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Effect of a targeted pain management protocol for the treatment of dermatitis interdigitalis contagiosa in Merino meat sheep in a tilt squeeze chute

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Introduction: The effect of a targeted pain management protocol consisting of sedation and local anesthesia on the stress response to treatment of dermatitis interdigitalis contagiosa (DINCO) was assessed in sheep placed in dorsal recumbency.

Methods: Blood cortisol concentrations were measured once a day (Day -3 to 2) and additionally on day 0, six times during the claw treatment (stress model). Twelve healthy sheep (control group; HEALTHY) and 36 sheep with DINCO, randomly allocated to one of three treatment groups, underwent the stress model with or without pain control: the XYLA-IVRA sheep were sedated with 2% xylazine hydrochloride (XYLA) and received retrograde intravenous regional anesthesia (IVRA); the IVRA sheep underwent IVRA and received a placebo instead of sedation; the PLACEBO sheep received placebos for sedation and IVRA. The HEALTHY sheep underwent sham claw treatment and received placebos.

Results and discussion: The cortisol concentrations were higher in sheep restrained in dorsal recumbency compared with the cortisol concentrations measured four hours later in standing sheep (HEALTHY 37.2 $\pm$ 3.3 ng/ml vs. 18.5 $\pm$ 3.3 ng/ml; DISEASED 34.0 $\pm$ 1.9 ng/ml vs. 17.6 $\pm$ 1.9 ng/ml; $p < 0.001$). The stress response of XYLA-IVRA (area under the curve; AUC = 34.9 $\pm$ 2.6 ng/ml) was reduced compared with the stress response of PLACEBO (AUC = 48.0 $\pm$ 2.6 ng/ml, $p < 0.01$) and HEALTHY sheep (AUC = 46.6 $\pm$ 2.5 ng/ml; $p = 0.01$). While cortisol concentration of XYLA-IVRA and HEALTHY sheep did not increase one day after the stress model (Day 1) compared with the day of the stress model

(Day 0), both PLACEBO (47.4 ± 3.3 vs. 35.6 ± 3.1 ng/ml, p = 0.02) and IVRA sheep (39.1 ± 2.8 vs. 28.6 ± 3.1 ng/ml, p = 0.01) had higher cortisol concentrations. The results confirm that fixation in dorsal recumbency in a tilt squeeze chute was a major stressor in sheep. The differences in the cortisol concentration of the PLACEBO sheep versus the XYLA-IVRA sheep during and after the stress model illustrate the effect and necessity of pain management protocols in practice.

KEYWORDS

cortisol, stress, dorsal recumbency, tilt squeeze chute, claw treatment, regional anesthesia, sedation, pain mitigation

1 Introduction

Husbandry and farm management procedures have been improved in recent years to address welfare issues in farmed animals. However, routine procedures such as castration, mulesing and tail amputation (Welsh et al., 1993; Shaofu et al., 2013; Hannemann et al., 2017) and locomotor disorders associated with lameness (Winter, 2008) are still common and remain important causes of pain (Fitzpatrick et al., 2006).

Painful conditions cause stress (Moberg and Mench, 2000). In addition to pain, other factors such as handling, transport and isolation from herd mates serve as stressors (Grandin, 1997). Stress results in activation of the sympathetic-pituitary-medullary system (Spencer and Deak, 2017) and the hypothalamic-pituitary-adrenal axis leading to an increase in the blood cortisol concentration (Mellor et al., 2002). The stress response has a negative effect on the health and well-being of farmed animals and on production levels (Hargreaves and Hutson, 1990; Grandin, 1997; Blokhuis et al., 1998; Wolf et al., 2020). Pain and stress should be prevented for economical as well as ethical reasons (Farm Animal Welfare Council, 2009).

Due to their interaction, the assessment of stress and pain is based on changes in the same physiological, biochemical and behavioral indices (Molony and Kent, 1997; von Borell, 2000; Fieseler et al., 2018). Stress and pain can each affect the well-being of an animal and both should therefore be evaluated (Stubsjøen et al., 2015). The measurement of cortisol concentrations in various matrices has been established for the assessment of stress and pain-induced stress in sheep (Miller et al., 1991; Stubsjøen et al., 2015; Wagner et al., 2021).

Analgesics and sedatives are used to mitigate stress and pain in animals undergoing surgical procedures or other painful manipulations (Anderson and Edmondson, 2013). An optimal pain management protocol should not be limited to the reduction of pain caused by a lesion but should also include steps to reduce movement-related pain and stressors such as defensive movements or other avoidance behaviors (Beloeil and Sulpice, 2016). This can be achieved through pain management protocols, which interrupt nociception at multiple sites of the nociceptive pathway (Stilwell et al., 2020). The combination of local anesthesia with sedation has been shown to be effective in mitigating pain in numerous surgical procedures not only in sheep (Hodgkinson and Dawson, 2007; Galatos, 2011) but also in cattle, dogs and horses (Searle et al., 1999; Greene, 2003; Cuzmar et al., 2019; Silva et al., 2020). Intravenous regional anesthesia (IVRA) is the technique of choice for pain mitigation in sheep undergoing surgery of the distal limb (Hodgkinson and Dawson, 2007; Galatos, 2011; Fieseler et al., 2019). Procaine hydrochloride is the only local anesthetic approved for sheep in the European Union. For sedation, xylazine hydrochloride is registered for use in food-producing animals in the European Union and other countries, but is not approved for livestock in many other parts of the world, for example the United States (Emmerich, 2011; Smith, 2013; Coetzee, 2013). Nevertheless, xylazine is often used off-label for sedation, analgesia and muscle relaxation in sheep (Hodgkinson and Dawson, 2007; Lizarraga and Chambers, 2012; Amin et al., 2022).

In sheep, *dermatitis interdigitalis contagiosa* (DINCO) or contagious footrot, is a painful claw disease (McLennan et al., 2016). Bacteriologic culture of typical DINCO lesions yield *Dichelobacter nodosus* as the primary etiological agent, *Fusobacterium necrophorum* and different species of *Treponema* (Frosth et al., 2015; Zanolari et al., 2021). Lesions of DINCO and treatment involving a tilt squeeze chute are a useful model for the assessment of acute and chronic pain and stress in sheep (Ley et al., 1989; Fieseler et al., 2019; Amin et al., 2022). Acute pain associated with treatment has also been shown to increase the cortisol concentration in sheep (Fell and Shutt, 1989). Fixation in a tilt squeeze chute in dorsal recumbency elicits a clear stress response even in healthy sheep (Wagner et al., 2021; Amin et al., 2022).

A literature search in pubmed (November 28, 2022) for studies investigating whether the stress responses differ between healthy and diseased sheep, using the search terms sheep, stress, cortisol, handling and footrot yielded negative results. An additional search (November 28, 2022) for studies on the effect of pain management on the stress response, determined using blood cortisol concentrations, during local treatment of DINCO in sheep also produced negative results (search terms: sheep, stress, cortisol, pain management, claw treatment, footrot).

The primary goal of this study was to determine whether a targeted pain management protocol that incorporates sedation and local anesthesia modifies the stress response to treatment of DINCO-associated corium lesions in sheep placed in dorsal recumbency in a tilt squeeze chute. A secondary goal was to measure changes in cortisol concentration during the adaptation phase before treatment and in the period when sheep were changed from a standing position to a recumbent position in the tilt squeeze chute.

2 Materials and methods

This study was part of a larger project focusing on pain management and individual stress response during treatment of DINCO in ewes. In addition to the data presented here, behavioral and physiological data were collected during the study (Fieseler et al., 2018; Fieseler et al., 2019). The study was approved by the State Administration Office (Landesverwaltungsamt, Referat Verbraucherschutz, Veterinärangelegenheiten Sachsen-Anhalt, Germany); permit number 42502-3-734 (Fieseler et al., 2018; Fieseler et al., 2019). The randomized, prospective, triple-blinded study was carried out on a commercial sheep farm with 700 Merino meat ewes in Central Germany between December 2014 and May 2015.

2.1 Animals

All sheep on the farm were checked daily, and suitable animals were included in the study based on the following criteria: normal demeanor, which was determined by observation (Jackson and Cockcroft, 2008), less than 100 days pregnant, and had not received non-steroidal anti-inflammatory drugs (NSAIDs) within 28 days before the start of the study or were not in a meat withdrawal period after antibiotic treatment. Only sheep with claw lesions in one hind foot suggestive of DINCO (Fieseler et al., 2018) and no other diseases of the locomotor system were included in the group DISEASED. Samples were collected from diseased sheep that were not included in the study but were housed in the same barn, before, during and after the study for culture of *Dichelobacter nodosus* (PCR; Office for food safety and animal health Graubünden, Swiss); all culture results were positive (virulent). For inclusion in the study, all sheep in the HEALTHY group had to be sound and free of claw lesions.

Forty-eight open ewes were included and used exclusively in this study. They were an average of 2.4 years of age (range, 1.1 to 8.4 years), weighed an average of 53.8 kg (range, 39.5 to 78.0 kg) and had a median body condition score (BCS) of 3.25 (range, 2.00 to 3.75).

2.2 Study design

Thirty-six sheep met the inclusion criteria for the group DISEASED, and the remaining 12 sheep fulfilled the criteria for the group HEALTHY. So-called *study groups* (n = 12) consisting of four sheep (3 diseased and 1 healthy) were formed and entered the study at the same time. Only after data collection from one study group was completed were the sheep of the next study group enrolled. The sheep of a study group were housed together in a straw-bedded, free-stall barn and had visual contact with the other sheep of the flock and with the nearby treatment area with an alleyway and a tilt squeeze chute (Biermann Eisenwaren Landtechnik, 31637 Rodewald). The sheep in the first eight study groups were an average of 1.2 years of age (range, 1.1 to 1.3 years), weighed an average of 49.5 kg (range, 39.5 to 64.5 kg) and had a median body condition score (BCS) of 3.30 (range, 3.00 to 3.75), while the sheep in the remaining four study groups had an average age of 4.9 years (range, 2.4 to 8.4 years), weighed an average of 62.2 kg (range, 47.5 to 78.0 kg) and had a median body condition score (BCS) of 2.97 (range, 2.00 to 3.50).

Within the DISEASED group, the treatment type was assigned randomly using a lottery procedure described by Schären et al. (2021):

- Group XYLA-IVRA: treatment of the lesion under retrograde intravenous regional anesthesia (IVRA) using 5 ml of 2% procaine hydrochloride (Procasel-2%®, Selectavet Dr. Otto Fischer GmbH) and sedation with 2% xylazine hydrochloride (0.1 mg/kg; Rompun®, Bayer Vital).
- Group IVRA: treatment of the lesion under IVRA using 5 ml of 2% procaine hydrochloride and a placebo (equivalent volume of isotonic saline solution; Isotone Kochsalz-Lösung 0.9% Braun®, B. Braun Melsungen AG) instead of xylazine hydrochloride.
- Group PLACEBO: treatment of the lesion using a placebo (5 ml isotonic saline solution) instead of procaine hydrochloride and a placebo (equivalent volume of isotonic saline solution) instead of xylazine hydrochloride.
- HEALTHY group: 12 healthy sheep formed a control group that underwent sham claw treatment and received 5 ml isotonic saline solution instead of procaine hydrochloride for the IVRA and an equivalent volume of isotonic saline solution instead of xylazine hydrochloride.

For all examinations and sample collections the sheep were calmly led from the group pen to the tilt squeeze chute along the alleyway. At the start of the study (Day -4), sheep selected based on the inclusion criteria underwent a complete physical examination, which included an orthopedic examination with the sheep standing and walking for 10 m on a level floor. After this, each sheep of a study group was led to the tilt squeeze chute to assess posture, behavior, physical condition, fleece, body condition, respiratory rate, pulse rate and body temperature. Sheep that met the inclusion criteria were tilted into dorsal recumbency, the limbs were secured and three feet were trimmed per sheep (Fieseler et al., 2018). In the three treatment groups, both front feet and the healthy hind foot of each sheep were trimmed. In the HEALTHY sheep, both front feet and the left hind foot were trimmed. After this procedure, sheep were moved back to the group pen. Two hours later, the sheep were restrained manually in a standing position in the group pen, and an indwelling Teflon catheter was placed in the external jugular vein (Figure 1; VWI Jugularis-Katheter, outer diameter 2.4 mm, length 10 cm, Walter Veterinär-Instrumente e.K., Baruth/Mark; Amin et al., 2022).

On days -3 to 2 between 08:00 and 10:00, the sheep of the study group underwent a brief physical examination to determine their health status. Blood was collected from the catheter with the sheep in dorsal recumbency in the tilt squeeze chute (Wagner et al., 2021). On day 0, between 11:00 and 15:00, the sheep of the treatment groups underwent claw treatment in the previously untreated hind claw with or without pain control (XYLA-IVRA, IVRA, PLACEBO), and the HEALTHY sheep underwent sham claw treatment in the right hind foot. Claw treatment compromised careful removal of undermined horn and necrotic tissue using curved claw trimming shears and a claw knife to create a clean transition to healthy tissue. Sham treatment of the right hind claws of HEALTHY sheep consisted of the careful removal of loose horn to restore the proper shape of the claws (Wagner et al., 2021). The procedures carried out between 11:00 and 15:00 on day 0 are referred to as the stress model. Days -3 to 0, before the stress model, represented the adaption phase (Figure 1).

The stress model on day 0 was conducted in a standardized fashion. Blood was collected immediately after each experimental procedure. The sheep were placed in the tilt squeeze chute and received the injections according to group allocation; 0.1 mg/kg 2% xylazine hydrochloride (Rompun®, Bayer Vital) or an equivalent volume of isotonic saline solution (Isotone Kochsalz-Lösung 0.9% Braun®, B. Braun Melsungen AG). The anconeus muscle was used for the injections. After this, the first blood sample of the stress model phase was collected (Min -30). The sheep were then left standing for 10 min without manipulation, after which time they were tilted into dorsal recumbency. The limbs were fixed, and the second blood sample was collected (Min -20). Preparations were then made for IVRA, and 10 min after tilting into dorsal recumbency, 5 ml 2% procaine hydrochloride (Procasel-2%®, Selectavet Dr. Otto Fischer GmbH) or 5 ml saline solution (Isotone Kochsalz-Lösung 0.9% Braun®, B. Braun Melsungen AG), depending on group allocation, was injected into the raised cranial branch of the lateral saphenous vein. A butterfly catheter (Surflo Winged Infusion Set 21G×¾" 0.8 x 19mm, 30 cm long, Terumo Europe N. V.) was used for this. This was followed by blood collection (Min -10). Claw treatment or sham treatment started 10 min after the IVRA-procedure. After claw treatment or sham treatment another blood sample was collected (Min 0). The exposed DINCO lesion was then treated with an oxytetracycline spray (Engemycin TM Spray 3.84 w/w, Intervet) and a bandage was applied to the foot 5 min after the start of treatment. Ten min after the start of treatment, the limbs were untied, the sheep were placed in a standing position, the tourniquet was removed and a blood sample was collected (Min 10). The sheep remained standing in the tilt squeeze chute for 10 min, after which time they were moved back to the group pen. At time point Min 30, the sheep were restrained manually

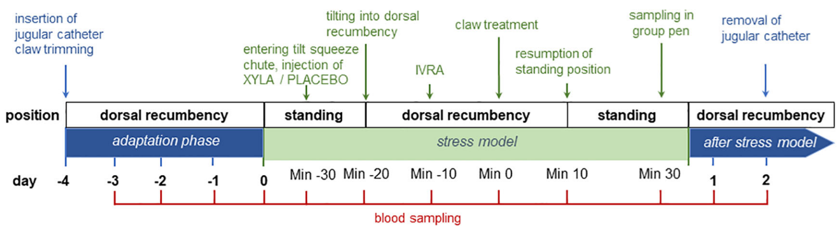

FIGURE 1
Illustration of the experimental design (Days -4 to 2): Day -4: Insertion of jugular catheter and trimming of both front feet and one hind foot; Days -3 to 0 (adaptation phase) and day 1 and day 2 (after stress model), blood sampling from jugular catheter in the morning; Day 0, midday: implementation of the stress model in a tilt squeeze chute: Min -30: sheep is placed in tilt squeeze chute and receives medication according to group allocation (XYLA = 0.1 mg/kg 2% xylazine hydrochloride; PLACEBO = equivalent volume of isotonic saline solution); Min -20: positioning of the sheep in dorsal recumbency; Min -10: administration of retrograde intravenous regional anesthesia (IVRA) according to group allocation; Min 0: claw treatment, actual or sham; Min 10: resumption of standing position; Min 30: blood collection in group pen; blood was collected after each procedure.

in a standing position, and the last blood sample of the stress model phase was collected (Figure 1).

All clinical examinations and blood collections were carried out by the same two veterinarians (RW, HF), and the actual and sham claw treatments and injection of drugs were done by a third veterinarian (MK, HM). The collection and storage of blood samples and the measurement of the cortisol concentration were done as described (Wagner et al., 2021). The cortisol concentration was determined using a ^{3}H-radioimmunoassay (label/tracer: [1,2,6,7-^{3}H]-cortisol; intraday variation coefficient 6.5%, interday 7.8%; sensitivity 0.5 ng/ml for serum, saliva, tears and milk) modified according to Abraham et al. (2005). The indwelling catheter was removed on day 2. Bandage changes and monitoring of wound healing were as described (Fieseler et al., 2019).

Criteria for exclusion from the study included a rectal temperature >39.5° C or a severely abnormal demeanor during the study period. An additional criterion for exclusion from the HEALTHY group was the development of DINCO. It was not necessary to exclude any sheep from the study. Sheep in which blood collection through the indwelling jugular vein catheter was not possible or sheep that developed thrombophlebitis of the catheterized jugular vein were excluded from the analysis of cortisol concentration data as soon as clinical signs occurred. Because of these complications the cortisol concentrations of 11 sheep (XYLA-IVRA, n = 4; PLACEBO, n = 4; HEALTHY, n = 3) were excluded from data analysis from day 1.

2.3 Statistics

Statistical analysis was carried out using linear models implemented in the MIXED procedure of SAS version 9.4 (SAS Institute 2013, Cary, NC, USA). Possible environmental or parity effects during the study period were considered by including fixed effects of the study groups in all models. The data of the sheep of the first eight study groups (= YOUNG; N = 32) and the data of the sheep of the last four study groups (= OLD; N = 16) were summarized and described descriptively in each case. The repeated measurements within an animal were taken into account by using random animal effects and (or) by the variance-covariance structure of the residual effects. The following competing structures were tested: compound symmetry (CS), heterogeneous compound symmetry (CSH), first order autoregressive (AR (1)), heterogeneous first order autoregressive (ARH (1)), unstructured (UN) and, toeplitz with two, four, or six bands (TOEP (2), TOEP (4), and TOEP (6)). The final correlation structure was chosen according to the lowest value of Akaike's information criterion (AIC) based on the REML method. The Shapiro-Wilk test was used to test the studentized residuals in all models for normality. Differences between treatment groups within time points (days or minutes) and differences between time points within treatment groups were analyzed using the Tukey test. The degrees of freedom were approximated according to Kenward and Roger (ddfm = KR in MIXED). Results were considered as statistically significant at $p \leq 0.05$, and a trend was declared at $0.05 < p \leq 0.10$.

2.3.1 Stress response during the stress model

Polynomial regression was used to analyze the effect of the targeted pain management protocol by describing the dependency of the cortisol concentration on time (minutes). The regression coefficients were considered to be specific to the four treatment groups. The degree of the polynomial was determined using the corrected Akaike's information criterion (AICC) based on the maximum likelihood method. To evaluate the model fit, the least square mean (LSM)-curves of the regression models were compared visually with trend curves calculated by procedure LOESS. Animal-specific regression functions (polynomials of second degree) were fitted simultaneously to the observations per minute and the following new traits derived (Heinrich et al., 2020): area under the curve (AUC) on the interval from minute -30 to 30 (divided by 60), maximum cortisol concentration from minute -30 to 30 (Ymax) and the time when maximum cortisol concentration occurred (Tmax). Area under the curve, Ymax and Tmax were analyzed using multifactorial ANOVA. For this purpose, the study and treatment groups were treated as fixed effects in the model. The polynomial model and the models of the three derived traits contain the cortisol concentration at the start of day 0 (before the stress model) as baseline cortisol concentration as linear covariate.

2.3.2 Stress response before and after the stress model

The factors groups and days and their interactions were represented by fixed effects in the models. The observations of the 11 sheep that had complications on days 1 and 2 were treated as missing values. For the analysis of data collected before the stress model, the treatment groups XYLA-IVRA, IVRA and PLACEBO were combined.

3 Results

3.1 Stress response of young and old sheep

YOUNG and OLD sheep had an analogous cortisol concentration pattern during the study period. While the YOUNG sheep had an average cortisol concentration of 47.2 ng/ml (range, 19.7 ng/ml to 76.2 ng/ml) on day -3, the average cortisol concentration of the OLD sheep was 39.4 ng/ml (range, 24.0 ng/ml to 62.9 ng/ml). On the following days (Day -3 to 2) and during the stress model (Day 0, Min -30 to 30) the YOUNG sheep had higher cortisol concentrations than the OLD sheep (Tables 1, 2).

3.2 Stress response to repeated dorsal recumbency and effect of the adaption phase

During the adaption phase, the cortisol concentration of DISEASED sheep was significantly higher on day -3 than on day -2 ($p < 0.0001$), day -1 ($p = 0.002$) and day 0 ($p = 0.0002$). After day -2, there was no further decrease in cortisol concentration in either group (Figure 2, Supplementary Table 1).

3.3 Stress response of sheep in dorsal recumbency and of standing sheep

The cortisol concentrations in the morning of day 0 before the stress model were higher in HEALTHY (37.2 ± 3.3 ng/ml; n = 12) and DISEASED sheep in dorsal recumbency (34.0 ± 1.9 ng/ml; n = 36) compared with measurements made four hours later (Min -30) in standing HEALTHY (18.5 ± 3.3 ng/ml) and DISEASED sheep (17.6 ± 1.9 ng/ml; $p < 0.001$). The concentrations did not differ between HEALTHY and DISEASED sheep (Table 3).

3.4 Effect of pain management on the perioperative stress response

The sheep of all groups had an analogous cortisol concentration pattern during the stress model (Figure 3). The AUC and the maximum cortisol concentration of the XYLA-IVRA sheep were lower than those of the PLACEBO ($p_{AUC} < 0.01$; $p_{Ymax} < 0.01$) and HEALTHY sheep ($p_{AUC} = 0.01$; $p_{Ymax} = 0.03$; Table 4). In all treatment groups, the cortisol concentration increased up to the time of claw treatment (Min 0), after which time it decreased (Figure 3). At the start of the stress model (standing, Min -30), the cortisol concentrations in the HEALTHY ($p < 0.01$), the IVRA ($p < 0.01$) and the PLACEBO sheep ($p < 0.001$) were lower than at the end of the stress model (standing, Min 20). After the administration of IVRA (Min -10) until the resumption of the standing position (Min 10), the cortisol

TABLE 1 Blood cortisol concentrations (mean, minimum, maximum; ng/ml) in YOUNG (n=32) and OLD (n=16) sheep before (Day -3 to 0) and after the stress model (Day 1 and 2).

Blood cortisol concentrations (mean, minimum, maximum; ng/ml)	sampling times (days)					
	-3	-2	-1	0	1	2
YOUNG	47.2 (19.7 – 76.2)	37.9 (11.2 – 58.3)	40.4 (8.7 – 62.4)	38.6 (8.8 – 56.5)	43.2 (26.5 – 60.6)	39.2 (16.9 – 55.1)
OLD	39.4 (24.0 – 62.9)	28.3 (10.7 – 65.8)	28.3 (7.6 – 64.0)	26.5 (9.1 – 41.2)	31.0 (8.2 – 49.5)	27.3 (14.3 – 39.1)

TABLE 2 Blood cortisol concentrations (mean, minimum, maximum; ng/ml) in YOUNG (n=32) and OLD (n=16) sheep during the stress model (in a tilt squeeze chute): Min -30: sheep standing in a tilt squeeze chute and receiving drugs according to group allocation; Min -20: placement and fixation of the sheep in dorsal recumbency; Min -10: application of IVRA according to group allocation; Min 0: actual or sham claw treatment; Min 10: resumption of standing position; Min 30: blood sampling in group pen; blood was also taken after each procedure.

Blood cortisol concentrations (mean, minimum, maximum; ng/ml)	sampling times (minutes)					
	-30	-20	-10	0	10	30
YOUNG	22.3 (7.1 – 51.3)	38.1 (15.3 – 61.8)	47.7 (23.8 – 70.6)	52.3 (28.6 – 76.8)	51.3 (21.3 – 78.9)	42.4 (9.1 – 84.8)
OLD	8.3 (3.3 – 16.6)	27.4 (14.9 –50.5)	38.9 (19.3 – 59.9)	46.7 (12.3 – 71.5)	46.7 (11.9 – 85.5)	33.2 (7.7 – 73.8)

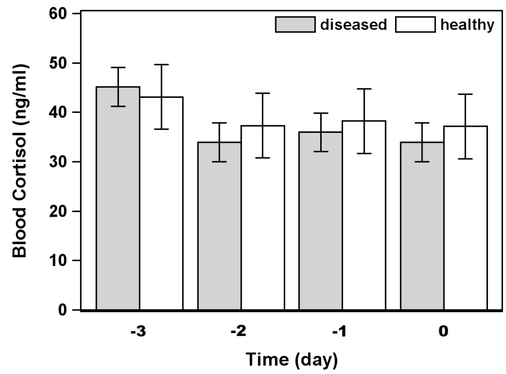

FIGURE 2
Blood cortisol concentrations (least square means; ng/ml; 95% confidence interval) in HEALTHY sheep (n = 12) and in DISEASED sheep (n = 36) on days -3 to 0 (before the stress model).

concentrations increased in the HEALTHY ($p < 0.01$), the IVRA ($p < 0.01$) and the PLACEBO sheep ($p < 0.001$) but did not change in the XYLA-IVRA sheep Table 5).

3.5 Comparison of the stress response before and one day after the stress model

On the day after the stress model (Day 1, Figure 1), the sheep of the IVRA and PLACEBO groups had higher cortisol concentrations (IVRA, 39.1 ± 2.8 ng/ml, $p_{IVRA} = 0.01$; PLACEBO, 47.4 ± 3.3 ng/ml, $p_{PLACEBO} = 0.02$) than before the stress model (Day 0, morning). The cortisol concentrations of the HEALTHY and XYLA-IVRA sheep at these time points did not differ (HEALTHY, 33.9 ± 3.1 ng/ml, $p_{HEALTHY} = 0.91$; XYLA-IVRA, 36.7 ± 3.3 ng/ml, $p_{XYLA\text{-}IVRA} = 1.00$). On the day after the stress model (Day 1), the cortisol concentration of the PLACEBO sheep was significantly higher than that of the group of HEALTHY sheep ($p = 0.02$), and tended to be higher than the cortisol concentration of the XYLA-IVRA sheep ($p = 0.10$). On day 2, the PLACEBO sheep tended to have lower cortisol concentrations than on day 1 (39.5 ± 3.2 ng/ml, $p = 0.07$; Figure 4, Supplementary Table 2).

4 Discussion

4.1 Effect of the targeted pain management protocol on the perioperative stress response

The targeted pain management protocol of this study consisting of sedation and local anesthesia successfully reduced the cortisol concentration during the stress model. Together with the results of the behavioral observations during the study (Fieseler et al., 2019), it can be concluded that the targeted pain management protocol of this study effectively mitigated stress, including pain-induced stress, associated with DINCO treatment in sheep in a tilt squeeze chute. Sheep that received xylazine and IVRA had significantly lower maximum blood cortisol concentrations, areas under the curve and cortisol concentration during the stress model than sheep that received a placebo. Claw treatment without pain management results in stress caused by the restraint (Fieseler et al., 2018; Amin et al., 2022) and handling (Wagner et al., 2021) and pain caused by treatment of the corium defect (Ley et al., 1989). The numerically higher cortisol concentration of the PLACEBO sheep compared with the HEALTHY sheep may suggest that the sheep of the PLACEBO group perceived perioperative pain-induced stress. However, it would be expected that the cortisol concentration of the PLACEBO sheep would be significantly higher than the cortisol concentration of the HEALTHY sheep because of the painful claw treatment. Chronic inflammation associated with DINCO decreases the pain threshold of the nociceptors in the corium (Ley et al., 1989). On the other hand, stressors can temporarily reduce nociception (Herskin et al., 2007; Raundal et al., 2015). It is possible that the perception of pain associated with claw treatment was reduced in the

TABLE 3 Blood cortisol concentrations (least square means (LSM) ± standard error (SE); ng/ml) in HEALTHY sheep (n = 12) and in DISEASED sheep (n = 36) measured in dorsal recumbency (morning of Day 0) and in standing sheep (Day 0, Min -30) before the stress model, and within-group comparisons (Tukey test).

Blood cortisol concentrations ng/ml (LSM ± SE)	Position during sampling		p dorsal recumbency vs. standing
	Dorsal recumbency	Standing	
HEALTHY	37.2 ± 3.3	18.5 ± 3.3	**< 0.01**
DISEASED	34.0 ± 1.9	17.6 ± 1.9	**< 0.01**
p HEALTHY vs. DISEASED	0.82	0.41	

All p-values that are significant or show a trend ($p \leq 0.10$) are bold.

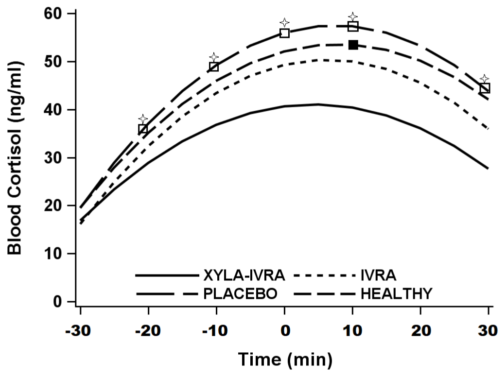

FIGURE 3
Blood cortisol concentrations (least square means; ng/ml) as second-degree polynomial function in 12 HEALTHY sheep and in 36 sheep with *dermatitis interdigitalis contagiosa* undergoing different pain management protocols (XYLA-IVRA = sedation and local anesthesia, n = 12; IVRA = local anesthesia, n = 12; PLACEBO, n = 12) in the stress model (in a tilt squeeze chute): Min -30: sheep standing in a tilt squeeze chute and receiving drugs according to group allocation; Min -20: placement and fixation of the sheep in dorsal recumbency; Min -10: application of IVRA according to group allocation; Min 0: actual or sham claw treatment; Min 10: resumption of standing position; Min 30: blood sampling in group pen; blood was also taken after each procedure. Within time points (minutes); LSM marked with a star differ from the concentrations of the XYLA-IVRA sheep ($p \leq 0.05$).

PLACEBO sheep because the sheep were stressed. The ceiling effect on the cortisol response may also have contributed to the lack of significant difference between the two groups; this effect describes the absence of an increase in a concentration above a certain level even though a higher concentration would be expected based on the prevailing stressors (Moberg and Mench, 2000). This may explain the mere numerical differences between the cortisol concentrations of the HEALTHY and PLACEBO sheep. Another explanation could be that the HEALTHY sheep experienced enormous stress during the sham claw treatment. It is unlikely that the sham claw treatment caused significant pain but it is possible that application of a tourniquet (Gregory, 2008), the injection of the placebo (Strazar et al., 2013) and reperfusion after removal of the tourniquet (Aihara et al., 2017) caused pain. In the IVRA sheep, on the other hand, nociceptive signals were disrupted by the regional anesthesia before they reached the central nervous system (Wiebalck and Zenz, 1997). The numerically lower cortisol concentration in the IVRA sheep compared with the HEALTHY and PLACEBO sheep suggests that this measure reduces pain in the distal limb (Hodgkinson and Dawson, 2007), in agreement with behavioral observations in the same population of sheep (Fieseler et al., 2019). The IVRA sheep had a low complication rate, and the technique was feasible under field conditions (Fieseler et al., 2019) making it a good choice for painful claw treatments.

However, cortisol concentration was numerically higher in the IVRA sheep than in the XYLA-IVRA sheep suggesting that sedation had an additional effect. Alpha-2 agonists are potent drugs for sedation and anesthesia in sheep (Kästner, 2006). Their muscle relaxant and sedative effects require the provision of good footing postoperatively to avoid injuries (Offinger et al., 2012). Alpha-2 agonists are also anxiolytic (Kästner, 2006) and potentiate the effect of local anesthesia (Chambers, 1993; Lizarraga and Chambers, 2012), which effectively reduced perioperative stress, including pain-induced stress, in the

TABLE 4 Area under the curve (AUC) ± standard error (SE) calculated using polynomial regression, maximum blood cortisol concentration ± SE, and time point ± SE of the maximum concentration in 12 HEALTHY sheep and in 36 sheep with *dermatitis interdigitalis contagiosa* undergoing different pain management protocols (XYLA-IVRA = sedation and local anesthesia, n = 12; IVRA = local anesthesia, n = 12; PLACEBO, n = 12) during the stress model (Day 0) in a tilt squeeze chute.

	Area under the curve (ng/ml × Min ± SE)	Maximum cortisol concentration (ng/ml ± SE)	Time point of maximum concentration (Min ± SE)
XYLA-IVRA	34.9 ± 2.6	42.3 ± 3.5	4.1 ± 2.7
IVRA	40.8 ± 2.7	51.5 ± 3.6	7.1 ± 2.8
PLACEBO	48.0 ± 2.6	60.0 ± 3.4	9.4 ± 2.7
HEALTHY	46.6 ± 2.5	56.4 ± 3.3	7.7 ± 2.6
$P_{XYLA\text{-}IVRA\ vs.\ IVRA}$	0.43	0.27	0.87
$P_{XYLA\text{-}IVRA\ vs.\ PLACEBO}$	**< 0.01**	**< 0.01**	0.49
$P_{XYLA\text{-}IVRA\ vs.\ HEALTHY}$	**0.01**	**0.03**	0.77
$P_{IVRA\ vs.\ PLACEBO}$	0.23	0.31	0.94
$P_{IVRA\ vs.\ HEALTHY}$	0.43	0.76	1.00
$P_{PLACEBO\ vs.\ HEALTHY}$	0.98	0.87	0.97

All p-values that are significant or show a trend ($p \leq 0.10$) are bold.

TABLE 5 Blood cortisol concentrations (least square means (LSM) ± standard error (SE; ng/ml) in 12 HEALTHY sheep and in 36 sheep with *dermatitis interdigitalis contagiosa* undergoing different pain management protocols (XYLA-IVRA = sedation and local anesthesia, n = 12; IVRA = local anesthesia, n = 12; PLACEBO, n = 12) in the stress model (in a tilt squeeze chute): Min -30: sheep standing in a tilt squeeze chute and receiving drugs according to group allocation; Min -20: placement and fixation of the sheep in dorsal recumbency; Min -10: application of IVRA according to group allocation; Min 0: actual or sham claw treatment; Min 10: resumption of standing position; Min 30: blood sampling in group pen; blood was also taken after each procedure.

Blood cortisol concentrations ng/ ml (LSM ± SE)	Sampling times (minutes)						p -30 vs. 30	p -10 vs. 10
	-30	-20	-10	0	10	30		
XYLA-IVRA	16.9 ± 2.8	28.9 ± 2.3	36.8 ± 2.6	40.6 ± 3.0	40.4 ± 3.4	27.7 ± 4.4	0.37	0.37
IVRA	16.1 ± 2.8	32.4 ± 2.3	43.4 ± 2.7	49.3 ± 3.1	50.0 ± 3.4	35.9 ± 4.4	**< 0.01**	**< 0.01**
PLACEBO	19.5 ± 2.7	37.0 ± 2.2	49.2 ± 2.6	56.0 ± 3.0	57.3 ± 3.3	43.8 ± 4.3	**< 0.001**	**< 0.001**
HEALTHY	19.6 ± 2.7	35.1 ± 2.2	46.0 ± 2.5	52.1 ± 3.0	53.5 ± 3.3	42.1 ± 4.3	**< 0.01**	**< 0.01**
p XYLA-IVRA vs. IVRA	1.00	0.71	0.31	0.21	0.20	0.55		
p XYLA-IVRA vs. PLACEBO	0.90	**0.05**	**< 0.01**	**< 0.01**	**< 0.01**	**0.05**		
p XYLA-IVRA vs. HEALTHY	0.90	0.21	**0.07**	**0.05**	**0.04**	**0.10**		
p IVRA vs. PLACEBO	0.83	0.46	0.40	0.41	0.42	0.58		
p IVRA vs. HEALTHY	0.82	0.83	0.90	0.92	0.89	0.75		
p PLACEBO vs. HEALTHY	1.00	0.92	0.80	0.79	0.84	0.99		

All p-values that are significant or show a trend (p ≤ 0.10) are bold.
Also shown are the comparisons of the groups within time points and the comparisons of the time points Min -30 and Min 20, and Min -10 and Min 10 within groups (Tukey test).

XYLA-IVRA sheep during the stress model. However, the numerical increase in cortisol concentration in these sheep during the stress model suggests that the sheep suffered some level of stress, which did not occur in healthy sedated sheep during sham claw trimming (Amin et al., 2022). The results of the study show that the perioperative pain protocol reduced peripheral and central nociception without completely eliminating it. Possible reasons for this include the occurrence of hyperalgesia (Ley et al., 1989), reduced effectiveness of alpha-2 agonists in sheep with chronic DINCO-associated pain (Ley et al., 1991b), reduced effectiveness of procaine hydrochloride (Tacke et al., 2012) or the release of cytokines during surgery (Desborough, 2000). Cytokines can bind directly to nociceptors causing chronic pain and hyperalgesia (Zhang and An, 2007). The release of cytokines during surgery can be prevented by preoperative administration of an NSAID (Fekry et al., 2019). While NSAIDs can provide postoperative analgesia, local anesthesia wears off immediately after the tourniquet is removed (Anderson and Edmondson, 2013). Sheep that had been pre-treated with an NSAID and underwent mulesing and tail amputation or castration had lower cortisol concentrations than sheep that had not received NSAIDs (Paull et al., 2007). Likewise, in goats undergoing experimental claw amputation, pre-treatment with an NSAID reduced nociception (Fekry et al., 2019). In cattle, the use of an NSAID during claw surgery is common (Becker et al., 2014), but a comparable protocol has not been established in sheep (Winter, 2011; Zanolari et al., 2021). Underutilization of NSAIDs may be because none are approved for use in sheep and, they have long withholding times for meat and increase treatment costs. In addition, unwanted side effects such as gastric ulceration and renal failure must be considered when administering NSAIDs (Lizarraga and Chambers, 2012). However, in other procedures and in other animal species, the benefits of an NSAID outweigh the risks (Anderson and Edmondson, 2013). Therefore, further studies are needed to investigate the additional effect of an NSAID in sheep undergoing painful claw treatment. Nevertheless, the results of

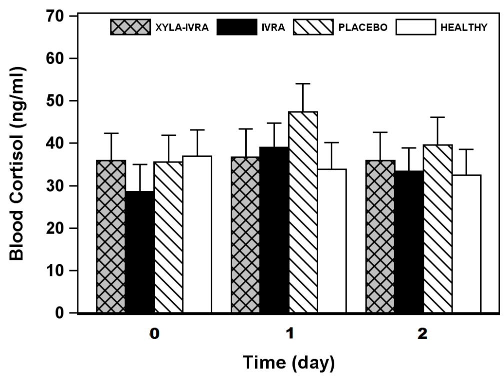

FIGURE 4
Blood cortisol concentrations (least square means (LSM); ng/ml) in 9 healthy sheep and in 28 sheep with *dermatitis interdigitalis contagiosa* under different pain management protocols (XYLA-IVRA = sedation and local anesthesia, n = 8; IVRA = local anesthesia, n = 12; PLACEBO, n = 8) on days 0 (before the stress model), 1 and 2.

the present study clearly show the positive effect of sedation and local anesthesia on the perioperative stress response in sheep undergoing claw treatment.

4.2 Adaptation phase

The cortisol concentrations did not decrease during the adaption phase, which indicated that the sheep did not habituate to the manipulations. The time required for adaptation depends on the type and duration of the stressors as well as on individual variation in stress vulnerability (Koolhaas et al., 2006). Sheep that were subjected to various handling treatments including partial shearing had significantly lower cortisol concentrations at the fourth treatment compared with the first (Hargreaves and Hutson, 1990). Sheep that underwent twice-daily venipuncture and once-a-day saliva collection had significantly lower cortisol concentrations after eight days compared with the first day. Since cortisol concentrations were not measured within the eight days and no behavioral data were collected, the authors were unable to determine the exact number of manipulations necessary to achieve habituation (Andanson et al., 2020). Sheep that received a grain reward in a tilt squeeze chute habituated to fixation in lateral recumbency after a few passes (Grandin, 1989). Dairy cows required four days to habituate to daily collection of blood, saliva, tears and feces (Heinrich et al., 2020). Although these studies show that the adaptation time chosen in the present study appeared to be sufficient, adaptation did not occur in any of the sheep within the four days. The sheep in the present study were kept on a conventional farm and had not been part of previous comparable studies. Nevertheless, it cannot be ruled out that the sheep had aversive experiences with routine handling procedures, such as shearing, blood sampling and claw treatment, before the start of the study. All study sheep maintained visual contact with each other and with the treatment area throughout the study. Although the sheep were always handled calmly and carefully, it is possible that observing the manipulation of another study sheep initiated a stress response in the remaining sheep. Observational learning has been successfully demonstrated in sheep and numerous other animals (Kilgour, 1987; Veissier, 1993; Veissier and Stefanova, 1993; Wechsler and Lea, 2007). This risk could have been minimized by completely separating the treatment area. However, isolation of the study sheep during the manipulation would have represented a massive stressor (Parrott et al., 1987) and was therefore not feasible. The selection of a sufficient adaption phase can be difficult (Kraetzl and Weiler, 1998; Andanson et al., 2020). Ideally, behavioral data would be collected and the cortisol concentration measured during the adaption phase, so the duration could be determined based on the cortisol profile and behavioral data (Fell and Shutt, 1989). In the present study, the behavioral data also indicated that the study sheep were not yet fully accustomed to the study design on the day of the stress model (Fieseler et al., 2018). However, extension of the adaptation time and thus the duration of the study would have been unacceptable due to the increased risk of thrombophlebitis (King et al., 2020). The significantly lower cortisol concentrations of standing sheep at the end of the adaptation phase indicated that sheep did not habituate to fixation in dorsal recumbency in the adaptation phase. This is in agreement with observations in healthy sheep (Wagner et al., 2021; Amin et al., 2022) and calves (Schulze et al., 2016). Sheep have been shown to remember a stressful event months later and appear to have long-term memory of unpleasant handling experiences (Rushen, 1986; Thórhallsdóttir et al., 1987). The cortisol concentrations measured at the start of the stress model (Min -30) in standing sheep were significantly lower than the concentrations measured in the morning of the same day with the sheep in dorsal recumbency. This leads to the conclusion, that the manipulation in the morning elicited a stress response but did not trigger a memory at blood sampling four hours later (Day 0, Min -30). Therefore, it seems that the sheep habituated to blood sampling but not fixation in dorsal recumbency. There have been no studies investigating the effect of dorsal recumbency on the adaptation phase in sheep (PubMed search November 28, 2022, search terms sheep, dorsal recumbency, habituation, stress). The significantly lower cortisol concentrations at midday could also be due to the circadian pattern of cortisol. While other mammals have a distinct circadian rhythm of cortisol secretion (Focke and Iremonger, 2020), such a rhythm has not been detected in sheep (Bassett, 1974; Brinklow and Forbes, 1984). Instead, previous studies have identified at most an ultradian rhythm with a concentration minimum in the morning (Fulkerson and Tang, 1979) and a possible effect of stress (Przekop et al., 1985), light (Brinklow and Forbes, 1984) and feeding (Murayama et al., 1986) on secretion. Furthermore, blood sampling was done at the same time of day in all sheep. Therefore, an effect of a circadian pattern on the measured cortisol concentrations is unlikely. In future studies that involve positioning of sheep in dorsal recumbency, the adaptation phase should be longer than four days if possible. However, this period was sufficient for adaptation to blood sampling from a jugular catheter and daily clinical examination.

4.3 Effect of the targeted pain management protocol on the stress response after the stress model – hyperalgesia and "memory of stress"

The significantly higher cortisol concentrations of the IVRA and PLACEBO sheep on the day after the stress model (Day 1) clearly showed that these sheep perceived more stress, including pain-induced stress, than the sheep of the XYLA-IVRA and HEALTHY groups. This may have been attributable to a

”memory of stress” or hyperalgesia. Animals can be accustomed to stressors (Reid and Mills, 1962; Fordham et al., 1989) through their ability to learn and remember (Liddell, 1925; Hutson, 1994). This allows the animal to foresee and, depending on the stressor, control a situation (Koolhaas et al., 2011). If negative experiences are collected, it is possible that the animal develops a “memory of stress”. In that case, the stress response can then be amplified by the recurrence of the stressor. Even when the actual stressor is no longer present, a stress response can be triggered (Rushen, 1986). Perhaps the sheep of the PLACEBO and IVRA groups remembered the stressors of the previous day and reacted to them. This could be an explanation for the higher cortisol concentrations in the sheep of these groups. Another possibility is that these sheep developed secondary hyperalgesia due to inadequate perioperative pain management. Hyperalgesia causes an increase in pain in response to a noxious stimulus (Sandkuhler, 2009) and may occur when a chronically inflamed lesion is treated without adequate pain management (Kaler et al., 2010). This is exacerbated by stress and pain associated with defensive movements in a tilt squeeze chute (Fieseler et al., 2018). While primary hyperalgesia occurs at the site of the injury, secondary hyperalgesia exists in an area adjacent to or remote from the site of the injury (Sandkuhler, 2009). Secondary hyperalgesia was prevented with local anesthesia in ram lambs up to 7 days of age undergoing castration (Mellema et al., 2007). On the day after the stress model, the cortisol concentration of the IVRA sheep tended to be lower than that of the PLACEBO sheep, but it was higher than on the previous day before the stress model (morning of Day 0). Thus, even the sheep of the IVRA group developed a memory of stress or secondary hyperalgesia. In the present study, IVRA was done with procaine hydrochloride, whereas lidocaine infiltration was used by Mellema et al. (2007). Compared with lidocaine, procaine hydrochloride is less effective and has a shorter duration of action (Tacke et al., 2012). The effect of IVRA subsides within 5 minutes after removal of the tourniquet (Weaver, 1972). The limited effect of the local anesthetic and the onset of pain after removal of the tourniquet may have led to a memory of stress or secondary hyperalgesia of the IVRA sheep. In contrast to the PLACEBO and IVRA sheep, the XYLA-IVRA sheep did not have increased cortisol concentrations on the day after the stress model, which indicated that stress and pain management prevented a memory of stress or secondary hyperalgesia. Similar observations were made in bulls that were sedated and received local anesthesia for castration (Ting et al., 2003). The lack of a stress response in the XYLA-IVRA sheep after the stress model emphasizes the positive effect of stress and pain management for the treatment of painful corium lesions in sheep with DINCO. Perioperative pain control using local anesthesia alone was considered inadequate.

4.4 Stress response in sheep with chronic pain and in healthy sheep

The cortisol concentration on day -3, which was one day after the initial examination, trimming of both front and one hind foot and catheter placement, was higher than on the following days (Day -2 to 0). The concentrations remained unchanged on days -1 and 0, and thus it can be assumed that the decrease in cortisol concentration from day -3 to day -2 was not due to habituation but rather due to the memory of the procedures on the day before (Day -4). The numerically higher cortisol concentration of the DISEASED sheep suggests that they perceived more stress, including stress-induced pain, during claw treatment than the HEALTHY sheep. Sheep with chronic pain are more sensitive to additional stressors (Jensen et al., 1996). Cortisol measurements on the first day the sheep were handled (Day -4) would have aided in the interpretation of the concentrations on the subsequent days in terms of habituation and memory of stress.

The cortisol concentrations of the DISEASED and HEALTHY sheep did not differ significantly during the adaptation phase. Other authors reported lower (Ley et al., 1991a) or higher (Ley et al., 1994) cortisol concentrations in sheep with DINCO compared with healthy sheep. By comparison, chronically lame cows had higher cortisol concentrations than healthy controls (Comin et al., 2013). Behavioral observations in the present study suggested that the sheep with DINCO suffered chronic pain (Fieseler et al., 2018). The measurement of blood cortisol should be combined with other criteria such as behavioral changes for optimal assessment of chronic pain (Lester et al., 1996). Future studies should consider using additional indices to assess chronic pain.

Both DISEASED and HEALTHY sheep had similar and significantly lower cortisol concentrations while standing compared with dorsal recumbency. Cows that were standing and in lateral recumbency on a tilt table had similar cortisol concentrations (Khraim, 2011). In the present study, fixation in dorsal recumbency constituted a massive stressor for the healthy sheep as well as for the sheep with DINCO. Nevertheless, fixation in dorsal recumbency is necessary to carry out zootechnical and medical procedures safely and without injury (Hargreaves and Hutson, 1997). However, such procedures usually take less than 60 minutes (Pesenhofer et al., 2006). The results of the study show that sheep should only be restrained for as long as absolutely necessary. The handling of sheep should be gentle and as stress-free as possible and the environment should be adapted to the needs of the animals (Grandin, 2021). When and whether sedation is necessary for longer procedures should be discussed critically by veterinarians.

4.5 Limitations of the study

There are some limitations in this study that could be addressed in future studies.

In the present study, sheep of the last study groups (OLD) showed lower cortisol concentrations than the sheep of the first study groups (YOUNG). The higher cortisol concentration in YOUNG sheep compared to the OLD sheep, could be due to age and lack of experience (Cook, 1996; Chniter et al., 2016). In contrast, studies in goats (Díaz et al., 2013) and cattle (Alhussien and Dang, 2018) determined higher cortisol concentrations in multiparous animals than in young animals. The present study was a field study, and thus the effects of environmental factors on cortisol concentrations cannot be completely ruled out (Schären et al., 2021). Based on the study design, it was not possible to distinguish between those factors. To consider the effects of both parity and environmental effects on the cortisol concentration, study group effects were considered as fixed effects in all models.

Thrombophlebitis in 11 sheep resulted in exclusion of their cortisol concentrations for analysis from day 1 onward. Thrombophlebitis causes pain, which can affect the cortisol concentration level (Rouleau et al., 2003). The prevalence of thrombophlebitis was lower than that reported in a study with hospitalized sheep (42%; King et al., 2020), but could not be completely avoided. Thrombophlebitis could have been prevented by using alternative matrices to determine the cortisol concentration. In sheep, saliva, tears and feces are particularly suitable for determining cortisol concentration (Stubsjøen et al., 2015; Wagner et al., 2021). However, measuring stress using the cortisol concentration in blood has been established as the gold standard (Hackney and Anderson, 2016) and was therefore selected to assess pain management and adaptation in the present sudy.

In the present study, the effect of a targeted pain management protocol consisting of sedation and local anesthesia was examined. However, even in the XYLA-IVRA sheep, the perioperative stress response could not be completely eliminated. Addition of an NSAID administered preemptively may have prevented the peri -and postoperative stress response in the study sheep. The positive effect of a multimodal pain management protocol including an NSAID on postoperative well-being has already been investigated in other procedures (Dinniss et al., 1997; Graham et al., 1997; Paull et al., 2007) and animal species (Banse and Cribb, 2017; Fekry et al., 2019; Kretschmann et al., 2020; Stilwell et al., 2020). Until now, there have been no studies analyzing the effect of a multimodal pain management protocol that incorporates an NSAID on claw treatment in sheep (PubMed search November 28, 2022, search terms sheep, multimodal pain management, antiphlogistic, claw treatment). The use of an NSAID as part of multimodal pain management should be examined in a follow-up study.

The cortisol concentrations were measured before, during and after the stress model. Determining the blood cortisol concentration is the gold standard for recording a stress response (Hackney and Anderson, 2016). Although the cortisol concentration is not a direct measure of pain, the noxiousness of the stimuli correlates with changes in cortisol concentration (Moberg and Mench, 2000).Therefore, determination of the cortisol concentration is an indirect but established method of assessing pain-induced stress, but should ideally be combined with physiological and behavioral traits (Mellor et al., 2000; Stilwell et al., 2010). Although only the stress response of the sheep was described in this study, it can be assumed that the DINCO sheep suffered from pain (Ley et al., 1991a; Winter, 2008). The results of the ethological assessment of the sheep have been published (Fieseler et al., 2018; Fieseler et al., 2019). A comprehensive description of the pain management and stress response in relation to behavioral and physiological data of sheep treated for DINCO is in preparation.

5 Conclusion

Fixation in dorsal recumbency represented a stressor to which the sheep failed to habituate within the four-day adaptation phase. The cortisol response to stress in the adaptation phase was similar in HEALTHY and DINCO sheep. If fixation in dorsal recumbency is necessary to carry out zootechnical procedures, sheep should only be restrained for as long as absolutely necessary. Corium lesions caused by DINCO are painful and lead to marked stress. The results of the present study show that a targeted pain management protocol, consisting of sedation and local anesthesia, reduced cortisol concentrations and therefore stress during and after the stress model. Local anesthesia alone was inadequate for the treatment of DINCO based on the cortisol concentrations during and after the stress model. For abolition of peripheral and central sensitization, the inclusion of an NSAID should be considered in future studies. However, the response after the stress model also underlined the benefits of the targeted pain management protocol that incorporates sedation and local anesthesia. Therefore, it would seem prudent to include sedation, in addition to local anesthesia, for the stress- and painful treatment of corium lesions in sheep.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by State Administration Office (Landesverwaltungsamt, Referat

Verbraucherschutz, Veterinärangelegenheiten Sachsen-Anhalt, Germany). Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

AS contributed to project acquisition. RW, HF, OP, AE, GM and AS contributed to trial and project design. RW, HF, MK and HM contributed to trial implementation and sample collection. JG and AE contributed to sample analysis. FR, RW, NM and AS contributed to data analysis and data interpretation. FR and RW contributed to writing of the manuscript. HF, MK, HM, OP, NM, JG, AE, GM, WB and AS contributed to revision of manuscript. All authors contributed to the article and approved the submitted version.

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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.2022.1021039/full#supplementary-material

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