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Perinatal behavioral patterns during and after human-animal interactions in rangeland breeding ewes

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Consistent individual behavioral differences (CIDs) among livestock are known to be inherent qualities of the animal that are repeatable over time and 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 than responses in an open testing environment. The current study investigated the relationship between several behaviors in multiparous ewes (n = 42) in two distinct contexts: 1) human-animal interactions, with no lamb present, during post-breeding, gestation, and weaning, which were performed three times per year for 2 years (six trials in total), and 2) HAIs, with the lamb present, after parturition once per year for 2 years (two trials in total). Tests without the lamb present included a Human Contact, Presence, and Approach test within each of the six trials, and tests with the lamb present included a Lamb Handling and Tie Down test. General additive models with a random term for individual were used to investigate the relationship between behaviors from outside of the lambing season to behaviors within the lambing season. The proportion of time in the 'peripheral zone' in the Human Presence test, at post-breeding, was a significant negative predictor of 'environmental vigilance' (i.e., being on look-out rather than attending to lamb) in the Lamb Tie Down test (P=0.02). A post-hoc negative relationship was found between 'environmental vigilance' and 'sniffing/grooming' the lamb in the Lamb Tie Down test. In addition, sheep who were more environmentally vigilant in the Lamb Tie Down test were less avoidant of the human in the Human Presence test (post-breeding). Weaning weights, yet not birth weights, were highly repeatable within ewes [R=0.70, *P*=0.001, CI(0.29, 0.91)], and weaning weight models were improved with the inclusion of time in the 'peripheral zone' in the Human Presence test and grooming and 'environmental vigilance' in the Lamb Tie Down test. Of note, the avoidance of the human, when the lamb was not present, was associated with weaning

weights and therefore could be considered as a metric to consider when selecting ewes. Ewe behavior in response to humans outside of the lambing season be useful in gauging future maternal behavior (i.e., grooming) and lamb birth and weaning weights.

KEYWORDS

animal behavior, ewe, human-animal interaction, maternal, personality, sheep, temperament

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 ('da Porciuncula et al., 2024), potential predation risk, and minimal assistance with raising lambs on the 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, 2008a). Lamb mortality is increasingly high 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, frequency of vocalizations (Nowak, 1996), and duration of sniffing (Alexander and Shillito, 1977; Alexander and Stevens, 1982), are crucial for bond development between the pair. Without the proper facilities in place or adaptive maternal behaviors performed (i.e., low-pitched bleating and allowing udder access), the lamb may suffer starvation or hypothermia and consequently poor welfare (Dwyer, 2008b), resulting in death.

Shepherds may select animals, based on observable behavioral responses, to improve lamb outcome. Behavioral responses toward 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, and transportation). According to previous literature, the response toward a human while ewes are restrained and not with offspring may be indicative of adaptive maternal behaviors, even over responses toward a human in the early postpartum period when the lamb is present (Everett-Hinks et al., 2005). Maternal behavior scores (MBSs), developed by O'Connor et al. (1985), showed promise as a tool for assessing ewes based on the retreat distance from the shepherd during lamb processing (i.e., individual tagging, castration, tail docking, and weighing/sexing); however, more current research indicates that this response may have little impact on lamb outcome (Lambe et al., 2001; Aydoğdu and Karaca, 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, and yard test), are negligibly related to the response toward a human in the early postpartum period or maternal behavior and are negligibly related to indicators of lamb outcome (Aydoğdu and Karaca, 2021; Peeva, 2009). On the other hand, ewe behavior toward a human during physical restraint tests (e.g., scale, squeeze chute, and 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 authors of the current manuscript would like to further validate these relationships (or lack thereof) between behaviors within and outside of the lambing season in a comprehensive design, using multiple types of human-animal interaction (HAI) that vary in intensity and length.

The current study used multiple contexts (restrained and unrestrained) to assess the relationship between responses toward the human when the lamb is not present (June to January) with responses when the lamb is present in the postpartum period (February to June). The first objective of the current study was to assess the relationship between restrained responses of the ewe (i.e., Human Contact test), when the lamb is not present, and compare them to behavioral responses when the lamb is present (i.e., Lamb Handling and Tie Down test) and to lamb outcome. Behaviors performed during a close Human Contact test in the raceway (e.g., 'stepping', 'head up', and 'head down') are expected to be related to adaptive maternal behaviors (i.e., 'udder access', 'closed mouth bleating', and 'sniffing/licking/nosing') and lamb outcome (birth, growth, and weaning weights). This prediction is based on previous literature indicating a connection between lamb outcome and ewe behavior in environments involving the restriction of movement (Dodd et al., 2012). The second objective of the current study was to assess unrestrained responses of the ewe (i.e., Human Presence and Approach test), when the lamb is not present, and compare them to behavioral responses when the lamb is present and to lamb outcome. The authors of the current study expect a weak to no association between behaviors in the Human Presence test (e.g., duration in 'zone with human', 'zone crossing', and 'investigating human') to 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 (i.e., birth, growth and weaning weights) (Dodd et al., 2012).

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 is a culmination of two separate studies carried out previously, one of which is published (Schiller et al., 2023). Study ewes were maintained within a flock of approximately 120 animals, on approximately 190 acres of total rangeland throughout the year. All ewes were rotated to paddocks ranging from 4 to 15 acres of available space every 5 days or when the land/vegetation became saturated. Ewes were flushed on alfalfa for the first year of the study (2019–2020) and a mixture of dry cob, corn oats, barley, and chia seed (1.25lbs/head/day) for the second year of the study (2020–2021). During mating season (October and November), rams of the same breed were introduced to the ewes (30 ewes to 1 ram) for natural cover. Shepherds managing the flock used low-stress handling methods, including a Bud-Box developed for sheep and cattle movement, non-aggressive dog use, minimal vocalizations, and a pressure-release system to encourage sheep in the desired direction. The Bud-Box is a simple box shape, specifically constructed to allow animals to see movement from other animals ahead of them, easing the ability of the farmer to guide the flock within an attached arena and toward a familiar home paddock (Section 2.2.1). All study ewes had previous experience with the raceway and Bud-Box, facilitating easy movement through the system.

HAIs were assessed during six repeated trials between June and January, when the lamb was not present, at two handling sites [Blue Oak Ranch (site A) and Belmantro Station (site B)] typically used for management procedures (vaccinations, foot trimming, weaning, etc.). The first trial of each year occurred at post breeding (November), the second during gestation (January), and the third directly after weaning (June). Unfortunately, the sixth and final trial in June had to be dropped due to extreme heat affecting the animal's behavior (>32.2°C). Each testing site contained a familiar wooden Bud-Box handling system with an adjacent raceway and modified open field test (mOFT) (Figure 1). Interactions between the lamb and the ewe, and the ewe and a human stimulus, were also assessed in a separate part of the year, after the lamb was born (February to April). Individuals who gave birth and could be observed on camera were those who made up the overall sample size (N=42) for the study, n=28 of which were present for both years. Within the first year (2019–2020), n=13 terminal Shropshire and n=19 blue-faced Leicester x white-faced (BLW) ewes were lambed and observed on camera. Within the second year (2020–2021), n=16 Shropshire and n=21 BLWs were present for data collection. Over the past 15 years, shepherds managing the study flock bred animals based on selection criteria within an EZ Care lambing system. These criteria was not used to select specific individuals of the study. The EZ Care lambing system included categories to score animals based on the perceived degree of attachment the ewe has to her lamb (i.e., moisture in lambs' mouth, fullness of belly, and distance from the birth site) and lamb vigor (i.e., activity levels). Shepherds managing the flock also used tags and an electronic tag reader to record the ewes' ID, number of lambs, lamb sex and weight, and any issues that occurred in the early parturition period.

2.2 Experimental setup

2.2.1 Human Contact, Presence, and Approach testing

Ewes were subjected to six HAI trials between 2019 and 2021 (June to January) as a part of a study performed previously by Schiller and Horback (2024) to explore consistent behavioral traits during HAIs. Each year, three trials were performed at the following times: post-breeding, gestation, and after weaning. The trials were repeated a year later at the same time points, for a total of six trials (3 per years × 2 years). The current study employed a typical wooden Bud-Box and raceway system to assess the interactions between three unfamiliar human handlers (Humans A, B, and C) and the flock (Figure 1). Humans A, B, and C were different humans within and between each trial. The Bud-Box consisted of an approximately 3.5 m × 4 m square holding pen, accompanied by a 1 m × 10 m raceway that led sheep in the direction of a familiar open pasture. The wooden sides of the Bud-Box were approximately 1.1 m high with a 1 m × 1 m wooden exit door that slid open to allow sheep into the raceway. Once in the raceway, animals (n=5 per group) were handled, by Human A, with one hand under the muzzle and one hand on the rump for 10 s during what was labeled the Human Contact test. Prior to the Human Contact test, Human A stood approximately 10 m perpendicular to the side of the arena and approached the last sheep in the raceway. At the same time, another researcher stood behind Human A with a camera to record the ewes behavioral response to handling. Once Human A made physical contact with the individual sheep, the test began and lasted for 10 s. If Human A broke physical contact with the sheep, the test continued and the breakage would be noted. Human A then moved forward, toward the next sheep. This was repeated until all sheep in the raceway received the contact treatment. After the Human Contact treatment, Human A opened the exit gate and moved from front to back on the raceway, eliciting movement toward the mOFT (10.5 × 10.5 m). Once all animals from the raceway entered the mOFT, a 5 min observation period began. The mOFT involved the constructed of wire mesh fencing (1.2 m height) with T-posts and cable ties at each junction to secure the fence. The mOFT consisted of a stationary human (Human B) placed in the center of the experimental area to evaluate the ewe's response to human presence in what was labeled the Human Presence test. After the duration of the 5 min mOFT test was met, animals were released by an approaching human (Human C) 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 ewes response to the stationary and approaching human.

2.2.2 Lamb Handling and Tie Down test

During lambing season (late February to early April), ewe behavior at the time of lamb processing (6–36 h after birth) and directly after was recorded during a Lamb Handling and Lamb Tie

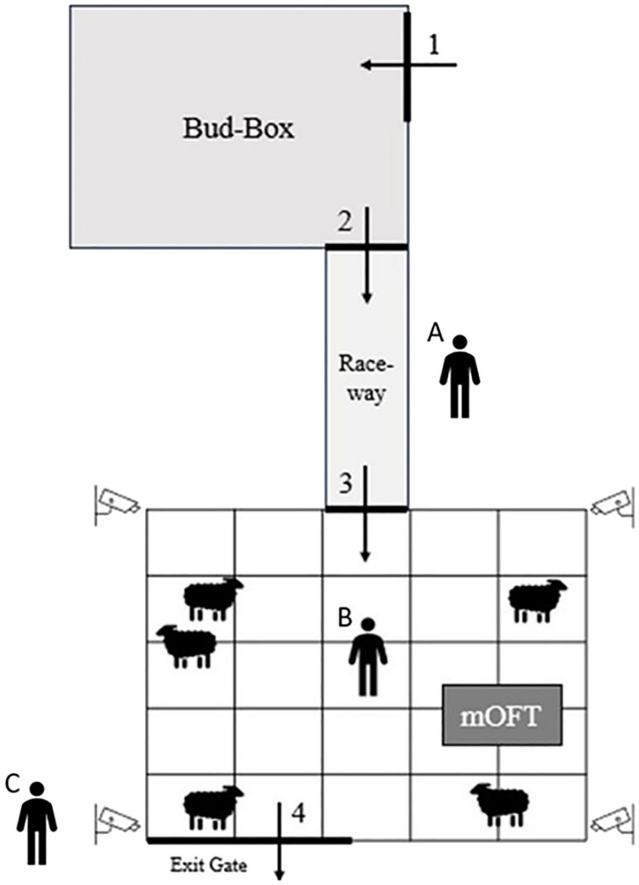

FIGURE 1
Illustration of the experimental setup for the six HAI trials when the lamb was not present. The 'Bud-Box', raceway and modified open field test were used to perform the Human Contact, Presence, and Approach testing. The flow of movement follows the numbers (1–4) indicated. Animals entered the Bud-Box (1), sorted into the raceway (2), exited the raceway into the mOFT (3), and finally were released through the exit gate (4). Human A performed the Human Contact treatment. Human A approached the sheep from the side of the raceway, starting with the last ewe, and placed one hand gently under the muzzle and another on top of the rump. After 10 s, Human A released contact, stepped away from the raceway, stepped back up to the raceway, and treated the next sheep in line. If the sheep broke contact with Human A, the test continued and the handler was tasked with reinstating contact. After all sheep were tested, they were moved into the mOFT for the Human Presence test with Human B. Human B was tasked with standing, stationary, in the center of the arena during the Human Contact test and before the Human Presence test. The zone bordering the fence line was the 'peripheral zone' and the zone with Human B was the 'zone with human'. After the 5-min duration of the Human Presence test, sheep were released by Human C, who would approach the exit gate and walk in a counterclockwise direction around the perimeter of the experimental area until all sheep exited. Humans B and C were instructed to not make direct physical contact with the sheep and not make audible noise during testing.

Down test. These tests were performed as a part of a study carried out earlier by Schiller and Horback (In Review) to assess behavioral traits of the ewe during lambing season. Recording of these two tests took place in familiar paddocks (on rangeland) where the ewes would undergo parturition. The actual location of each test depended on where the ewe was located at the time of observation. As the Lamb Handling and Tie Down tests took place soon after parturition, the ewe was typically separated from conspecifics as per normal behavior to allow for isolated bonding with the lamb. Cameras were set up on tripods, approximately 10 m away from the shepherd and lamb, to record the ewe's response to the lamb being handled (i.e., tail docking, castration, near tagging, individual marking, and iodine treatment) in what was labeled the Lamb Handling test. During the Lamb Handling test the lamb(s) was secured by the shepherd allowing for little movement. The Lamb Handling test was performed to assess the ewe's response toward the human handler. Behaviors of interest from this test included 'open-mouth bleating', 'pacing', 'walking', 'grazing', 'environmental vigilance', 'investigating the human' and 'vigilance at human/lamb'. After processing, the lamb was tied down by the back leg with twine (0.5 m) and a camping stake for a 10 min recording session during what was labeled the Lamb Tie Down test. The length of the twine was such that it would allow for the lamb to stand and take a few steps yet not move out of the frame of the camera. During the Lamb Tie Down test, the shepherd and researcher walked >30 m away to leave the ewe and lamb undisturbed for the duration of the test. The purpose of the Lamb Handling test was to record separate ewe-lamb interactions and maternal behavior without a human present. Behaviors of interest from this test included 'grazing', 'pacing', 'walking', 'open-mouth bleating', 'closed-mouth bleating', 'sniffing/grooming' lamb, and 'allowing udder access'. After the 10-min recording session, the

shepherd or researcher would approach the lamb(s) and gently release them from the twine. If the ewe was not near the lamb(s) at the time of 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) before the behavioral annotation of video data using The Observer XT v. 11 (Noldus Information Technology, Wageningen, The Netherlands). See the full ethogram with behaviors and operational definitions in the Supplementary Materials and Table 1 below for a description of tests and time periods.

2.2 Statistical analysis

Data from the various HAI tests (Human Contact, Presence and Approach) and lambing season (Lamb Handling and Lamb Tie Down) were from two companion studies (Schiller and Horback, 2024; In Review). Repeatability estimates were considered important in the context of this paper; however, owing to the nature of the data, they were not performed on behavioral data. Repeatability estimates using the 'rptR' package (Stoffel et al., 2019) can be carried out on data demonstrating non-Gaussian distributions; however, continuous proportional data on an interval could not be modeled with this approach. The same issues arose when considering assessing repeatability by estimating within-subject and between-subject variation using the *lme4* package. To combine years 1 and 2 into a single analysis, relationships identified through Multiple factor analysis (MFA) were further analyzed using general additive models or *gams* from the 'mgcv' package (Wood, 2006) fit by the restricted effects maximum likelihood method (REML). Behavioral data from the Human Presence, Lamb Handling, and Lamb Tie Down tests were largely proportional in nature and therefore were modeled with a beta family and 'logit' link function (Douma and Weedon, 2019). The models with a binary term for 'investigate human' were checked by modeling linearity between the predictor variable and its own residuals. Collinearity of predictors was also considered for this model. Proportional data from the lambing season was fit as a response and data from outside of the lambing season was fit as a predictor term with an individual ID as a smoothed random term. Data from lambing was fit with a covariate term for lamb activity when needed. When required, a fixed term for the 'group' the ewe was in from the Human Presence model was fit; however, this likely led to overfitting due to the low sample size. Variables including 'zone with human' from outside of the lambing season and 'udder access' from within the lambing season were dropped from the analysis as they contained many 0s or 1s, making data analysis and interpretation challenging. The duration of 'zone with human' was replaced with 'peripheral zone' and the authors decided to model 'sniffing/grooming' lamb and drop 'udder access' and 'close proximity' to the lamb. Finally, behaviors of interest from the Human Contact and Presence test, including the duration of 'head down' and 'peripheral zone' were analyzed against lamb birth and weaning weights. Model residuals were checked with Q-Q norm plots. Birth and weaning weights from twin lambs were averaged and compared with weights from singles. Predictors were considered significant at an alpha level = 0.05. Cumulative model weight based on Akaike information criterion (AIC) values (Akaike, 1973) were also reported. Models carrying more cumulative weight are considered a better fit. R^2 adjusted values are also reported to describe variance explained in the models. Best-fit null models are described in Table 2.

TABLE 1 Description of the terms used to designate tests, variables, and time periods in 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 × 10.5 m) used to measure the 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 × 2 years); unrestrained
Human Contact test	Alleyway (1 × 15 m) where ewes line up (n=5) single file, prior to the Human Presence ^{mOFT} test; the human places one hand under the muzzle and one hand on the rump of the focal ewe; responses were recorded for 10 s; 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
Lamb Handling test	Rangeland area used to record/observe ewes' behavior while the human handler is processing (tail docking, castrating, and identifying) the lamb (~5 min); unrestrained; human stimulus was close to the lamb (s) (<1 m)
Lamb Tie Down test	Rangeland area used to record/observe ewes' behavior after the human handler has processed the lamb (~10 mins); unrestrained; human stimulus was far from the lambs (>30 m)

3 Results

3.1 Repeatability

Repeatability could not be assessed on continuous proportional data from lambing or on non-normal continuous data from the Human Contact, Presence, and Approach tests. The repeatability of

TABLE 2 Best fit null models for important behavioral variables from the human contact and presence tests and Lamb Handling and Tie Down tests.

Response	Predictor	Estimate	Std. Error	Z value	*P*-value
Environmental vigilance ^{LTD}	Age	0.13106	0.05486	2.389	0.02
	Year	0.10961	0.19582	0.560	0.58
Sniffing/grooming lamb(s) ^{LTD}	Age	-0.17898	0.05398	-3.315	<0.001
	Lamb vocals	-9.57484	4.29850	-2.227	0.03
Investigate human (binary) ^{LHT}	Age	0.7172	0.2789	2.571	0.01
	Breed	-1.4370	0.8843	-1.625	0.10
Lamb birth weights	Age	0.09448	0.26646	0.355	0.73
	Lamb vocals	-7.20243	10.89248	-0.661	0.51
Lamb weaning weights	Age	-1.5509	0.7660	-2.025	0.05
	Day of birth	-0.7459	0.1491	-5.004	<0.001
	Birth weight	3.0588	0.6656	4.595	<0.001

Null model construction based on AIC values for best fit. LTD, Lamb Tie Down test; LHT, Lamb Handling test.

weaning and birth weights was assessed. Birth and weaning weights could be modeled with a normal Gaussian distribution and a random term for the subject. Weaning weights had a high repeatability estimate [R=0.70, *P*=0.001, CI(0.29, 0.91)]. Birth weights had a low repeatability estimate [R=0.21, *P*=0.24, CI(0, 0.756)].

3.3 Cross-contextual analysis

3.3.1 Human contact vs. lambing behavior

The duration of 'sniffing/grooming' the lamb and 'environmental vigilance' were important variables from the Lamb Tie Down test and were therefore assessed against behaviors from the Human Contact and Human Presence tests. The model for 'sniffing/grooming' and 'environmental vigilance' included a term for the age of the ewe and year. The frequency of head posture changes in the Human Contact test (gestation ^{Y1}) were significant positive predictors of 'sniffing/grooming' the lamb in the Lamb Tie Down test (*P*=0.03). The frequency of head posture changes were a significant positive predictor of 'sniffing/grooming' the lamb at weaning ^{Y1} (*P*<0.05), when a term for position in the raceway was included in the model. The frequency of head posture changes at post-breeding was an insignificant positive predictor of 'sniffing/grooming' the lamb(s) (*P*=0.40) and did not carry more cumulative weight than the null model. The inclusion of the frequency of head posture changes at post-breeding, gestation, and weaning did not carry more cumulative model weight than the null model; however, it did explain more variance at gestation and weaning [($R^{2adjusted}$ = 28.8% $_{gest}$, 40.2% $_{wean}$ vs. 26.6% $_{null}$)]. The duration of 'head down' in the Human Contact test was modeled as a binary predictor (0=did not occur and 1=occurred). In the model for 'environmental vigilance', a binary term for 'head down' in the Human Contact test was a significant negative predictor at gestation ^{Y2} (*P*=0.04) and a nearly significant negative predictor at weaning ^{Y1} (*P*=0.10). The inclusion of the binary term for 'head down' at weaning ^{Y1} and gestation ^{Y2} did not carry more cumulative model weight than the null model; however, it did explain more variance than the null model [(R^2 $_{adjusted}$ = 13.7% $_{wean}$, 23% $_{gest}$ vs. 8% $_{null}$)]. Data were limited for the Human Contact test, as gestation from year 1 and weaning from year 2 were missing. Owing to the limited number of individuals, a few individuals influenced the results from this test. Furthermore, although a factor term for position in the raceway was needed, it likely resulted in overfitting given the low sample size.

3.3.2 Human presence vs. lambing behavior

A negative relationship was identified between 'investigate human' in the Lamb Handling test and proportion of time in the 'peripheral zone' at gestation $^{Y1\ \&\ Y2}$ of the Human Presence test. The null model for 'investigate human' in the Lamb Handling test as a binary response included a term for lamb vocal activity and age of the ewe. The term for the proportion of time in the 'peripheral zone' was a significant negative predictor (*P*<0.05), explaining more variance than the null model ($R^{2adjusted}$ = 10.2 vs. −5.5%), and this model carried more cumulative model weight than the null model (71 vs. 29%). Terms for the proportion of 'peripheral zone' in the Human Contact test at post-breeding $^{Y1\ \&\ Y2}$ and weaning $^{Y1\ \&\ Y2}$ were insignificant positive predictors in the binary 'investigate human' model; however, they did carry more cumulative model weight than the null model.

The proportion of 'sniffing/grooming' and 'environmental vigilance' were involved in patterns of association with behaviors from the Human Contact and Human Presence tests. The P

proportion of time in the 'peripheral zone' at gestation was a significant positive predictor of 'sniffing/grooming' the lamb(s) (*P*=0.01) (Figure 2). The model with a term for the proportion of time in the 'peripheral zone' at gestation $^{Y1\ \&\ Y2}$ carried more cumulative weight than the null model (cumulative weight= 84 vs. 16%) and explained more variance ($R^{2adjusted}$= 36 vs. 26.6%). The proportion of time in the 'peripheral zone' at weaning $^{Y1\ \&\ Y2}$ was a significant positive predictor of 'sniffing/grooming' the lamb when a term for the group (Human Presence test) was included (*P*=0.01).

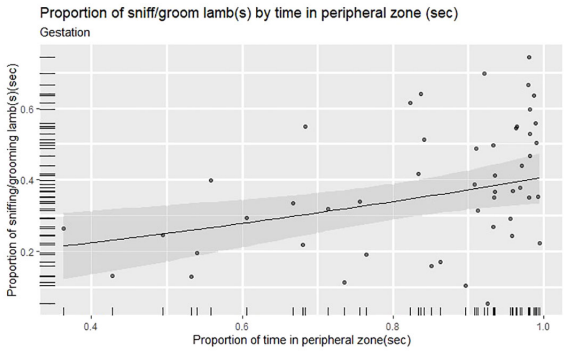

FIGURE 2
Proportion of 'sniffing/grooming' the lamb in the Lamb Tie Down test by time in the 'peripheral zone' in the Human Presence test. The proportion of time in the 'peripheral zone' at gestation was a significant positive predictor of 'sniffing/grooming' the lamb(s) (P=0.01). The model with a term for the proportion of time in the 'peripheral zone' at gestation $^{Y1\ \&\ Y2}$ carried more cumulative weight than the null model (cumulative weight= 84 vs. 16%) and explained more variance ($R^{2adjusted}$ = 36 vs. 26.6%).

The model including 'peripheral zone' carried less cumulative model weight than the null model (3 vs. 97%) yet explained slightly more variance than the null model (30.9 vs. 26.6%). The proportion of time in the 'peripheral zone' at post-breeding was an insignificant positive term in the model for 'sniffing/grooming' lamb(s) (P=0.22). The null model carried more cumulative model weight (80%), with a lower REML value than the hypothetical model (cumulative model weight = 20%). The null model also explained more variance than the hypothetical model ($R^{2adjusted}$= 25.3 vs. 26.6%).

The proportion of time in the 'peripheral zone' in the Human Presence test, at post-breeding, was a significant negative predictor of 'environmental vigilance' in the Lamb Tie Down test (P=0.02) (Figure 3). The model including a term for the 'peripheral zone' at post-breeding explained more variance than the null model ($R^{2adjusted}$ = 14.2 vs. 8%); however, it carried less cumulative model weight than the null model (69 vs 31%). Time in the peripheral zone at gestation and weaning was not a significant predictor of environmental vigilance in the Lamb Tie Down test, and this term did not improve model fit. A term for the peripheral zone at weaning explained more variance than the null model, but not at gestation [($R^{2adjusted}$ = 7.2% $_{gest}$, 12.1% $_{wean}$ vs. 8% $_{null}$)].

3.3.3 Cross-contextual analysis and lamb outcome

The null model for birth weight included a term for the age of the ewe and vocal activity of the lamb(s). The proportion of time in the 'peripheral zone' at post-breeding and weaning was an insignificant positive predictor term in the model for birth weight, and the inclusion of this term carried more model weight than the null model. The proportion of time in the peripheral zone at weaning was a nearly significant positive term in the model for lamb birth weights (P=0.06), when a factor term for group was included. The proportion of time in the 'peripheral zone' at gestation was an insignificant positive predictor of lamb birth weights, and the inclusion of this term did not carry more cumulative model weight than the null model (21 vs. 79%). The birth weight models including a term for 'peripheral zone' had negative $R^{2adjusted}$ values. The null model for weaning weights included a term for the age of the ewe, birth weight, and day of

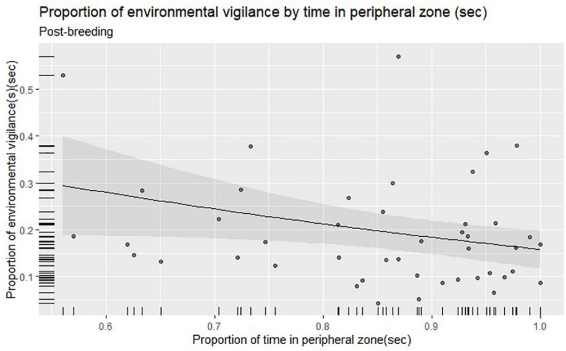

FIGURE 3
The proportion of 'environmental vigilance' in the Lamb Tie Down test by time in the 'peripheral zone' in the Human Presence test. The proportion of time in the 'peripheral zone' in the Human Presence test, at post-breeding, was a significant negative predictor of 'environmental vigilance' in the Lamb Tie Down test (P=0.02). The model including a term for 'peripheral zone' at post-breeding explained more variance than the null model ($R^{2adjusted}$ = 14.2 vs. 8%) but carried less cumulative model weight than the null model (69 vs. 31%).

birth. The proportion of time in the peripheral zone at post-breeding, gestation, and weaning was an insignificant positive predictor of lamb weaning weights, and the inclusion of this term resulted in a more cumulative model weight than the null model (100 vs. 0%) and explained similar variance to the null model ($R^{2adjusted}$ = 38.7% $_{post}$, 36.7% $_{gest}$, 37.2% $_{wean}$ 38.2% $_{null}$).

3.4 Post-hoc findings

Although not cross-contextual, a *post-hoc* relationship was found between 'sniffing/grooming' the lamb(s) and 'environmental vigilance' in the Lamb Tie Down test. The model for 'sniffing/grooming' the lamb included a term for the age and breed of the ewe. The proportion of 'environmental vigilance' was a significant negative term in the model for 'sniffing/grooming' the lamb(s) (P<0.01) (Figure 4). According to Schiller and Horback (In Review), the proportion of 'sniffing/grooming' the lamb(s) was an insignificant positive term in the model for lamb birth and an insignificant negative term for lamb weaning weights. The inclusion of 'sniffing/grooming' carried more cumulative model weight than the null model for birth weights and weaning weights. The proportion of 'environmental vigilance' in the Lamb Tie Down test was a nearly significant (P=0.06) negative predictor of lamb birth weights (Figure 5) and an insignificant positive predictor of lamb weaning weights. The inclusion of the proportion of 'environmental vigilance' in the hypothetical model carried more cumulative model weight than the null model for birth and weaning weights. The inclusion of environmental vigilance explained a similar amount of variance to the null model for weaning weights ($R^{2adjusted}$ = 37.6 vs. 39.2%). The variance explained in the birth weight models was very low (approximately 0).

4 Discussion

The selection for desirable phenotypes, by observing behavioral expression, may be carried out on farms to promote successful ewe-lamb bonding and consequently improve lambing success and increased performance in extensively farmed sheep. The objectives of the current study were to observe a gradient of HAIs (Human Contact, Presence and Approach) when the lamb was not present and compare them to behavioral responses toward a human directly after parturition, when the lamb was present. 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 postpartum behavior and lamb outcome. Interestingly, a *post-hoc* negative relationship was identified between environmental vigilance and grooming the lamb(s) in the Lamb Tie Down test, indicating the presence of a response that may have been the basis of other relationships found outside of the lambing season. Sheep who groomed the lamb more had increased frequencies of head posture changes in the Human Contact test and stayed in the zone furthest from the human in the Human Presence test (gestation and weaning), compared with sheep who groomed less. Increased grooming has been observed in sheep after human encounters who have a negative perception of the human handler (Hild et al., 2011), and therefore it is possible that frequent head posture changes and the avoidance of humans also indicate a negative arousal state during human exposure. Sheep who were more environmentally vigilant in the Lamb Tie Down test were less likely to assume a head down posture in the Human Contact test (gestation and weaning) and were less avoidant of the human in the Human Presence test (post-breeding). Head down posture and human avoidance may be signs of stress in the ewe (Hemsworth et al., 2011, 2019; Beausoleil et al., 2008, 2012). Environmental vigilance is known to reflect anxiety states in sheep (Monk et al., 2019, 2020); however, the biological meaning within the context of the lambing season is unknown. Furthermore, sheep who investigated the human more in the Lamb Handling test were less avoidant of the human in the Human Presence test, only at gestation. No statistically significant relationships were observed between birth and weaning weights with behavioral measures; however, grooming the lamb and environmental vigilance did improve the model fit for these responses. Larger birth weights were explained by more grooming (Schiller and Horback, In Review) and less vigilance, and larger weaning weights were explained by less grooming (Schiller and Horback, In Review) and more vigilance. More research is needed to explore this grooming/vigilance response in relation to HAIs outside

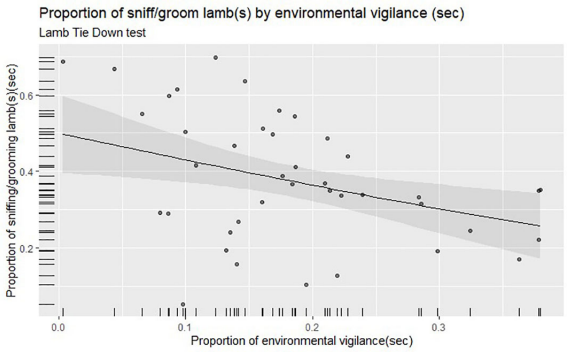

FIGURE 4
The proportion of 'environmental vigilance' by the proportion of time 'sniffing/grooming' the lamb in the Lamb Tie Down test. The proportion of 'environmental vigilance' was a significant negative term in the model for 'sniffing/grooming' the lamb(s) (P<0.01).

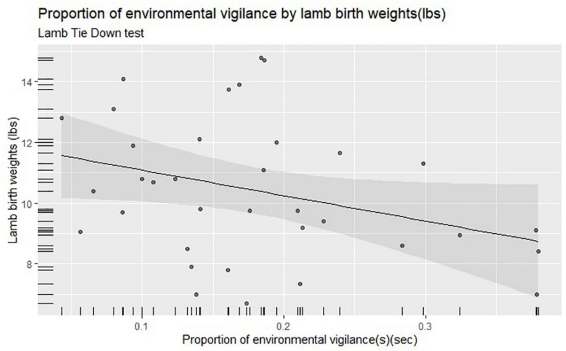

FIGURE 5
The proportion of environmental vigilance in the Lamb Tie Down test by lamb birth weights (lbs). The proportion of 'environmental vigilance' was a nearly significant negative predictor of lamb birth weights. The inclusion of 'environmental vigilance' in the birth weight model improved the fit over the null model and carried 100% of the cumulative model weight.

of the lambing season and their relationship with lamb outcome. Given the relationship with other behaviors, it seems that grooming may have been a coping response after a stressful encounter with the human.

4.1 Cross-contextual relationships

Given the negative relationship between environmental vigilance and grooming the lamb in the Lamb Tie Down test, it is likely that these two behaviors were a part of a perinatal, context-specific lambing response that connected to other relationships outside of the lambing season. Unfortunately, repeatability estimates could not be gathered on these variables, which would be ideal to identify a stable behavioral trait. The authors theorize that this context-specific response was characterized by vigilance and grooming behavior immediately after the lamb was born, and also involved avoidance of the human across the perinatal period (gestion to weaning). Ewes who groomed the lamb more in the Lamb Tie Down test also avoided the human more in the Human Presence test, which was most poignant at gestation and weaning. This relationship did not persist through post-breeding. At first, the authors postured that grooming behavior was pronounced in individuals who experienced more stress during HAIs during the perinatal period. Increased grooming behavior has been observed after stressful encounters with a human handler in ewes that were treated aversely compared with gently treated ewes (Hild et al., 2011). Ewes in the current study were not treated aversely however, individuals who groomed the lamb more may have experienced more stress/anxiety during the processing event and performed compensatory grooming behavior as a coping strategy. More non-maternal oral grooming was observed in ewes following a stressful event (shearing) than in control ewes (Emeash et al., 2008). To support this context-specific response theory, individuals who avoided the human in the Human Presence test at gestation also avoided the human in the Lamb Handling test, which did not occur at weaning and post-breeding.

Stress-induced grooming could have been present and supported through other behavioral relationships in the current population of ewes. Grooming behavior was 1) highest in ewes that avoided the human during gestation and weaning, 2) highest in ewes who frequently changed head posture during human contact at gestation and weaning, and 3) highest when lambs were heavier at birth. Grooming behavior was not positively associated with lamb weaning weights. Schiller and Horback (In Review) hypothesized that grooming was driven by maternal investment in heavier lambs; however, with more information now, it also seems plausible that these relationships were moderated by the stress response, and ewes who were more susceptible to stress groomed their lambs more and gave birth to heavier offspring. Roussel et al. (2004) reported that ewes who underwent more prenatal stress (social isolation) gave birth to heavier lambs than control ewes. There was no difference in daily weight gain between prenatally stressed and control lambs and no difference in weight after 8 months of age (Roussel et al., 2004). Roussel-Huchette et al. (2008) found that lambs born to ewes who experienced isolation stress during pregnancy had greater live weights at 3 months of age than control lambs. Although a somewhat inconsistent finding, shearing (a stressful procedure) during pregnancy has been associated with increased lamb birth weights (Kenyon et al., 2003; Wei et al., 2023). Authors of the current manuscript frame stress as a trait of the ewe and site research that has imposed stress on the ewe; however, the impact on lamb outcome may be the same. In terms of human avoidance, Aydoğdu and Karaca (2021) found that ewes who spent less time in the zone with the human in an arena test also separated less from the lamb and yielded lambs of heavier live weights at days 30 and 45, yet this difference was not observed at weaning. Aydoğdu and Karaca (2021), however, found no differences in the duration of grooming the lambs between avoidant and less avoidant ewes. Murphy et al. (1994) conducted an isolation box test and arena test to compare behavioral responses against maternal care after lambing. Behavior in the presence of a human was not related to the duration of time licking the lamb after birth, however, and reactivity in isolation was negatively associated with the licking time after

birth. These results are somewhat relevant under the framework of the current study; however, grooming behavior immediately after birth is different to grooming behavior after a stressful event. If there is a positive relationship between grooming, stress, and lamb birth weights with the current population of ewes, results would align with Aydoğdu and Karaca (2021) and Roussel et al. (2004) in that ewes who are more susceptible to stress (and consequentially avoidant of the human) may realize differences in early postpartum lamb live weights that do not persist throughout the lambing season. Leedy and Alexander (2007) found that after ewes were separated from their lambs for 60 s, there was a reduction in maternal behavior (nosing, udder allowance, and low-pitched grunting) compared with ewes who were not separated from the lamb, which would contradict the theory that stress increases grooming behavior. Leedy and Alexander (2007) did combine nosing, udder allowance, and low-pitched grunting into one maternal score, which was not carried out in the current study. Stress-induced grooming seems like a potential explanation for the findings in this study, yet the variance explained for birth weights was quite low and therefore there may have been a variable that went unaccounted for. The hour of birth was not included as a term in the model, as it was hard to estimate in the rangeland ewes and could have affected birth and grooming behavior. Data were collected 6+ h after birth; therefore, initial grooming to clean the amniotic fluid and allow the lamb to thermoregulate would have already occurred. Additionally, the hour of birth could be a covariate of grooming behavior but is likely not related to human avoidance and head posture changes in the Human Contact test. Finally, grooming was negatively associated with weaning weights. The working theory behind this relationship is that grooming the lamb after a stressful event is likely not representative of the maternal care provided throughout the entire lambing season and is more just a coping strategy. Once the ewe and lamb were left undisturbed, the ewe likely resumed her normal mothering behavior, which may have been repeatable, as evidenced through the repeatable weaning weight measures. Grooming is normally considered an adaptive maternal behavior that should benefit the lamb and foster a stronger ewe-lamb bond. Grooming behavior in the current study seems to be less of an adaptive response and more one moderated by the stress response.

Other results of the study support a notion that stress-induced grooming behavior occurred after lamb processing. More frequent head posture changes in the Human Presence test, at gestation and weaning, were related to increased levels of grooming in the Lamb Tie Down test. Tamioso et al. (2018) reported that ewes who underwent brushing by a human handler showed fewer head posture changes than ewes simply experiencing human presence. The same study found that highly reactive ewes (defined as frequent vocalizations and reduced vigilance posture) changed head posture more during human presence than brushed ewes. Findings from Tamioso et al. (2018) suggest that fewer head posture changes could be related to positive valence in sheep and a more positive experience with the human handler. Tamioso et al. (2020) also reported that individuals who demonstrated fewer head orientation changes experienced a more positive valence state during brushing, supported through heart rate and parasympathetic nervous system activity. With the current population, sheep that changed head posture frequently may have been more fearful of the human during the Human Contact test and during/after the Lamb Handling test.

Environmental vigilance was negatively related to grooming behavior in the Lamb Tie Down test. The biological relevance of vigilance during the lambing season is underreported. Typically, vigilance is thought to reflect a negative high arousal state in ewes such as anxiety (Monk et al., 2019; 2020). Monk et al. (2023) also found vigilance in sheep to be repeatable and perhaps evident of an underlying temperament trait. Relationships between environmental vigilance in the Lamb Tie Down test and those outside of the lambing season suggest that vigilance may have been moderated by a neutral valence state or at least one separate from heightened stress. This is evidenced through the 1) negative relationship with head down behavior in the Human contact test (gestation and weaning) and 2) the negative relationship with time in the 'peripheral zone' in the Human Presence test (post-breeding). Hemsworth et al. (2011, 2019) found that increased head down prior to slaughter was associated with greater serum cortisol concentrations in sheep. Multiple studies have found that sheep who avoid the human more in an arena test are less bold and have greater serum cortisol levels than sheep who approach the human (Beausoleil et al., 2008, 2012). The relationship between the avoidance of human and environmental vigilance was most discernable at post-breeding and less so at gestation.

Results from the weaning weight models were more challenging to interpret. Lamb weaning weight models were improved by time in the 'peripheral zone' during the Human Presence test, and the association between these two variables was positive. More research is needed to explain the relationship behind the avoidance of human and lamb weights. In visualizations of weaning weights, they appeared to share a very slight positive relationship with time in the peripheral zone within the Human Presence test. Previous research has mostly assessed the avoidance of the human in relation to weaning weights by using a maternal behavior scoring system (1=flee lamb and shepherd and 5=stay near lamb and shepherd) during lamb processing (O'Connor et al., 1985). Moraes et al. (2016) found that higher MBSs (ewe stays close to the lamb/shepherd) were associated with greater lamb survival but were not strongly related to other maternal behaviors. Lambe et al. (2001) reported that weaning weights were not affected by the MBS of the ewe. Everett-Hinks et al. (2005) and Brown et al. (2015) found that these maternal behavior scores had low repeatability, and Lambe et al. (2001) reported a moderate repeatability estimate on the MBS. Oddly, Everett-Hinks et al. (2005) found that the heritability of lamb survival was greater for ewes who avoided the human more at processing, which is typically the opposite of what is expected, which was in conflict with Lambe et al. (2001) finding that MBSs of 1 were related to the increased mortality of the lamb. Inconsistent findings on MBSs may decrease the functionality of this scoring system under differing environmental conditions. Schiller and Horback (In Review) found a potential fear response related to the human, during lamb processing, that could override normal maternal care. Some research has corroborated a

relationship between lamb outcome and ewe reactivity in restraint. Pajor et al. (2010) found that ewes who are less reactive in restraint, on a weight, produced more milk and had lambs of a higher preweaning weight gain and weaning weights than nervous ewes, and Gavojdian et al. (2015) observed that calmer ewes had improved lamb performance (i.e., lamb growth rates). Greater durations in the zone furthest from the human had a positive association with weaning weights, birth weights, and environmental vigilance in the Lamb Tie Down test. We cannot claim here that human avoidance would be useful in a selection tool; however, the avoidance of the human, when the lamb was not present, was associated with weaning weights (a repeatable measure) and therefore could be considered in future investigations.

5 Limitations

During the Human Contact test, the position in the raceway was not standardized and ewes assumed any position by self-sorting. A fixed term for the position in the raceway was included in the models with a Human Contact response outcome (when needed); however, this was likely not enough to entirely account for the effect of crowing in the back of the raceway. Ewes were group tested in the Human Contact, Presence, and Approach test, which may have diminished individual behavioral responses toward the human stimulus. Group (n=5) was included as a fixed factor term for some models, particularly those during weaning. Weaning seems to have affected group behavior most, which makes sense as that is when the lamb is separated from the ewe. Observations during the lambing season for the Lamb Handling and Lamb Tie Down test were made on rangeland, wherever the ewe was spotted. Ewes that were not being tested that day were not restrained and could interrupt the behavior of the focal ewe. Lambs were restrained for the Lamb Tie Down test to keep them in camera view while the researcher and shepherd were not in close proximity; however, this treatment seemed more stressful than expected. Lamb behavior was recorded and used as a covariate for the ewe's behavior, yet a different method to keep the lamb on camera would be preferred for future studies. The day on which the lamb was born was recorded; however, the hour could not be precisely recorded and may have been an important covariate for some of the ewe-lamb interaction models. Lambs were processed between 6 and 36 h after birth and that time difference could have resulted in differences in grooming behavior. Fortunately, grooming was related to behaviors outside of the lambing season that would not need the hour of birth as a covariate term.

6 Conclusion

The current study identified some patterns of association between behaviors within and outside of the lambing season in rangeland ewes. A context-specific perinatal response seemed to manifest strongest between post-breeding and weaning. This response was characterized by levels of grooming and environmental vigilance when the ewe and lamb were alone together and avoidance of the human during close contact and presence. Processing can be a stressful event for the ewe, and grooming may have been a strategy to cope after the human intervention, when the lamb was tied down, or a way to calm the lamb. It is likely that the ewes' behavior during this event was not representative of their regular maternal care throughout the rest of the lambing season. Weaning weights were repeatable, within ewe, and positively associated with the avoidance of human in the Human Presence test and environmental vigilance in the Lamb Tie Down test and negatively associated with grooming behavior. More research is required to validate these relationships; however, the avoidance of human when the ewe is unrestrained could be considered as a behavior to focus on in terms of improving lamb outcome.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal studies were approved by University of California, Davis Institute of Animal Care and Use Committee (protocol #20926). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

KS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. KH: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2024.1366211/full#supplementary-material

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