs. Temperatures in the lambing season from February – early April ranged from 7.2 – 12.8 °C at 0600 – 0800 hours. Shepherds managing the study flock practiced low stress handling techniques in the ‘Bud-Box’ and selected ewes based on a subjective EZ Care system developed to score ewes at processing. This score included criteria for how well the mother followed the young when the shepherd picked up the lambs, lamb vigor and other aspects of maternal care. Shepherds had been using this system for 10 + years.

2.2 Experimental setup

Ewes were subjected to HAI trials between 2019 – 2020 when the lamb was not ‘on the ground’, which were repeated a year later between 2020 – 2021. Using a ‘Bud-Box’ set up, animals (n=5 per group) were handled with one hand under the muzzle and one hand on the rump in the raceway (1 x 15 m) for 10 sec during what was labeled the Human Contact test. This was followed by a 5 min observation period, using the same 5 animals, in the modified open field test (mOFT; 10.5 x 10.5 m) with a stationary human placed in the center to evaluate ewe response to human presence (Table 2) and labeled the Human Presence test. Animals were released by an approaching human who entered the mOFT and walked counterclockwise around the zone closest to the fence line until all individuals exited during what was labeled the Human Approach test. Four cameras (Sony Handycam DCR SX85; Sony Corporation of America, New York, NY, USA) stabilized on tripods were placed at every corner of the mOFT to video record the ewe’s response to the stationary human and approaching human. All ewes were familiar with handling in the raceway and mOFT prior to testing.

During lambing season (late February – early April), ewe behavior at the time of lamb processing (6 – 36 hours after birth) and directly after were recorded during a Lamb Handling and Lamb Tie Down test. Cameras were set up on tripods to record the ewe’s response to the shepherd handling her lamb, approximately ~10 m away from the shepherd and lamb pair during the Lamb Handling test. After lamb processing, the shepherd and researcher walked >30m away once the lambs were tied down by the back leg with twine and a camping stake for 10 min to video record ewe-lamb interactions during the Lamb Tie Down test. After the 10 min recording session, the shepherd or researcher would approach the lambs and gently release them from the twine. If the ewe was not near the lambs at the time or release, the shepherd would carry the lambs to wherever she was in the paddock.

For all tests in the dry, gestational and lambing season, interobserver reliability was established (Cohen's kappa = 0.80) prior to behavioral annotation of video data using The Observer XT v. 11 (Noldus Information Technology, Wageningen, Netherlands). See Chapters 2 and 3 (Table 1) for a full ethogram including operationally defined behavioral variables.

2.3 *Statistical Analysis*

Statistical analysis followed a similar structure to that of Chapters 2 and 3, in terms of residual data collection. Guidelines on data reuse were taken from Ranganathan et al. (2016). Ranganathan et al. (2016) advices a mixture of adjusting for potential Type I errors when reusing data and avoiding Type II errors when applying alpha level corrections (i.e., Bonferroni correction). Ranganathan also suggests a level of common sense be applied when assessing effect level and data reuse. In brief, using R statistical Software Version 4.2.1 (R Core Team 2018) distributions of variables were checked for normality. Data from when the lamb was not present in the Human Contact, Presence and Approach tests were found to be non-normal, zero-inflated or having a log/sqrt Poisson error distribution. Using the *glmmTMB* (Brooks et al., 2017) package in RStudio, raw data was controlled for extraneous (i.e., replicate, position in raceway) and endogenous factors (i.e., breed, age, parity, pregnancy status) (Table 1) and controlled for the random effect of group membership (n=5). Generating residual data using a linear mixed effect approach for repeated measures is similar to what Diess et al. (2009) used in order to enter residual data into factor/component analysis. Models also included restricted effects maximum likelihood (REML) and an autoregressive component for the repeated individual testing when necessary (e.g., duration of vigilance).

Data from when the lamb was 'on the ground' (February – April) was proportional in nature and therefore entered into general linear models with various fixed (i.e., year, litter size, parity, breed) or covariates (i.e., age, lamb activity, day of birth) included with a quasibinomial distribution. Lamb activity included variables that were durational in nature and involved lamb standing or lying down and whether or not they were active (legs moving) while doing so.

2.3.1 *Multiple Factor Analysis*

To assess cross-contextual relationships between when the lamb was not 'on the ground' (Human Contact, Presence and Approach tests) to when the lamb was 'on the ground' (Lamb Handling and Tie Down tests), multiple factor analysis (MFA) was performed within each year of the study on residual data. Multiple factor analysis is an advanced data reduction technique, used in the current study to explore for patterns of interrelated variables with one or more factor groups. MFA is similar to PCA, however, this analysis can allow you to introduce different grouping levels and allow you to look at the relationship between them. MFA allows data on different levels to be analyzed together to describe clusters of information. Groups in the current MFA included "Lambing" (Lamb Handling and Lamb Tie Down test) and "Gestation/Dry" (Human Contact, Presence and Approach tests) periods, for each year. Initially, all variables were entered into MFA to get a general idea of where clusters of inter-relatedness within the data set were present. Only select variables that achieved appropriate clustering adequacies, according to Kaiser-Meyer Olkein (KMO) values equal to or greater than 0.6, were kept and used to determine cross contextuality of relationships. Dimensions extracted were considered important if they achieved an eigenvalue >1. Loading values were used as a measure of association between individual variables and the dimension, and were considered important if > 0.40. Unfortunately, not all variables of interest could be entered into multiple factor analysis. Some variables, such as 'zone with human' and 'head down' in the raceway at gestation in the second year had to be omitted do to missing data points due to human error. MFA runs on a correlation matrix and cannot handle missing information.

Finally, general linear models using the *glmmTMB package* (Brooks et al., 2017) were used to look at the relationship between clusters of related behaviors from the MFA and lamb outcome variables including necessary covariate and fixed terms (Table 1). Lamb outcome variables were also analyzed against responses from the Human Contact, Present and Approach tests. Models with lamb outcome variables (birth, growth, and weaning weights) included a fixed term for sex and a covariate term for day of birth as these two variables were important terms to consider.

3. Results

3.1 *Year 1: Cross contextual multiple factor analysis*

Behaviors including 'open-mouth bleating', 'close proximity', and 'allowing udder access' from the Lamb Tie Down test, 'head down' from the Human Contact test, and 'zone with human' from the Human Presence test met an adequate clustering value and were kept for MFA. Using these behaviors, multiple factor analysis revealed two dimensions with eigenvalues >1 in the first year explaining 49.3% of the variance amongst the variables entered (Table 3). The first dimension (27.0%) is characterized by a positive loading for 'allowing udder access', negative loading for 'open-mouth bleating' , and positive loadings for 'head down' in the Human Contact test from post-breeding. The second dimension (22.4%) was characterized by positive loadings for ‘close proximity’ in the Lamb Tie Down test and ‘zone with human’ from the Human Presence test at gestation, and a negative loading for ‘zone with human’ during human presence test at post-breeding (Figure 3).

3.2 *Year 2: Cross contextual multiple factor analysis*

For the second year the same variables were entered into MFA as the first year (excluding behaviors from gestation). Multiple factor analysis revealed two dimensions with eigenvalues > 1, explaining 63% of the variance in the data. The first dimension explained 33.7% of the variance in the data and was characterized by high positive loadings (>0.70) for ‘close proximity’ in the Lamb Tie Down test, high negative loadings for ‘open-mouth bleating’ in the Lamb Tie Down test, positive loadings for ‘head down’ in the Human Contact test at post-breeding and positive loadings for ‘zone with human’ in the Human Presence test at post-breeding (Table 3). The second dimension explained 29.1% of the variance in the data variables and was characterized by positive loadings for ‘allowing udder access’ in the Lamb Tie Down test, a negative loading for ‘head down’ from the Human Contact test at post-breeding and a positive loading for ‘zone with human’ from the Human Presence test at post-breeding (Figure 4).

3.3 Year 1 & 2: Cross contextual analysis

Dimensions from MFA were unrelated to indicators of lamb performance/outcome across both years of the study (Table 4). The ‘pacing/ avoidance of human’ response (Chapter 3) from the Lamb Handling test was also unrelated to behavioral responses from the dry season. According to results from Chapter 2, duration of ‘head down’ in the raceway was indicative of consistent individual behavioral differences in the raceway and was therefore evaluated against indicators of lamb outcome measures. Duration of ‘head down’ in the raceway was also a contributor to dimensions from MFA and therefore considered an important behavior in this population of ewes. Duration of ‘head down’ at weaning was related to lamb growth rates for multiples in year 1 (z= -5.6, P<0.0001). Duration of ‘head down’ did not predict growth rates an any other time of the year, in year 2. Duration of ‘head down’ at weaning was a significant predictor of lamb birth weights in twins (z= -1.99, P=0.05) in the first year. Duration of ‘head down’ in the raceway was also a significant predictor of combined birth weights for twins at post-breeding in the second year (z= -3.59, P<0.001). Duration of ‘head down’ in the raceway was unrelated to weaning weights for singles and multiples in both years of the study.

4. Discussion

Selection based for desirable phenotypes, by observing behavioral expression, may be done on farms to promote successful ewe-lamb bonding and consequently reduced lamb mortality and increased performance in extensively farmed sheep. Sheep under controlled, more intensive conditions, may not face the same biological/fitness consequences of exhibiting a certain CIDs when human intervention is more readily available (Bickell et al., 2010; 2011), however, sheep reared extensively (in outdoor conditions) could realize these consequences based on different behavioral types (Porcuicula et al., 2022). Previous work done suggests that behavior towards a human within the lambing season when the animal is unrestrained are seldom associated with responses towards a human outside of the lambing season or indicators of lamb outcome in the early-postpartum period (Aydogdu and Karaca, 2021; Dodd et al., 2012; Dwyer and Lawrence, 2005). In addition, behavior in an arena, outside of the lambing season and without the human present, have been identified to be associated with maternal behavior scores (proximity to shepherd) at lambing time (Porciucula et al., 2022). Pajor et al. (2010) and Gavojdian et al. (2015) found lamb growth rates and post weaning growth to be associated with responses in restraint (scale test) during human interventions. Given these previous reports, restrained responses towards a human are promising as indicators of maternal care and lamb outcome in sheep (Dodd et al., 2012).

Results of the current study indicate that both unrestrained and restrained responses towards a human, when the lamb is not present, can be indicative of maternal behavior. Additionally, restrained responses, particularly head position in the raceway, is reflective of CIDs (Chapter 2) and related to lamb performance/outcome measures, as predicted. Duration of ‘head down’ behavior while being handled in the raceway was related to frequency of ‘open mouth bleating’, duration of ‘allowing udder access’ during the Lamb Tie Down test and lamb birth weights for multiple across both years of the study. Proximity during the Human Presence test was repeatedly found to be associated with proximity to the lamb during the Lamb Tie Down test, suggesting that there may be a domain-general response in terms of distance maintained from the human and the lamb across the year in sheep. In contrast, behavioral variables (multiple, covarying behaviors) during the Human Approach test and Lamb Handling test did not show consistent patterns of association to maternal behaviors or other measures of lamb outcome. In fact, variables used from the Lamb Handling test were not related to indicators of lamb outcome (Chapter 3) nor were they related to responses towards the human outside of the lambing season. Previous work suggests that ewe response to humans within and outside of the lambing season may not be consistently expressed across time, nor is ewe response to a human at lambing time always indicative of degree of maternal care (Porciuncula et al., 2022; Everett-Hinks, 2005). It is possible that the methods of this study elicited a different behavioral response outside of lambing season compared to previous studies targeting behaviors during visual isolation (i.e., isolation box test and physical isolation (i.e., arena tests) from the flock. Ewes in the current study were tested in groups of five, possibly triggering a different "alertness" (Chapter 2) response towards humans that may be separate from behavioral traits related to activity level or sociality observed in the arena test. It is also possible that previous studies did not identify a trait associated with maternal care because variables from both the isolation box test and arena tests culminated into one temperament trait or coping style. For future studies, caution should be placed on aggregating responses together from IBT and arena tests to identify a salient trait in sheep as the two tests may be measuring different dimensions of the individual's temperament or coping style (Murphy et al., 1994; Atkinson et al., 2022).

4.1 Ewe response to human contact as it relates to maternal behavior and lamb outcome

The duration of time the ewes spent with their head below their shoulder while in restraint (year 1: post-breeding and weaning; year 2: post-breeding) negatively related to 'open mouth bleating' during the Lamb Tie Down test within both years of the study. In other words, sheep that spent more time with their head down while being handled in the raceway performed less open mouth bleating when alone with the lamb. In the same population of sheep as the current study, duration of 'head down' in the raceway was the most repeatable behavior (Chapter 2) compared to other measures observed during group testing. To date, there is evidence from Hemsworth et al. (2011; 2018) that increased 'head down' behavior may be a sign of negative arousal states in sheep. Variation in post-mortem cortisol concentrations can be attributed to a number of variables in sheep including intensity of handling and head position while single file in a raceway, prior to slaughter (Hemsworth et al., 2011). Hemsworth et al. (2011) states that increased head-down behavior during interactions with the human handler was associated with increased cortisol concentrations, suggesting that head-down behavior may reflect a heightened stress state in sheep or some other high arousal response. More work is needed to validate head postures as indicators of negative arousal states in sheep, however, it makes sense that lowering the head reflects increased stress during handling as sheep may be trying to reduce the level of contact exposure with the human or reduce the amount of stimulation, they are receiving by hiding within the gates of the raceway. There is a current lack of literature exploring head postures in sheep as suitable indicators of CIDs or as meaningful behavioral variables in terms of performance outcomes. This is somewhat surprising given that head posture is an important consideration when observing vigilance and attention related behaviors in sheep. Further, greater levels of locomotory behavior and vocalizations are associated with longer durations of interacting with a novel stimulus (Atkinson et al., 2022), approaching a human in the arena (Beausoleil et al., 2008; 2012; Tamioso et al., 2018) and risk taking in sheep less than 11-month-old (Yu et al., 2021). Diess et al. (2009) found a negative correlation between "high-pitched" vocalizations and reactivity to humans, and Beausoleil et al. (2012) reported that "more active" sheep in an arena test had lower cortisol levels following a human-interaction compared to "less" active sheep, or individuals that had lower levels of movement and vocalizations.

It may be that greater frequencies of open-mouth vocalizations are associated with reduced stress and increased boldness. Though most studies have not reported ewe behavior at the time of lambing, vocalizations are often thought to be a generally performed response incited during arousing situations, across multiple contexts (Beausoleil et al., 2012; Atkinson et al., 2022). Bickell et al. (2010) found lambs that moved and bleated less during arena testing also spent less time performing exploratory behavior. Results from Bickell et al. (2010) are possibly related to the boldness spectrum in sheep and support the notion that greater levels of boldness (i.e., exploration) are associated with increased frequencies of vocalizing compared to less bold sheep.

Duration of ‘head down’ behavior during the Human Contact test (Year 1: gestation and weaning) was also related to greater durations of ‘allowing udder access’ during the Lamb Tie Down test in the first year. This finding indicates that ewes that had greater durations of ‘head down’ behavior, possibly experiencing greater levels of stress during handling, allowed access to the udder more than ewes assuming different head postures in the raceway. In the second year, analyses revealed the opposite pattern: duration of ‘head down’ behavior at post-breeding was negatively related to ‘allowing udder access’ during the Lamb Tie Down test. This means that sheep that spent more time with their head down while being handled allowed less udder access compared to those that spent more time with their head in another position. The biological meaning of head postures in sheep is under-researched and therefore hard to interpret with regard to maternal care. It is possible that the lamb was the major driver of this behavior, separate from the ewe, however, most variance in ‘allowing udder access’ behavior explained by the lambs’ activity level was removed. O’Connor et al. (1992) found lamb activity and ewe behavior to have no direct relationship with lamb sucking success. Ewes designated with “calm” temperaments in the arena and isolation box test, or those displaying reduced movement/agitation, tended to have longer latencies to accept their lamb to the udder (Bickell, 2010). The exact meaning of “calm” according to Bickell (2010) is somewhat challenging to decipher since both the arena and isolation box test were used to determine CIDs, however, both could be measuring different aspects of temperament in sheep (Murphy et al., 1994). In contrast, Aydogdu et al. (2021) found no differences in maternal behavior between sheep categorized into ‘proactive’ and ‘reactive’ groups according to responses in an arena and scale test, corroborated by Murphy et al. (1994).

Allowing udder access can be an indication of selectivity and preference (Nowak et al., 1997; Levy and Keller, 2008) for the ewe’s own lamb therein being an adaptive behavior to promote lamb health and survival (Nowak, 1996; Nowak and Poindron, 2006; Dwyer et al., 2008; 2014). Interestingly, ewes subjected to an isolation box test, and arena test showed little differences in their preference towards their own lamb when also exposed to a foreign lamb (Bickell, 2009), perhaps meaning that the context of the HAI is important to identify these relationships. Further, Peeva (2009) identified selectivity issues that were more common in “nervous” phenotypes in dairy ewes assessed for reactivity (increased kicking and movement) at milking. Though Bickell et al. (2010) did find that “calm” ewes tended to have longer latencies to accept their lamb to the udder, rates of terminating sucking bouts and survival rates were no different between “calm” and “nervous” ewes. Ewes designated into high or low categories based on serum cortisol concentrations after isolation testing displayed differences in udder refusals (Coulon et al., 2014). Individuals that were termed ‘low responders’ for decreased levels of cortisol concentrations after testing showed more udder refusals compared to ‘high responders’ (Coulon et al., 2014). For studies that did find a difference in sucking measures between behavioral groups, there seems to be contradictory evidence over the consequences of having a “nervous” or “reactive” behavioral type in terms of the costs to lamb survival. Notably, there does appear to be some coherency in evidence providing that “calmer” ewes, have enhanced milk properties including IgG in their colostrum, boosting the health the lambs especially in multiples (Hart et al., 2009) and reduced somatic cell counts in the milk (Toth et al., 2017) resulting in improved health of the ewe and lamb. Hart et al. (2009) did, however, describe great variation in IgG levels within behavioral groups. Comparing results between IBT, arena, scale and raceway tests is also perhaps precarious, as the tests could be measuring different dimensions of CIDs as previously mentioned.

Patterns of association were also identified between durations of 'head down' and lamb birth weights for multiple bearing mothers. Combined birth weights for multiple bearing mothers had a negative relationship to duration of 'head down' at weaning in the first year and at post-breeding in the second year. In other words, ewes that had longer durations of 'head down' in the raceway gave birth to lighter lambs relative to ewes that had shorter durations of 'head down'. This could be an important consideration for shepherds seeking to select ewes that give birth to lambs that are able to survive the first few days of life. Birth weights for twin-bearing ewes is an important factor when addressing starvation risk in the lambs that could result in major incidents of mortality in the first three days of life (Dalton et al., 1980). Low birth weights can also be a cause of dystocia, possibly due to ineffective contractions and slow birth (Dalton et al., 1980). Given the small sample size and infrequent lamb loss in the current study, we cannot draw a strong conclusion about the potential influence of selecting ewes based on head posture in restraint in terms of lamb survival.

Rates of lamb survival can also be highly dependent on weather conditions (Nowak and Poindron, 2006), year of birth and management style (Sawalha et al., 2007), so temporary environmental effects should be an additional concern. To date, there is little coherent information on the relationship between head postures in restraint as they relate to maternal care. Though it is difficult to draw a strong conclusion about ewe temperament in the raceway as it relates to behaviors in the early post-partum period, responses during close contact HAIs in sheep are thought to be reflective of underlying arousal states (Pajor et al., 2008; Schiller et al., 2020), metabolic profiles (Toth et al., 2017), immunity (Dimitrov et al., 2005; Caroprese et al., 2009) and growth rates (Pajor et al., 2013) in sheep. More research and detailed observation of frequency of head and body posture changes may be relevant when inferring negative or positive arousal states in sheep (Tamioso et al., 2018). In sum, reactivity during handling or in restraint may or may not have consequences on maternal care and lamb outcome, however, selecting directly for lamb survival at the time of lambing is likely to be more beneficial (Plush et al., 2011) as selection based on the ewe's temperament may be ineffective (Bonato et al., 2021) or highly dependent on the test used.

4.2 Ewe response to human presence as it relates to maternal behavior and lamb outcome

Previous research has found negligible or contradictory associations between response towards a human in an unrestrained environment (e.g., arena or OFT) and maternal care or lamb outcome. Ewes categorized as 'proactive' or 'reactive' based on response to humans in the arena test showed little distinction in early postpartum maternal behaviors (Aydogdu et al., 2021). The current study did find that response towards a human in the Human Presence test was associated with the amount of time the ewe spent in 'close proximity' to her lamb during the Lamb Tie Down test, and not the Lamb Handling test. Durations of 'close proximity' to the shepherd during the Lamb Handling phase of testing are similar to MBSs (maternal behavior scores) developed by O'Connor et al. (1985) to assess the strength of attachment the ewe has to her lamb during processing. MBS has also been demonstrated to be related to aspects of the ewe's temperament including exploratory behavior and risk taking (Porciuncula et al., 2022). Research that has been published since the development of this scoring system suggests that this may not be an effective tool for gauging degree of attachment to the lamb or lamb survivability and performance (Everett-Hinks, 2005; Lambe et al., 2001), especially in multiparous ewes that have been pre-selected for maternal care. Further, Schiller and Horback (Chapter 3) found a unique trait in response to the human that may be triggered during the lambing season and is separate from regular maternal care.

According to results from the first years MFA, ewes that spent more time in 'close proximity' to the lamb during the Lamb Tie Down test spent less time in the zone with the stationary human at post-breeding. Ewes that spent more time in 'close proximity' to the lamb in the first year then spent more time in the zone with the human at gestation. In contrast to the first year, ewes that spent more time in 'close proximity' to the lambs during the Lamb Tie Down test spent longer durations of time next to the stationary human at post-breeding in the second year. Given these results, it is possible that some habituation occurred over the course of testing that reduced the distances the ewe were willing to maintain between themselves and the stationary human stimulus. Durations in 'close proximity' to the lamb and shepherd during the Lamb Handling test were uninvolved in patterns of association with duration of time spent near the stationary human in the Human Presence" test. Murphy et al. (1994) found time at the birth site in the first hour after birth was positively related to time spent in each zone of the arena test, implying that the ewe's spatial behavior amongst human presence had some association to her distance maintained from the lamb in the early postpartum period. Kilgour (1998) found that ewes that moved and vocalized less in the presence of a stationary human in the arena were more successful at rearing lambs. When ewes were subjected to an OFT without a human present, there were no differences found between groups (Kilgour and Szantar-Coddington, 1995). Given these findings from Kilgour (1998) and Kilgour and Szantar-Coddington (1995), it does seem that the presence of a human is important for drawing out behavioral expression associated with maternal care and spatial behavior at lambing. More investigation is required to understand the underlying response moderating spatial behavior from the lamb during the postpartum period and a human during the dry season, however, perhaps this response is related to levels of comfortability or docility under semi stress-inducing situations. The fact that proximity to the human during the postpartum period did not share a relationship with proximity to the human in the dry season is curious, and possibly due to other factors affecting the ewe's behavior during the Lamb Handling test including perception of the level of stress or pain that the lamb was undergoing.

4.3 Ewe response to human approach as it relates to maternal behavior and lamb outcome

There were no behavioral patterns observed between the response to a moving human in the dry period to maternal care or lamb outcome. Fewer researchers have looked at the effect of an approaching or moving human compared to a stationary human in sheep. Studies that have used a 'moving human' to observe different behavioral types in sheep have found that this stimulus may elicit behaviors reflective of a different temperament trait than that found in isolation (Atkinson et al., 2022) or may cause a more heightened arousal state compared to the stationary human stimulus (Goddard et al., 2000). In group-tested sheep, Goddard et al. (2000) found that there was an increase in heart rate above normal resting rates when the human stimulus entered the test pen and when the human began to move after having been stationary above a non-moving human. Finally, Deiss et al. (2009) did use an approaching human stimulus in a corridor test to observe contextuality of behavioral reactivity in sheep, however, they did not observe vigilance related behaviors during this test.

4.4 *Temperament testing*

Temperament assessments outside of lambing season may be more promising for gauging the ewe's ability to rear lambs until weaning in extensively farmed settings. Temperament in farm animal literature is typically assumed to be an inherent quality or trait of the animal associated with response to unpredictable and stress-inducing situations (i.e., human handling).

These responses to unpredictable or stress-inducing situations may infer differential survival or reproductive consequences (Real et al., 2007). This response is also assumed to be consistent across time, denoting that measuring temperament traits of the ewe at one stage in life will be harmonious with later responses in life. Porcuicula et al. (2022) suggests that ewe's CID may have more influence over maternal behavior and lamb performance in extensive systems as intensively reared sheep receive more assistance from the human handler, diminishing the "negative" consequences of carrying certain CIDs. Common assays for measuring CIDs include subjecting the ewe to an arena test that places a human between the subject ewe and conspecifics, allowing the ewe freedom of movement and reactivity to the separation event. Another common assay is known as the isolation box test (IBT), employed for recording agitation/movement when the ewe is visually isolated from the flock and granted less freedom of movement. Behavioral responses from arena tests are found to be repeatable (Cakmakci, 2022; Murphy et al., 1994; Kilgour and Szantar-Coddington, 1995) and may be reflective of the ewe's level of sociability or willingness to explore. Though this procedure, and others that involve freedom of movement, are relatively common for exploring CIDs related to exploration (i.e., locomotion, sniffing human) or sociability, there is conflicting evidence if this assessment can be used as a proxy for maternal behavior and lamb outcome. Some authors have reported that arena behavior can be used to assess maternal performance in ewes selectively breed to successfully rear lambs (Kilgour, 1998; Kilgour and Szantar-Coddington, 1995), while other authors have reported no such relationship between arena behaviors and maternal care (Aydogdu et al., 2021; Murphy, 1994; Bickell et al., 2009; 2011) or lamb survival (Bickell et al., 2010). On the other hand, assessments in squeeze chutes (Schiller et al., 2020) or weight crates (Pajor and Poti, 2007) may be effective at assessing CIDs of the ewe in restraint and these responses could indicate maternal behavior and lamb growth (Gavojdian et al., 2015; Pajor et al., 2010).

5. Limitations

Assessments in the raceway and modified open field test involved group tested sheep which can diminish behavioral responses towards an unpredictable or novel stimulus (Real et al., 2007). During raceway and modified open field testing, sheep could also see their flock mates that were not being tested, which could further influence their behavioral response towards the human stimulus. During the Human Contact test, position in the raceway was not standardized and ewes assumed any position by self-sorting. A random term for position in the raceway was included in models with a Human Contact response variable, however, this was likely not enough to entirely eliminate crowding in the back of the raceway. Linear mixed effects models for repeated measures used did include a random effect term for individual and group membership, however, this may not have been enough to completely nullify the effects of group testing. Group membership could not be nested within the random effect of individual since group configuration was not completely balanced across the study. The final weaning test in the second year of this study had to be dropped due to extremely high seasonal temperatures affecting behavioral responses of the ewes. During observation in the lambing season, ewes and lambs were recorded while together on range, exposed to other groups of ewe-lamb pairs and yearlings. The current study worked with a relatively small population of privately-owned sheep. Future studies with greater sample sizes of more commercial flocks may find different results using similar methods.

6. Conclusion

Most patterns of association in the current study were observed between duration of 'head down' in the Human Contact test and 'zone with human' in the Human Presence test to responses during the Lamb Tie Down test when the ewe and lamb were alone together. Durations of the 'head down' behavior seemed to be involved in the most patterns of association to maternal care (i.e., udder access) and lamb outcome (i.e., birth weights in multiples), and are considered a reliable indicator of individuals differences in ewes (Chapter 2). Direct human contact is known to be more aversive than stationary human presence, and able to elicit unique CIDs related to biological consequences in sheep. Even so, there were responses from the Human Presence test in the modified open field, namely duration in the 'zone with human', that showed a pattern of association to maternal behaviors and indicators of lamb outcome. There were no patterns of association identified between behaviors during the lamb processing phase of testing to behaviors in the dry season. More research is needed to explore the association between behaviors expressed when the lamb is on the ground and when not on the ground, however, it appears that response towards a human in group tested sheep can be a viable method to gather information about certain maternal characteristics (i.e., udder allowance, proximity to lamb) that may be helpful for selection purposes for multi-year breeding ewes. Further investigation should be aimed toward exploring the validity of head or body postures in restraint as indicators of arousal and individual differences in sheep. Observations in the raceway and 'Bud-Box' are easier to incorporate into current extensive farming systems compared to arena and isolation box tests. Based on comprehensive results of this dissertation, observing the ewe during handling or simply during ewe-lamb interactions, as opposed to scoring the ewe during lamb processing, may be more helpful for inferring lamb outcome in rangeland ewes.

7. Figures

Figure 1. Illustration of trial/testing protocol including the Human Contact, Presence, and Approach tests from when the lamb was not 'one the ground' and the Lamb Handling and Lamb Tie Down tests from when the lamb was 'on the ground'. Flags illustrate each time point when a trial was conducted. All trials were performed between 2019 – 2020 and repeated a year later between 2020 - 2021. Figure 1 was adapted from Figure 1 in Chapter 2.

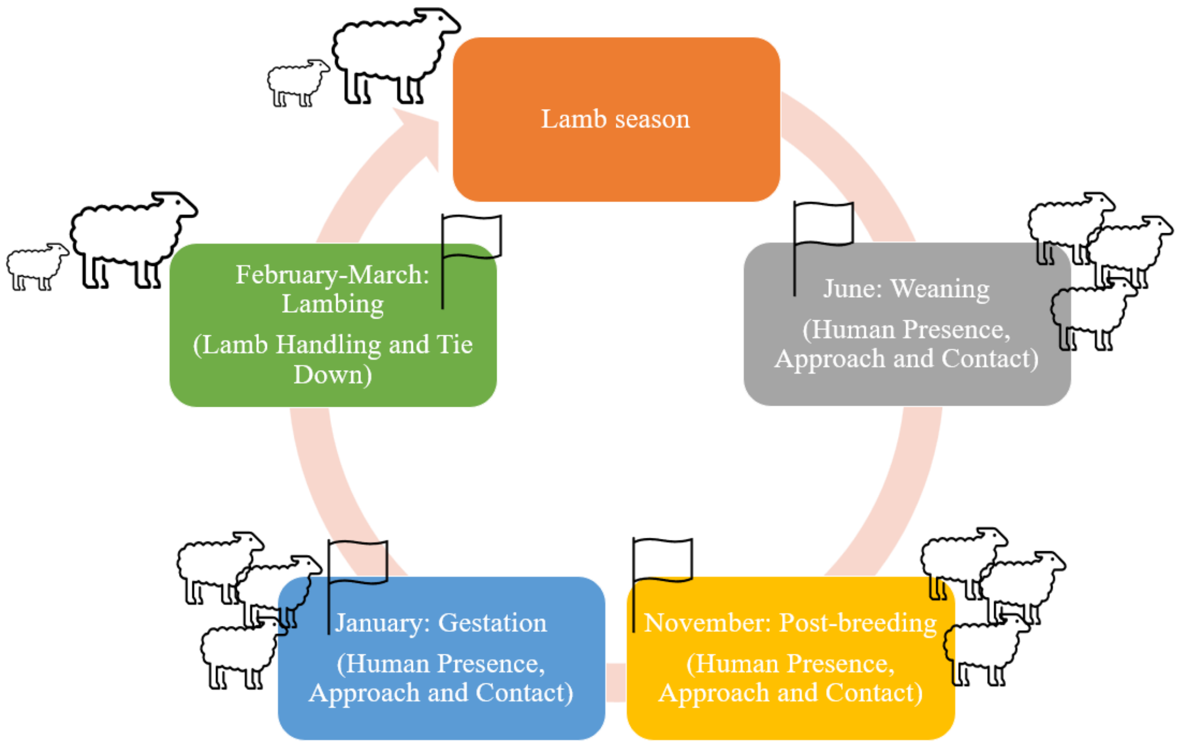


Figure 2. According to MFA, rate of 'open-mouth bleating' during the Lamb Tie Down HF test was negatively related to duration of 'head down' behavior in the raceway within the first year at post-breeding and weaning and within the second year at post-breeding.

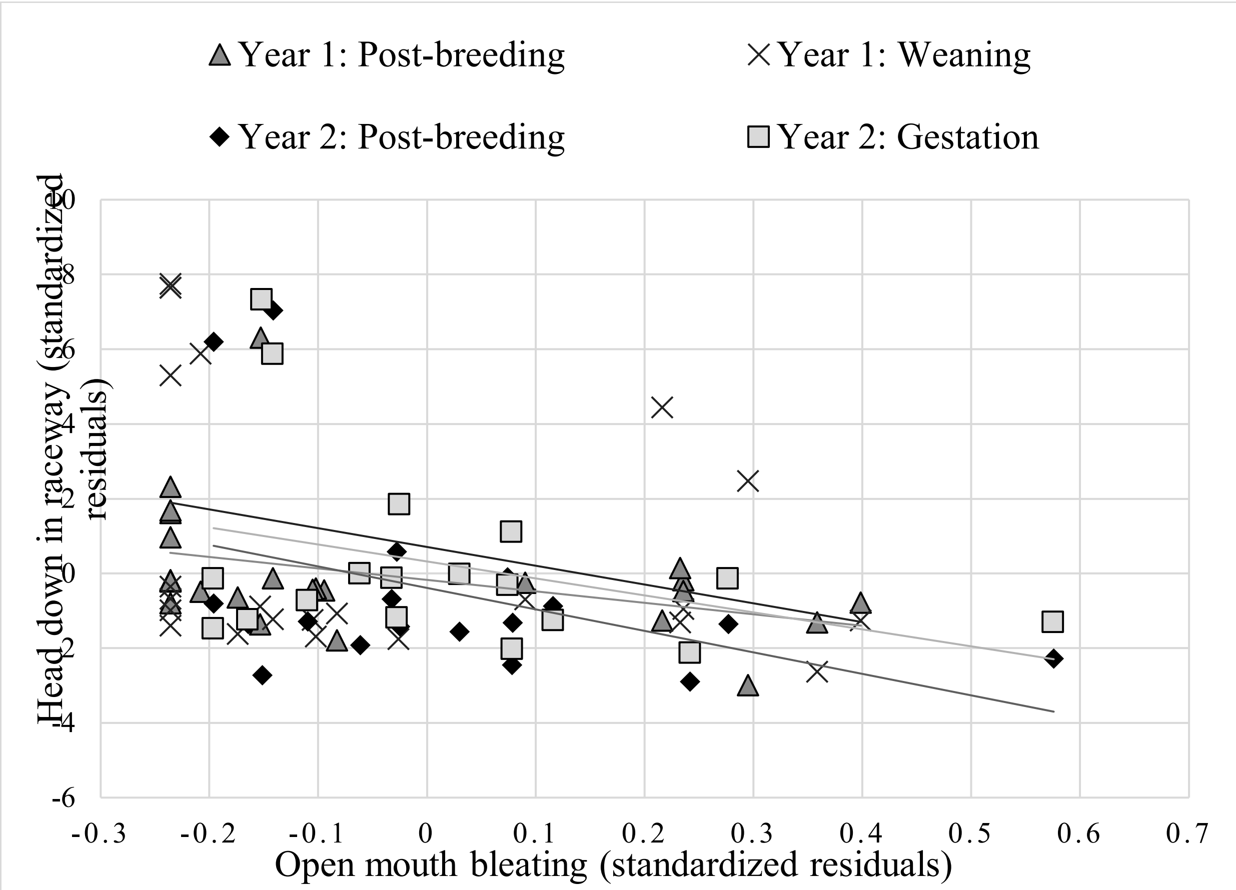


Figure 3. Coordinates of quantitative variables from the multiple factor analysis in year 1. The first dimension explained 27 % of variance in the data and is characterized by positive loadings for 'allowing udder access', and duration of 'head down' at post-breeding and gestation, and, a negative loading for 'open-mouth bleating'. The second dimension explained 22.4% of variance in the data and is characterized by positive loadings for 'close proximity' to the lamb, 'zone with human' at gestation and negative loadings for 'open mouth bleating' and 'zone with human' at post-breeding.

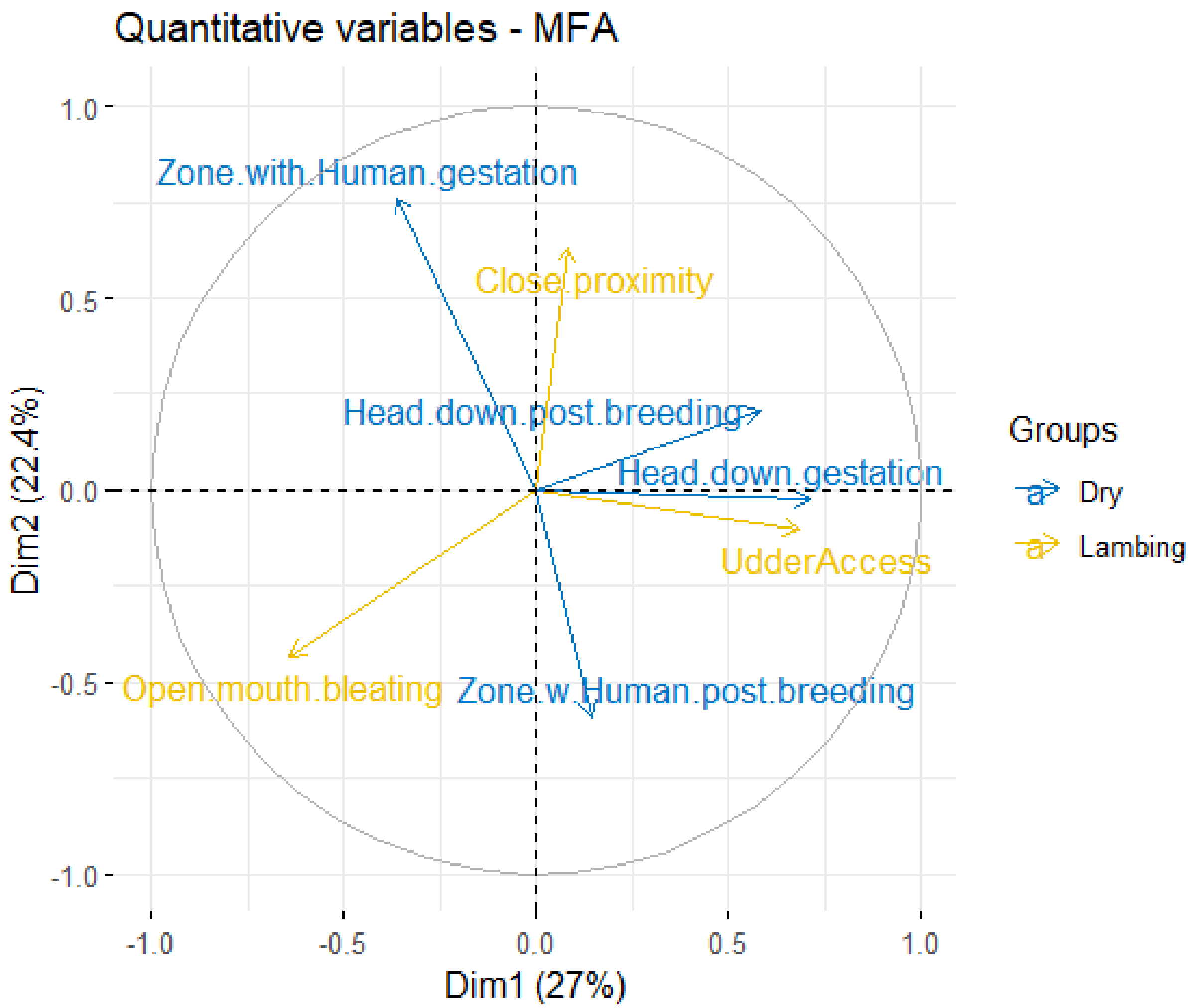
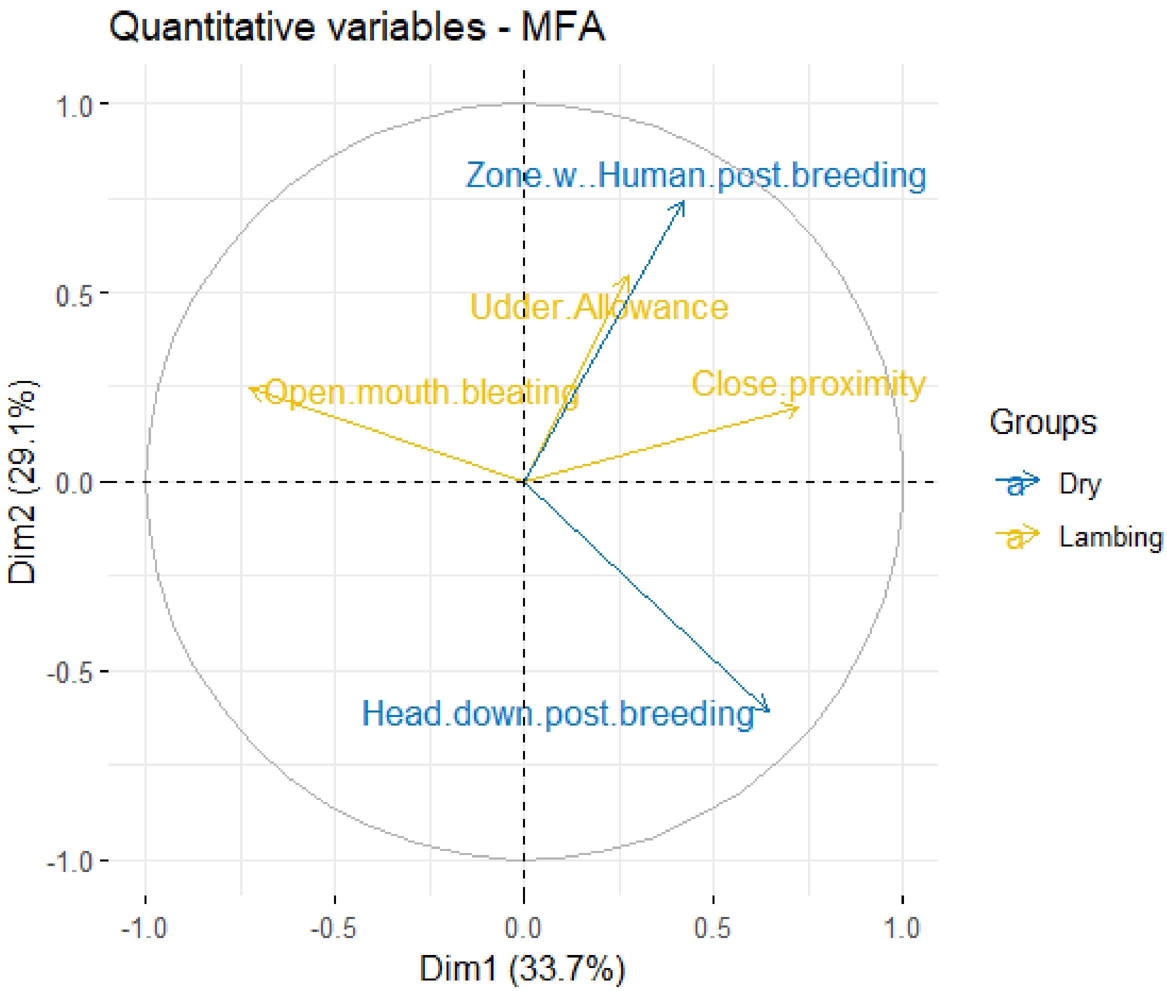


Figure 4. Coordinates of quantitative variables from the multiple factor analysis in year 2. The first dimension explained 33.7% of variance in the data and is characterized by positive loadings for 'close proximity' to the lamb, 'zone with human' at post-breeding and 'head down' at post-breeding. The first dimension was also characterized by a negative loading for 'open-mouth bleating'. The second dimension explained 29.1 % of variance in the data and is characterized by positive loadings for 'allowing udder access', 'zone with human' at post-breeding and a negative loading for 'head down' at post-breeding.



8. Tables

Table 1. List of covariate and fixed terms for behavioral variables within the Human Contact, Human Presence, Human Approach, Lamb Handling and Lamb Tie Down tests. The Human Approach and Lamb Handling test were not included as they did not meet suitable clustering cutoffs for MFA. Single bearing (SB) and multiple bearing (MB) ewes are included.

Fixed and Covariate terms	
Behavior	**Fixed or Covariate Term**
Human Contact test	
Head down	Weaning Status
Humna Presence test	
Zone with human	Weaning Status
Lamb Tie Down test	
Proximity to the lamb	Litter Size, Breed
Allowing udder access	Breed, Day of Birth
Sniffing/Grooming/Nosing lamb	Day of Birth, Parity, Breed
Closed-mouth vocalizing	Lamb Lying/Inactive
Open-mouth vocalizing	Lamb Vocalizing
Lamb Outcome $^{\text{SB and MB}}$	
Birth Weigh	Day of Birth, Lamb Sex
Growth Rate	Day of Birth, Breed
Weaning Weight	Day of Birth, Lamb Sex

Table 2. Description of terms used to designate tests, variables, and time periods of the study.

Test/ Variable/Time Period	Description
Lamb not present	When lambs have been weaned (mid-June) through the end of gestation (mid-February)
Lamb present	When ewes begin to lamb (late February) through weaning (mid-June)
Human Presence test	Modified open field test (mOFT); Fenced in experimental area (10.5 x 10.5m) used to measure response to a stationary human stimulus standing in the center; ewes were tested in groups of n=5 for 5 mins; individuals were tested at post-breeding, gestation, and weaning events (3 times per year x 2 years); unrestrained
Human Contact test	Alleyway (1 x 15m) where ewes line up (n=5) single file, prior to the Human Presence ^{mOFT} test; human places one hand under muzzle and one hand on rump of focal ewe; responses were recorded for 10 sec; restrained
Human Approach test	Modified open field test (mOFT); A moving human approached the experimental area and opened a single panel to release ewes (n=5); the approaching human walked c
Lamb Handling test	Rangeland area used to record/observe ewes' behavior while the human handler is processing (tail docking, castrating, identifying) the lamb (~5 min); unrestrained; human stimulus was close (HC) to the lamb(s) (<1m)
Lamb Tie Down test	Rangeland area used to record/observe ewes' behavioral after the human handler has processed the lamb (~10mins); unrestrained; human stimulus was far (HF) from the lambs (>30m)

Table 3. Multiple factor analysis was performed on variables from the Lamb Tie Down (LTD), Human Contact (Raceway) and modified open field test (mOFT). Behavioral variables were entered if they had a minimal amount of missing data points (<5 points), as MFA cannot handle missing data. For dry season variables, within each year the '1' = post-breeding, '2' = gestation and '3' = weaning. Variables from weaning are not well represented in MFA since this is around the time animal dropout occurred. Dimension 2 from year 2 had a value of 0.98.

Multiple Factor Analysis		
Year 1		
Behavior	**Dimension 1 (27%)**	**Dimension 2 (23.3%)**
close proximity (LTD)	0.08	**0.63**
udder access (LTD)	**0.69**	-0.10
open-mouth bleating (LTD)	**-0.64**	**-0.43**
zone with human (mOFT) [1]	0.14	**-0.59**
zone with human (mOFT) [2]	-0.36	**0.76**
head down (Raceway) [1]	**0.58**	0.21
head down (Raceway) [3]	**0.71**	-0.02
Year 2		
Behavior	**Dimension 1 (33.7%)**	**Dimension 2 (29.1%)**
close proximity (LTD)	**0.72**	0.19
udder access (LTD)	0.27	**0.54**
open-mouth bleating (LTD)	**-0.72**	0.24
zone with human (mOFT) [1]	**0.42**	**0.74**
head below (Raceway) [1]	**0.64**	**-0.61**

Table 4. Relationship between MFA dimensions and lamb outcome variables

Multiple Factor Analysis			
Year 1			
	Single Bearing		
Dimension	Birth weights	Growth Rates	Weaning Weights
1	z= NA, P= NA	z= 0.04, P= 0.97	z=-0.90, P=0.37
2	z= NA, P=NA	z= -0.81, P=0.42	z=-0.08, P=0.94
	Multiple bearing		
1	z value= -1.18, P=0.24	z=0.69, P=0.49	z=-1.00, P=0.32
2	z value = 1.02, P=0.31	z=1.00 , P=0.32	z=-1.01, P=0.31
Year 2			
	Single Bearing		
Dimension	Birth weights	Growth Rates	Weaning Weights
1	z=0.132, P=0.89	z= NA, P= NA	z=-0.35, P=0.73
2	z=-0.11, P=0.91	z=NA, P=NA	z=0.23, P=0.82
	Multiple bearing		
1	z= -1.54, P= 0.12	z=0.47, P=0.64	z=-0.36, P=0.72
2	z=0.89, P=0.37	z=0.34, P=0.734	z=-1.10, P=0.27

Chapter 5

General Discussion

Rangeland breeding ewes are subject to numerous environmental challenges including infrequent human handling that may induce stress or anxiety, predation risk, extreme environmental events and raising offspring with little assistance. Responses observed during and after HAIs may be indicative as to how the ewe manages the stress of human management, and other stressors including caring for lamb(s) from birth to weaning on rangeland. Using HAIs as a tool to select breeding ewes would be convenient for the farmer as it could be implemented into current handling practices and would potentially reduce the risk of lamb mortality due to poor or maladaptive mothering. Lamb mortality can be high, exceedingly so in tumultuous environments with minimal shepherd input, leading to poor welfare outcomes for the lamb due to starvation and hypothermia (Dwyer et al., 2008; Nowak et al., 1996). Previous research indicates that some HAIs are useful for gauging mothering ability while others (i.e., arena test) are seemingly ill equipped (Bickell et al., 2010; 2011). Current literature points to brief durations in a restraint device that limit freedom of movement (e.g., squeeze chute, milking parlor, weight crate) are the most promising environments for identifying ewes that carry adaptive maternal characteristics. Though maternal behavior scores (MBS) (O'Connor et al., 1985) have been considered as a potential tool for assessing quality of maternal care and lamb outcome, the repeatability of this score can be low (Yilmaz et al., 2011) and negligibly related to lamb performance (Lamb et al., 2001) or survivability (Everett-Hinks et al., 2005). This scoring system would be ideal, as ewes can be quickly scored at the time of lamb processing, however, there may be another CID trait elicited during that time that is unrelated to regular maternal care in the absence of the human handler. HAI tests outside of the lambing season (i.e., scale test) may be more suited for estimating maternal qualities compared to MBSs (Aydogdu and Karaca, 2021). Findings of this dissertation do suggest that behaviors under brief and more restrained interactions with the human are related to indicators of maternal care and lamb outcome, and, unrestrained responses such as 'proximity' to the human in a group setting may also be promising (chapter 4). Even observing simple ewe-lamb interactions can be useful in this respect (Chapter 3). Though group testing the ewes did not indicate a strong temporally stable trait, the functionality of group testing ewes should be considered as the human stimulus may still be eliciting biologically relevant responses despite social buffering (Chapter 2), and it is possible that these responses may not otherwise be viewed in isolation of the group.

Human Animal Interactions (HAIs)

Behavioral expression during and after human-animal interactions (HAIs) may be recorded or observed when desiring to gather information regarding biological outcomes and CIDs in sheep. HAIs are more practical to employ on farm settings compared to tests such as the isolation box test (IBT) and may be observed in restrained (e.g., weight crate, squeeze chute, raceway) or non-restrained environments (e.g., yard, pen, pasture). Responses in restraint such as a weight crate or squeeze chute have been identified to be repeatable across time (Schiller et al., 2020) and valuable for looking specifically at performance parameters (Pajor et al., 2010; Gavojdian et al., 2015). Behavioral performance within unrestrained arena tests are also repeatable (Dodd et al., 2012; Murphy et al., 1994), even despite management or environmental differences (Wolf et al., 2008), and have utility when assessing CIDs or personality traits such as 'docility', 'boldness' or 'exploration' (Yu et al., 2021; Beausoleil et al., 2012). Biological outcomes that have been identified to be related to response towards a human include propensity to become mastitic (Toth et al., 2017), oxidative stress and immune competence (Zhang et al., 2012), weight gain and postweaning weight (Gavojdian et al., 2015; Pajor et al., 2010), metabolic profiles (Pajor et al., 2013) and some aspects of maternal care (Brown et al., 2016; Peeva, 2009; Everett-Hinks et al., 2005). Though these responses have the potential to be valuable to shepherds seeking to select breeding ewes for flock productivity and health improvement, the type, timing, intensity (Hemsworth et al., 2011) and duration (Erhard et al., 2006) of the interaction does have to be considered. For example, unrestrained behavioral responses of sheep that underwent an arena scale test were not related to potential immune reactivity, recorded a year prior (Schiller et al., 2023) and a growing body of literature suggest unrestrained responses (i.e., in the arena) have weak to no relationship with maternal care and lamb performance (Cakmakci, 2022; Atkinson et al., 2022).

Another pressing issue with respect to HAIs is the inconsistent evidence towards a temporally stable trait (two or more co-varying behaviors). Though the arena test can be used to observe transient co-varying behavioral and physiological patterns of activity (Yu et al., 2021), this response is time sensitive and vulnerable to habituation or sensitization (Destrez et al., 2012; Erhard et al., 2006). To be able to understand the functionality of using HAIs as an indicator of performance, biological outcome or CIDs, it is required that we know which and when HAIs should be observed, to what duration, and what behaviors are biologically relevant to the animal in that context. The current dissertation provides evidence for the existence of behavioral traits within and outside of the lambing season during a variety of HAIs, with some clarity on which behaviors and types of HAIs are important to consider in rangeland breeding ewes in terms of selecting for adaptive maternal characteristics. According to findings from chapters 2 - 4, brief yet more intense interactions with sheep in the raceway (i.e., during human contact) can elicit responses that are associated with maternal behavior and lamb outcome. Further, responses in the presence of a human during lamb processing may be less effective at reflecting maternal behavior and more indicative of a response related to the stress or anxiety induced when the lamb is collected by the human handler. More investigation is needed to understand the underlying mechanisms of the 'pacing/ avoidance of human' response that manifested during lamb processing as it was unrelated to other HAI responses outside of the postpartum period.

Relevance of HAI responses

Behavioral traits consisting of two or more interrelated variables, during HAIs, were identified in the 'Bud-Box' raceway test, modified open field test (mOFT), and during the lambing season. Though the current study only observed two lambing seasons, there was strong evidence of a response elicited by the presence of the human handler at processing, and another during ewe-lamb interactions (Chapter 3). There was weak to no evidence for a temporally stable trait manifesting in the mOFT amongst group tested sheep (Chapter 2). Sheep subjected to group testing in the ten second raceway test and five-minute mOFT demonstrated consistency in singular behaviors including duration of 'head down' and frequency of 'environmental vigilance'. Of the five behavioral variables entered into PCA from the mOFT including (grazing, environmental vigilance, vigilance at human, contacting fence, and walking) it appeared that 'grazing' and 'environmental vigilance' were the most salient contributors to the transient 'alertness' response. It makes sense that duration of 'grazing' and 'environmental vigilance' would load together onto an 'alertness' response in group tested sheep, and were nearly correlated to the latency to 'look at approaching human' since this is a group-living species that is evolutionarily adapted to use mechanisms such as the "many eyes" or "dilution" effect to avoid predation while grazing. These mechanisms allow single individuals to fluctuate between levels of grazing and vigilance, and from previous literature in ungulates we know that certain individuals may participate in more levels of one or the other behavior. The 'alertness' response was unrelated to behavioral responses occurring in the raceway, and the 'pacing/ avoidance of human' and 'probing' traits occurring at lambing season. This is likely due to the distinction in testing protocol as the raceway involved more intense human interaction which is known to stimulate greater levels of cortisol (Hemsworth et al., 2011), and ewes were tested apart from their conspecifics during the lambing season. In terms of relevance to the farmer and consumer, it is possible that the 'alertness' response could be related to levels of stress or anxiety experienced by individuals, inferring possible differences in welfare state during unrestrained HAIs in rangeland sheep. Sheep administered an anxiogenic showed lower motivation to feed (Doyle et al., 2015) and greater levels of vigilance behaviors compared to control sheep when exposed to a dog stimulus (Monk et al., 2018), suggesting that reduced grazing and increased vigilance is indicative of anxiety in sheep. Though physiological biomarkers of anxiety were not collected in this study, it is possible that individuals that are repeatedly more prone to become vigilant compared to grazing in a group are experiencing increased levels of anxiety. It also makes sense that most studies looking at arena behaviors would find responses that reflect locomotor and vocal behavior (Beausoleil et al., 2008; 2012; Cakmakci, 2022; Atkinson et al., 2022; Kilgour and Szantar-Coddington, 1995) since sheep are tested alone and likely trying to reinstate contact with their flock mates. This 'alertness' response was not related to maternal behaviors or indicators of lamb outcome, so observing 'alertness' as a way to infer lambing success would likely be ineffective.

Duration of ‘proximity’ to a human in the Human Presence test did, however, show a pattern of association to proximity to the lamb from the Lamb Tie Down test at post-breeding in the first year and second year and gestation only in the first year (Chapter 4). In other words, sheep that spent more time in the zone with the human stimulus in the Human Presence test during gestation the first year and post-breeding the second year also spent greater durations in close proximity to their lambs during the Lamb Tie Down test. The opposite relationship was observed between proximity to the human and the lamb in the first year at post-breeding. There is evidence that spatial proximity to a human stimulus in the arena test is related to time spent at the birth site in the early postpartum period (Murphy, 1994), which may help explain findings within Chapter 4. According to Chapter 4 results, it seems that the willingness to approach and be near the lamb is associated with the ewe’s willingness to be near the human in the mOFT, the exact mechanism behind this association is unknown. The reason that the direction of this relationship changed after post-breeding in year 1 is also unknown. This could be due to a mixture of degree of attachment to the lamb and the physiological influence over CIDs or personality traits during gestation. Gestation is a period of allostasis resulting in immense hormonal changes for the ewe. CIDs and personality traits can alter in expression due to endogenous activity (Biro and Stamps, 2010), so this may be one explanation for the differences seen in proximity to the human stimulus across time.

Behavioral expression, particularly head posture, in the raceway showed the most promise as an indicator of CIDs and biological outcome in this population of sheep. Duration of ‘head down’ in the raceway showed a pattern of association with ‘open mouth bleating’ during the Lamb Time Down test and combined birth weights (Chapter 4). Duration of ‘head down’ was the only behavior in the Human Contact test that showed moderate or even low repeatability

(Chapter 2). Duration of 'head down' could be an important measure to observe for gaining information related to other contexts, however, time of the year in which it is observed should be considered. Interestingly, the post-breeding event seemed to be the most reliable time to record 'head down' behaviors as they relate to maternal characteristics and lamb outcome. As discussed in Chapter 4, 'head down' behavior may indicate stress levels during close human contact (Hemsworth et al., 2011) and due to its negative association with postpartum 'open mouth bleating', it could also reflect an underlying personality trait, such as 'boldness' or 'sociability'.

During the lamb processing, a unique pacing/ avoidance of human' response (Chapter 3) was identified that was independent of behavioral responses during separate ewe-lamb interactions and unrelated to the 'alertness' response in the mOFT. This response was present during lamb processing, which would usually be the time to score ewes based on their proximity to the shepherd and willingness to be near the lambs. This 'pacing/ avoidance of human' response may have been stimulated by the fear or stress of human presence and/or the act of collecting the lamb for processing. Interestingly, ewes showed no difference in behavior when their lambs were in a state of stress due to isolation yet they did show differential responses when the lamb was in pain due to castration and tail docking (Hild et al., 2011). Though it is possible the ewes were responding to the lambs' pain during the Lamb Handling test, the processing event was quite fast (usually less than 5 min) and so they would have had to pick up on the lambs' pain immediately. Further, the authors would assume that if this response were related to the experience of the lambs' pain or stress, it would be related to the 'probing' trait, or other behaviors occurring during the longer Lamb Tie Down test. The 'probing' trait was contextually specific to ewe-lamb interactions and may have been elicited as a way to encourage the lamb to move after receiving a painful procedure. The lamb's pain and the ewe's perception of the lamb's pain should be considered when observing maternal care, as this likely affected the ewes normal maternal response.

Maternal care and lamb outcome

Maternal behavior scores (MBSs) have been moderately explored as a system for estimating qualities of maternal care and lamb outcome. More recent research coming out suggests that it is likely more beneficial to simply observe ewe-lamb interactions without a human close or present. Coordinated behaviors from the ewe towards the lamb are vital for the lamb's survival (Dwyer, 2003) in the early postpartum period when anywhere from 5 to 50% of lambs may die due to a number of factors including weather events and management practices (Everett-Hinks and Dodds, 2008). Behavioral performance of the ewe during separate ewe-lamb interactions drew the most significant correlations to other indicators of lamb outcome including duration of 'sucking', 'nosing/licking/sniffing' the lamb, 'closed mouth bleating', birth weights and weaning weights (Chapter 3), however, these relationships were not in the expected direction. The negative relationship observed between adaptive maternal behaviors and indicators of lamb outcome was likely a result of the testing methods. The lamb Tie Down test was perhaps stressful for the ewe and lamb pair, and ewes that had formed a stronger bond with their lamb may have abstained from adaptive maternal care during the test due to this level of stress they were experiencing. During ewe-lamb interactions, there were inter-variable relationships that would be expected to exist when the ewe is establishing a bond with her lamb (see supplementary materials), mostly in year 2 of the study. Such relationships include a positive relationship between 'closed mouth bleating', and 'sucking'. Positive relationships were also seen between duration of 'close proximity' to the lamb, and duration 'sucking' and 'nosing/licking/sniffing' the lamb. Behaviors such as sucking are important for bond development and preference between the ewe and lamb (Nowak, 1997) and more accepting ewes will perform licking and grooming behavior while the lamb is at the udder (Vince, 1993). Licking and grooming behavior and orientation of the ewe is also important for lamb sucking success (Alexander and Williams, 1964).

6. Conclusion

This dissertation set out to explore for consistent individual differences (CIDs) among ewes during pre-and postpartum human animal interactions. Ewes demonstrated repeatability in singular behaviors (i.e., duration of 'head down', and frequency of 'environmental vigilance') when the lamb was not present and a 'pacing/ avoidance of human' response when the lamb was present during processing. Behavioral responses of the ewe while the lamb is being processed are commonly thought to reflect the strength of the relationship between the ewe and lamb, however, results of the current study indicate that the ewe's response was seemingly driven by level of maternal investment (according to lamb birth weight) and a CIDs trait separate from adaptive maternal care that may have been triggered by the presence of the human and/or act of handling the lamb (Chapter 3). Ewes of this study also demonstrated behavioral patterns between periods when the lamb was and was not present. Duration of 'head down' when ewes were being handled, without the lamb present, was repeatable (Chapter 2) and related to frequency of 'open-mouth vocalizing' after processing and lamb birth weights (Chapter 4). Little research has been done on the relevance of head posture in sheep, however, this behavior deserves more focus as a potential and important species-specific indicator of stress or arousal. In sum, human-animal interactions occurring when the ewe is restrained or unrestrained can provide valuable, biologically relevant insight into the ewe's fitness and suitability under human management.

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