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Postoperative Severity Assessment in Sheep

Eva Zentrich[a] Laura Wassermann[a] Birgitta Struve[a] Kristin Selke[a] Manuela Buettner[a] Lydia Maria Keubler[a] Janin Reifenrath[b, c] Nina Angrisani[b, c] Merle Kempfert[b, c] Annika Krause[d] Olaf Bellmann[d] Marcin Kopaczka[e] Dorit Merhof[e] Marion Bankstahl[a] André Bleich[a] Christine Häger[a]

[a]Institute for Laboratory Animal Science, Hannover Medical School, Hannover, Germany; [b]Department of Orthopedic Surgery, Hannover Medical School, Diakovere Annastift Hospital, Hannover, Germany; [c]Lower Saxony Centre for Biomedical Engineering, Implant Research and Development (NIFE), Hannover Medical School, Hannover, Germany; [d]Research Institute for Farm Animal Biology (FBN), Dummerstorf, Germany; [e]Institute of Imaging and Computer Vision, RWTH Aachen University, Aachen, Germany

Keywords
Sheep · Telemetry · SGS · Pain

Abstract

Introduction: Sheep are frequently used in translational surgical orthopedic studies. Naturally, a good pain management is mandatory for animal welfare, although it is also important with regard to data quality. However, methods for adequate severity assessment, especially considering pain, are rather rare regarding large animal models. Therefore, in the present study, accompanying a surgical pilot study, telemetry and the Sheep Grimace Scale (SGS) were used in addition to clinical scoring for severity assessment after surgical interventions in sheep. ***Methods:*** Telemetric devices were implanted in a first surgery subcutaneously into four German black-headed mutton ewes (4–5 years, 77–115 kg). After 3–4 weeks of recovery, sheep underwent tendon ablation of the left M. infraspinatus. Clinical scoring and video recordings for SGS analysis were performed after both surgeries, and the heart rate (HR) and general activity were monitored by telemetry. ***Results:*** Immediately after surgery, clinical score and HR were slightly increased, and activity was decreased in individual sheep after both surgeries. The SGS mildly elevated directly after transmitter implantation but increased to higher levels after tendon ablation immediately after surgery and on the following day. ***Conclusion:*** In summary, SGS- and telemetry-derived data were suitable to detect postoperative pain in sheep with the potential to improve individual pain recognition and postoperative management, which consequently contributes to refinement.

Introduction

In recent years, approximately 20,000 sheep have been used every year in biomedical research across Europe [1]. Sheep are used in a wide variety of experimental fields, for

Eva Zentrich and Laura Wassermann: shared first authorship. André Bleich and Christine Häger: shared senior authorship.

Karger@karger.com
www.karger.com/esr

Correspondence to:
Christine Häger, haeger.christine@mh-hannover.de

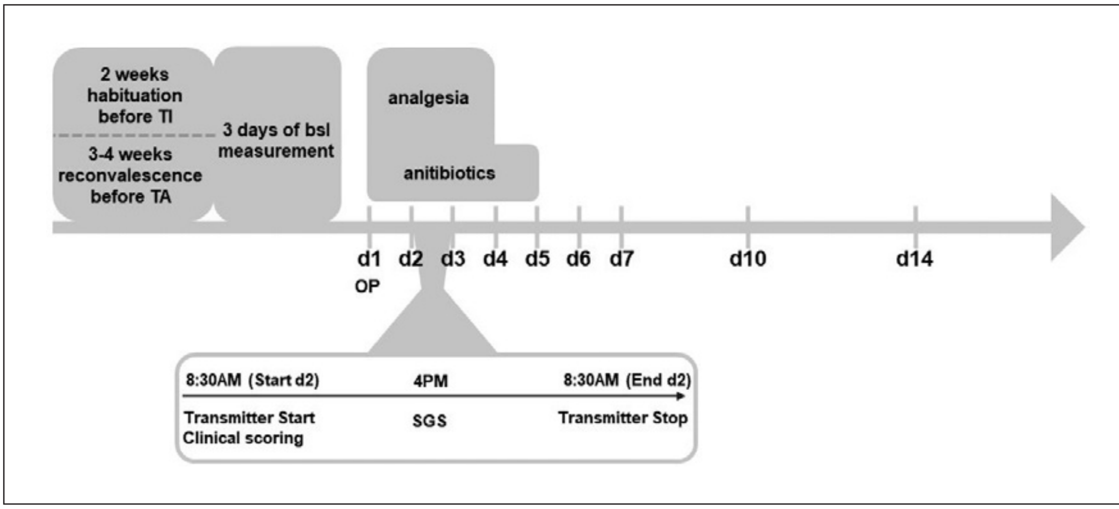

Fig. 1. Experimental setup. On the assigned days, after both surgeries' telemetrically derived data and video data were collected and clinical scoring (except d1) was performed. bsl, baseline; d, day.

instance in studies on cardiovascular [2, 3] and gastrointestinal diseases [4], as well as in studies on respiratory diseases [5, 6]. Especially orthopedic studies frequently rely on sheep [7–9] since the weight and size of the animal, as well as the regeneration time of healing is similar to that of humans [9]. Here, surgical interventions are commonly used, and researchers are obliged to minimize any kind of suffering to the minimum according to the 3R principle [10] and animal welfare legislation [11, 12]. This in turn implies that any form of suffering must be recognized. However, the lack of adequate and objective methods to assess the severity of experimental procedures makes this challenging [13].

Regarding severity assessment in such studies, clinical scoring is still the gold standard [14]. However, the assessment of pain, suffering, or fear may be biased, especially in the case of escape animals like sheep, since the animals may not show their natural behavior in the presence of humans [15]. Therefore, contactless and observer-independent methods are needed. In this context, telemetric devices enable monitoring vital parameters of animals 24/7 without the need of human interaction [16]. Another contactless and observer-independent method to detect pain during experiments is the Grimace Scale (review: [17]), which has been adapted for sheep [18].

Focus of the present study was the assessment of postoperative pain in laboratory sheep. We therefore joined an orthopedic pilot study investigating surgically induced tendon defects in sheep and analyzed whether telemetry-derived parameters and the SGS improved severity assessment. In comparison to the orthopedic surgery, we also assessed the postoperative pain after the surgical transmitter implantation itself. The aim of this study was to evaluate the suitability of telemetry-derived parameters and the SGS in addition to clinical scoring as methods for an improved severity assessment of surgical procedures in laboratory sheep.

Material and Methods

Animals

All experiments were approved by the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES, license 33.12-42502-04-18/2837). Four female black-headed mutton sheep (referred to as: #13, #14, #28, and #72) at the age of 4–5 years and 77–115 kg of body weight (individual values are given in online suppl. Table 1; see www.karger.com/doi/10.1159/000526058 for all online suppl. material) were obtained from the country Sheep Breeders' Association of Lower Saxony, Hannover, Germany. In each case, two animals were delivered at the same time and kept in this pairing for the entire course of the experiment with carrying out the experiments once in spring and once in autumn. The ewes were housed in groups of two, provided with tap water and hay ad libitum and fed pelleted sheep feed (Mawel Schaffutter, EquoVis GmbH, Münster, Germany) by hand at 8:30 a.m. and 4:00 p.m., to get the animals used to the handling procedures and experimenters. During the experiments, the sheep were housed in a covered stable with straw (2.60 m × 3.20 m). The health status of the sheep was examined at the arrival day by a veterinarian and regularly checked during the course of the experiments. The sheep were handled only by staff members wearing the same colored protective clothing.

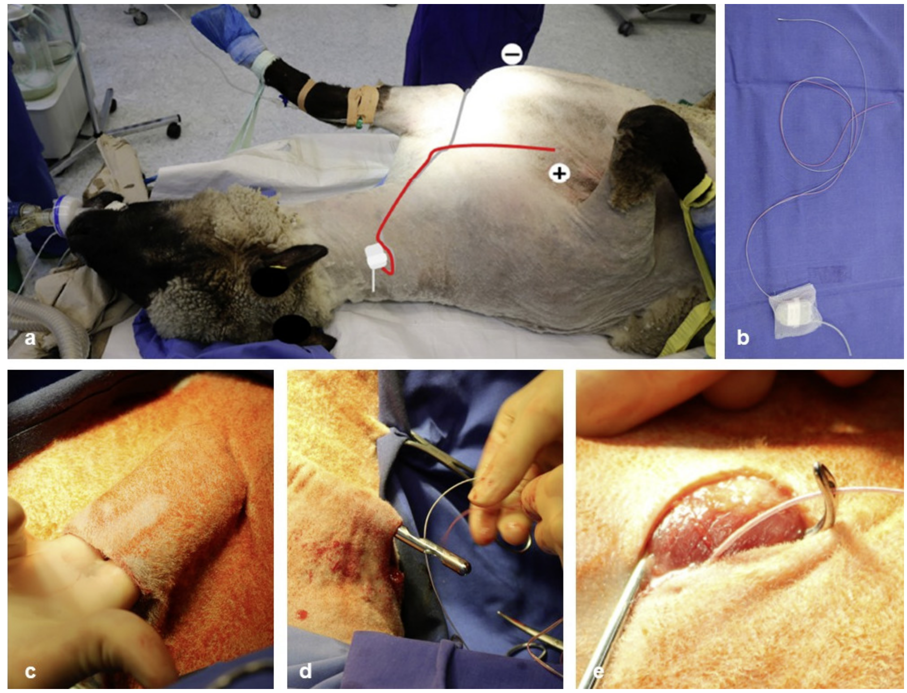

Fig. 2. TI. **a** Position of the sheep during surgery and the transmitter including the electrodes shown schematically. **b** A transmitter sewn into a nonabsorbable mesh. **c** The created pocket in the right neck region. **d** The tunneling of the electrodes using a urinary catheter. **e** The placement of the Deschamps Ligature Needle underneath the muscle to embed the electrodes into the musculature.

Experimental Setup

After arrival, the animals were given 2 weeks of habituation to the staff members and regular feeding from the hand. Subsequent to habituation, the transmitter implantation (TI) was performed. Three to four weeks after the first surgery, the sheep underwent the second surgery for the tendon ablation (TA) of the left musculus infraspinatus. Prior to surgery, sheep were fasted for 24 h, while still having access to water ad libitum. The clinical condition was monitored daily, and on preselected days, telemetric as well as video data were collected (Fig. 1).

Anesthesia and Analgesia

The sheep underwent general anesthesia for both surgeries. Therefore, sheep were pretreated with midazolam (0.2 mg/kg, i.v., Midazolam-ratiopharm®, Ratiopharm, Ulm, Germany) before general anesthesia was induced by propofol (5 mg/kg, i.v., Narcofol®, CP-Pharma GmbH, Burgdorf, Germany). Following orotracheal intubation and insertion of a stomach tube, anesthesia was maintained with 1.9–2.4 vol % isoflurane (Isofluran CP®, CP-Pharma GmbH, Burgdorf, Germany), 1 L/min of oxygen, and 1 L/min of ambient air by inhalation. The sheep were artificially respirated, and they received Ringer's lactate solution (5–10 mL/kg/h, i.v., Ringer-Lactat-Lösung ad us. vet®; WDT, Garbsen, Germany). Oxygen saturation, temperature, heart rate (HR), and respiratory rate were monitored continuously, and no abnormalities were observed. For local anesthesia, 2% lidocaine (max. 4 mg/kg, Xylocaine®, AstraZeneca GmbH, Wedel, Germany) was administered along the incision sites. Additionally, fentanyl (0.01 mg/kg, i.v., pre-OP; 0.001–0.005 mg/kg, i.v., every 20–40 min, Fentanyl®, Rotexmedica, Trittau, Germany) and buprenorphine (0.01 mg/kg, i.m., Buprenovet®, Bayer Vital GmbH, Leverkusen, Germany) were given during and at the end of the TA surgery, respectively. As soon as the animals breathed spontaneously, they were extubated, and the rumen tube was removed.

Regarding pain management, carprofen (4 mg/kg, s.c., Rimadyl®, Pfizer GmbH, Berlin, Germany) was administered pre-operatively as well as the following 3 days post-surgery (2 mg/kg, s.c., once a day [d2–4]). In case of surgery-related pain detection after TA, carprofen or metamizole (40 mg/kg/daily, s.c., Vetalgin®, MSD Tiergesundheit, Unterschleißheim, Germany) were administered additionally. All animals received antibiotics (amoxicillin, ca. 15 mg/kg, s.c., [d1, d3, d5], Duphamox® LA, Zoetis Deutschland GmbH, Berlin, Germany) until day 5 post-surgery.

Table 1 shows a summary of the analgesics and antibiotics used. Additionally, an overview of analgesia each animal received during the course of the experiments can be seen in online supplementary Table 2.

Transmitter Implantation

The telemetric device (M01; PhysioTel Telemetry System DSI; Harvard Bioscience, Inc.) with a weight of 13.9 g and a volume of 11 cm (Fig. 2b) was subcutaneously implanted in the right neck region of the ewes under general anesthesia as described above. For this purpose, the sheep were placed in supine position, and the right forelimb was tied back (Fig. 2a). After shaving and disinfecting the field of operation, a 5-cm incision was made on the right side of the neck, and a pocket was formed for the transmitter (Fig. 2c). To build a derivation after Einthoven n°II, the electrodes connected to the device were tunneled subcutaneously to the left caudal (negative electrode) and right cranial (positive electrode) pectoral region by using a urinary catheter and additional small skin incisions at the apertura thoracis and the designated positions (Fig. 2d, e). Both electrodes were embedded into the musculus pectoralis major and fixed by using a single, nonabsorbable, subcutaneous suture. The transmitter, sewn into a nonabsorbable mesh, and the antenna were fixed with the help of nonabsorbable suture material (Fig. 2b). At the end of surgery,

Table 1. Overview of analgesia and antibiotics used

Anesthesia	
	Midazolam (0.2 mg/kg, i.v.), Midazolam-ratiopharm®, Ratiopharm, Ulm, Germany
	Propofol (5 mg/kg, i.v.), Narcofol®, CP-Pharma GmbH, Burgdorf, Germany
	Isoflurane (1.9–2.4 Vol %), Isofluran CP®, CP-Pharma GmbH, Burgdorf, Germany
	Local: lidocaine (2%, max. 4 mg/kg), Xylocaine®, AstraZeneca GmbH, Wedel, Germany
Analgesia	
	Pre- and perioperative
	Carprofen (4 mg/kg, s.c.), Rimadyl®, Pfizer GmbH, Berlin, Germany
	Only during TA surgery: fentanyl (0.01 mg/kg i.v. pre-OP; 0.001–0.005 mg/kg i.v. every 20–40 min), Fentanyl®, Rotexmedica, Trittau, Germany
	Only at the end of TA surgery: buprenorphine (0.01 mg/kg i.m.), Buprenovet®, Bayer Vital GmbH, Leverkusen, Germany
	Postoperative
	Carprofen (2 mg/kg, s.c., once a day [d2–4]), Rimadyl®; Pfizer GmbH, Berlin, Germany
	Due to surgery-related pain detection, additional injections of carprofen or metamizole (40 mg/kg/daily, s.c.), Vetalgin®, MSD Tiergesundheit, Unterschleißheim, Germany
Antibiotics	
	Amoxicillin (ca. 15 mg/kg s.c. [d1, d3, d5]), Duphamox® LA, Zoetis Deutschland GmbH, Berlin, Germany

Table 2. Clinical score

Parameter	Clinical signs	Score
Vocalization	None	0
	Occasional teeth grinding	1
	Frequent teeth grinding	2
Activity	Sleeping and resting	0
	Frequent change of position	1
	Restless, directionless walking	2
Food/water intake	Normal, rumination	0
	Reduced	1
	Inappetence, no rumination	2
General appearance	Listless, sniffing and looking for straw, hay, or water	0
	Downcast, turning head to the wound	1
	Flehming, apathetic	2
Maximum score		8

absorbable sutures and suture clips closed the incisions, and aluminum spray was used for wound covering.

Tendon Ablation

The present study accompanied a surgical pilot study. As part of the pilot study, the TA of the musculus infraspinatus was performed under general anesthesia as described above. Briefly, after preparation of the surgical field by covering with sterile drapes and disinfection, the corresponding area of the shoulder joint and scapula was opened, and the musculus infraspinatus was dissected. After intervention on the tendon, the wound was closed in layers using absorbable suture material and covered with aluminum spray.

Severity Assessment

Clinical Score

Throughout the experiment three non-blinded observers monitored the general health status according to the clinical score sheet, respectively (Table 2; Fig. 1). Baseline score was determined by averaging the score values from the 3 days before surgery. On the day of surgery no clinical scoring was performed because rumination was impaired by fasting before surgery and general appearance could not be assessed because of inactivity.

Sheep Grimace Scale

As indicated (Fig. 1), videos of the sheep were recorded to analyze the SGS according to Häger et al. [18]. In the afternoon around 4:00 p.m., a digital video camera (Sony High Definition Handycam® Camcorder; HDR-CX100) was placed directly in front of the grid, and videos were recorded for 45 min, without the presence of an observer.

For evaluation, faces of the sheep were detected with a HOG-SVM detector [19] automatically. In the case of poor quality of the images, they were replaced manually using Microsoft Windows Media Player and Snipping Tool. In total, 640 pictures (eight pic-

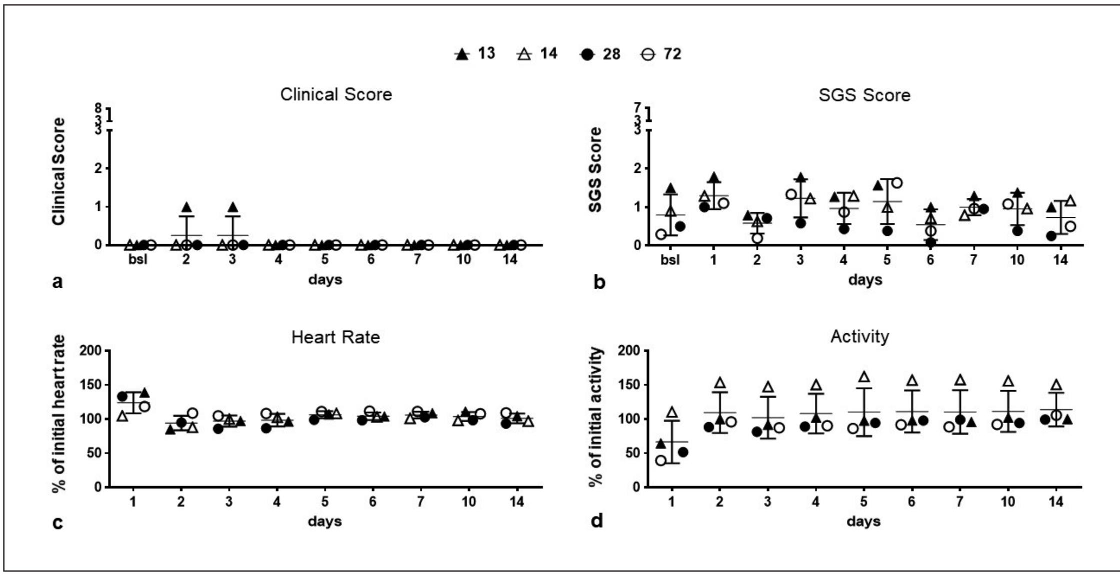

Fig. 3. Clinical examination, telemetrically derived data, and SGS after TI. $N = 4$. **a** Clinical score. **b** SGS score. **c** % change of the initial HR. **d** % changes of initial activity.

tures per day and sheep) were provided for randomized scoring by three blinded scorers, one experienced, one half-experienced, and one non-experienced.

For the analysis of the SGS, the mean of all score values from the three scorers was calculated. When more than one scorer was unable to score an image due to poor quality, that image was excluded from the analysis.

Transmitter Measurement

Before surgery, all electrical components were checked for functionality. To receive telemetric data, two receivers were attached to the walls of the stable (90° angle), and the respective animals were annotated to the receivers by using the data acquisition and analysis software Ponemah data 6.41 (component of the PhysioTel Telemetry System by DSI, Harvard Bioscience, Inc.). After TI, data of the telemetric devices was sent to the computer via a Data Exchange Matrix and was collected on the preselected days (Fig. 1).

Electrocardiograms were recorded for the analysis of the HR in beats per minute (bpm). Furthermore, activity data of the animals were provided in counts per minute (cpm). The activity was calculated in a three-dimensional space via accelerometry and using the location of the transmitter on hypothetical X-, Y-, and Z-axes. Therefore, changes in activity could also be detected in the vertical direction.

Telemetry-derived data (11:00 a.m.–9:00 p.m.) were evaluated with a logging rate of 5 min, with the HR provided as means and activity as sum. Since sheep are diurnal, animal's telemetric data of the active (light) phase were analyzed. To ensure that only periods were taken for analysis in which animals were not disturbed by human activity, the time for regular maintenance until 11:00 a.m. was omitted.

Baseline was created at the end of the recovery period of the TI (recovery period: 21 days for sheep 13 and 14; 28 days for sheep 28 and 72) and just directly before TA surgery by calculating the mean of 3 days in a row of the respective parameter (HR and activity). This baseline was used for the analysis of both operations.

Statistical Analysis

Data are shown as mean ± SD. All statistical analyses were performed using GraphPad Prism 8 software (La Jolla, CA, USA). All values used for the analysis can be found in online supplementary file 1. For SGS and telemetry data, repeated-measures (RM) one-way ANOVA was carried out followed by Dunnett's multiple comparisons post hoc test against baseline. Clinical score data were analyzed using the Friedman test followed by Dunn's multiple comparisons post hoc test against baseline. Strength of correlation was determined by Pearson's correlation coefficient. $p \leq 0.05$ was considered significant. * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$, and *** indicates $p \leq 0.001$. It must be noted here that the investigations were carried out as part of a pilot study, and therefore, no previous power analysis was carried out. The applied analyses should therefore be considered with regard to the small sample size.

Results

Severity Assessment after TI

The animals' health status was evaluated using the clinical score provided in Table 2. A baseline score of 0 was determined on the 3 days before surgery. On days 2 and 3 post-surgery, one sheep showed teeth grinding, resulting in a slightly increased clinical score with a maximum of 1 out of 8 score points (Fig. 3a, sheep #13). In the

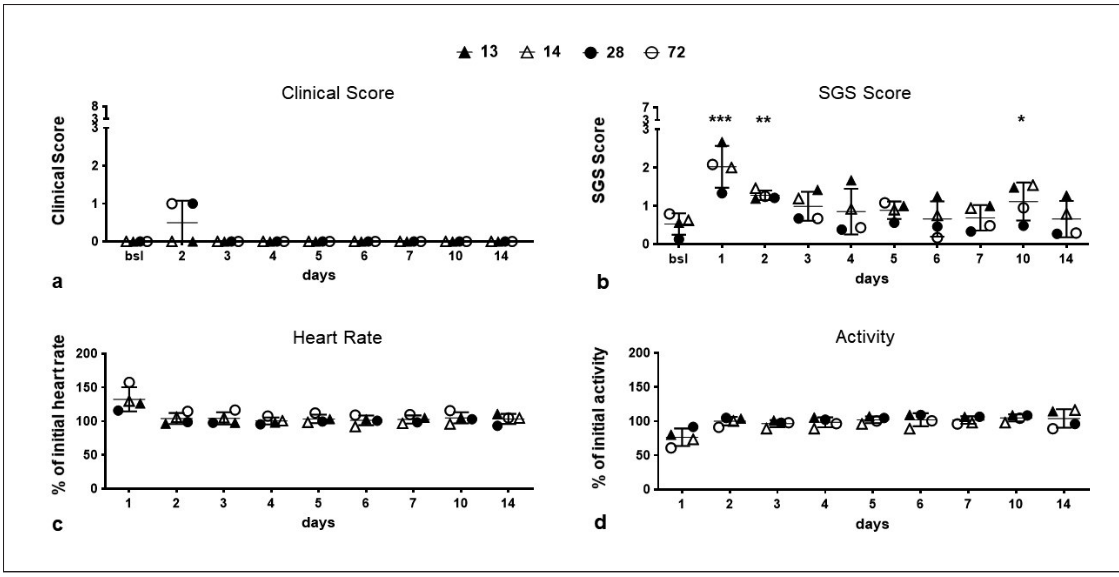

Fig. 4. Clinical examination, telemetrically derived data, and SGS after TA. $N = 4$. **a** Clinical score. **b** SGS score. **c** % change of initial HR. **d** % changes of initial activity.

rest of the postsurgical phase, the clinical score remained at a value of 0 for all animals.

Facial expressions of the sheep were analyzed using the SGS as an indicator of pain (Fig. 3b). Three days before surgery, a mean baseline score of 0.8 ± 0.5 was determined. On the day of surgery, there was only a slight increase of the SGS with a mean value of 1.3 ± 0.4. During the rest of the experiment, SGS values varied between values of 0.5 ± 0.4 and 1.2 ± 0.5.

Analysis of telemetry-derived data revealed a relative increase in the HR of 23.9 ± 15.5% on the day of surgery (d1), but no significant changes until the end of the experiment (Fig. 3c; RM one-way ANOVA, $F[1.672, 5.015] = 5.186$, $p = 0.063$). In line with this, the general activity decreased by 33.55 ± 31.1% on the day of surgery but returned to the baseline level during the rest of the experiment (Fig. 3d; RM one-way ANOVA, $F[1.327, 3.980] = 6.878$, $p = 0.056$).

Severity Assessment after TA

After TA, no statistically significant changes of clinical scores were observed. On day 2 post-surgery, teeth grinding resulted in a slightly increased clinical score (1 out of 8 score points) in two sheep (Fig. 4a, sheep #28 and #72). Analysis of facial expressions revealed significant increases of the SGS compared to baseline on d1 ($p < 0.001$), d2 ($p < 0.01$) and again on d10 ($p < 0.05$) after surgery (Fig. 4b).

The telemetry-derived HR was slightly increased on the day of surgery (d1) with an increase of 32.4 ± 17.81% (Fig. 4c). General activity was reduced by 23.46 ± 12.88% on the day of TA but returned to baseline level on the next day (Fig. 4d). Until the end of the experiment, activity remained at baseline level.

Correlation Analysis

Correlation of HR and activity data revealed a significant negative correlation of both parameter with a Pearson´s correlation coefficient of −0.40 ($p = 0.01$) for TI surgery (Fig. 5a) and −0.65 ($p < 0.0001$) for TA surgery (Fig. 5b). Indication of SGS values on data points with a high HR and low activity demonstrated that an elevated HR unrelated to higher activity was accompanied by a higher SGS. Furthermore, data points of low activity but a high HR and SGS solely originates from d1 after TI or TA surgery.

Discussion/Conclusion

According to the 3R principle [10] and legislation [11], it is imperative to minimize animal suffering during experiments. In this context, a profound assessment of the severity of the performed interventions is important and contributes to improving the well-being of the animals. An evaluation of possible variables for severity assess-

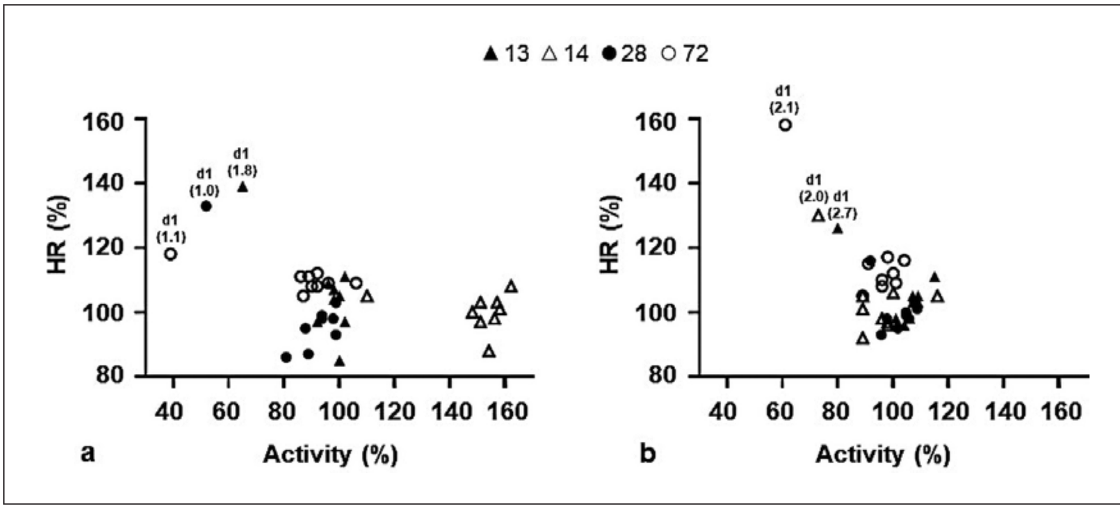

Fig. 5. Correlation of HR and activity data of all sheep. **a** Data points of d1–d7, d10, and d14 after TI. **b** Data points of d1–d7, d10, and d14 after TA. Data points with a high HR/reduced activity were provided with the associated SGS values.

ment is important to determine whether they can serve as objective methods for pain and distress assessment [14, 20]. Therefore, in the present study, clinical score, SGS, and telemetry-derived HR and activity were evaluated for severity assessment after TI and TA in sheep.

After a total of eight surgeries, we observed only slight increases of the applied clinical score in 3 cases after surgery. The telemetry-derived HR was increased after each surgery, and animals showed reduced activity when compared to baseline. Increases in the SGS indicated slightly elevated levels of severity immediately after each surgery, especially after TA. Correlation analysis of HR and activity data revealed significant negative correlation of both parameters. In case of a high HR and low activity, it turns out that especially these data were accompanied by higher SGS scores. This supports the concept that telemetry-derived data in combination with the SGS substantially improve postoperative severity assessment.

The clinical score sheet applied in this study did not reveal pronounced signs of suffering or pain in the animals. This might be due to the fact that sheep are prey animals and try to hide any "vulnerability" to predators [21, 22]. Since scoring requires the presence of an investigator, this may lead to less pronounced scores. Furthermore, the clinical scoring was performed in a non-blinded fashion; thus, bias due to subjectivity cannot be ruled out. Furthermore, scoring only took a few minutes, during which the pain-related behavior is probably not shown, which can be another reason for missed detection of pain [23]. This is underlined by the fact that flehming was only detected in video recordings of the sheep in the present study. Another drawback of clinical scoring is that immediately after surgery, the prior fasting and effects of anesthesia impede the assessment, especially with regard to pain detection. For a reliable detection of all levels of pain, a supplementation to clinical scoring by additional contactless and objective methods, such as HR monitoring or analysis of facial expressions, might be advisable.

In recent years, telemetry has been increasingly used as a contactless method for severity assessment. Especially in mice [21] or guinea pigs [24], telemetry was used to perform postoperative pain assessment. The first attempts have also been made with sheep. In wildlife, long-term observations of the HR were performed in bighorn sheep using a transmitter placed at the dorsolateral thorax [25]. In another study with free ranging ruminants, per os applied transmitter was tested [26]. Using subcutaneously implanted transmitters, it is possible to record physiological parameters such as HR or activity. However, when using telemetry as a method for severity assessment, it must be taken into account that the implantation itself represents an additional burden for the animals.

An increased HR which is not associated with increased activity is a good indicator of pain after surgery [27]. In a study investigating the effects of verapamil on the autonomic reaction to visceral pain in sheep after duodenal distension, operated animals showed tachycardia without therapy [28]. In the present study, the animals demonstrated an elevated HR only immediately after surgery, suggesting a very short perception of pain on these days. This is corroborated by short-lasting elevated SGS values for 1 day after TI and 2 days after TA, suggesting good pain alleviation due to the applied multimodal analgesia.

Grimace scales have been established as observer-independent and objective indicators for postoperative pain in various species (review: [17]). In 2010, the Mouse Grimace Scale was developed [29] and followed by grimace scales for rats [30], rabbits [31], cats [32], horses [33], and sheep [18]. Recently, our working group demonstrated the SGS as a reliable and valuable indicator for pain detection in laboratory sheep after surgery [18]. Furthermore, the authors showed that the inter-rater reliability of the SGS was high (ICC:0.92, accuracy: 68.2%; false positives: 22.7%, false negatives: 9.1%) even with half-experienced scorers. In the present study, the SGS was utilized to reveal pain-related behavior, especially after TA, demonstrating the good applicability of this method for the assessment of pain. Automation and real-time analysis of the SGS is desirable for its routine use, and there are already automation approaches initiated that aim at contributing to a reliable assessment of distress and pain in the future [34, 35].

We have previously observed SGS values that ranged around a score of 2 for up to 1 week in a study where sheep were submitted to an osteotomy of a hind leg [18]. Compared to that study, the degree of pain and suffering was probably considerable lower in the present study. The slightly longer lasting elevation of the SGS after TA might be due to the localization of pain and the ability of avoidance. The telemetric device is located at the neck, likely enabling that the relieving posture is possible for the protection of the forelimb.

Nevertheless, it should be considered that stress due to food withdrawal before surgery or anesthesia might impact the SGS and telemetry-derived parameter. Cardiac arrhythmias and tachycardia are frequent complications during surgical intervention in humans [36]. However, in sheep, such complications are poorly reported. Guidelines on anesthesia and analgesia in sheep report tachycardia as a sign of pain [37], confirming the findings of this study. Because we did not perform further analysis of the blood, including volume status, catecholamine levels, or electrolyte misbalance, we cannot finally rule out that an elevated HR might be due to adverse effect of the applied anesthesia. However, during surgical procedure, no tachycardia or arrhythmic events were detected.

It was observed that two sheep (13, 14) that did not receive prolonged pain treatment after TA surgery had higher SGS values at d7, d10, and d14, in contrast to the other two sheep (28, 72). This suggests that the prolonged administration of the analgesics caused the sheep 28 and 72 to experience less pain, as demonstrated by the SGS values. In the future, further investigations are necessary.

Another issue to consider in relation to the HR is the body weight of the animals, especially after TA surgery. For example, sheep 72 is significantly heavier compared to the other three sheep. The HR as well as the SGS is also highest in this animal after TA surgery, which can be attributed to greater pain due to the greater burden on the limb from the greater body weight. In line with that, after TI surgery (in which weight does not play such a big role), the sheep 72 is not the animal with the highest scores.

The results presented in this study revealed that clinical scoring as performed in this study failed to reliably detect pain in sheep. In contrast, especially the SGS but also HR and activity monitoring were well suited to indicate postoperative pain in sheep. Therefore, video recordings or methods for contactless HR monitoring might be valuable tools for the detection of pain, supporting refinement in large animal studies.

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Statement of Ethics

Experiments were approved by the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES, license 33.12-42502-04-18/2837). All efforts were made to minimize pain or discomfort of the used animals. All animal experiments were carried out in accordance with the EU Directive 2010/63/EU for animal experiments, including approval by local authorities and an animal ethics committee.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Christine Häger, André Bleich, and Marion Bankstahl: study conception, design, and supervision. Laura Wassermann, Eva Zentrich, Birgitta Struve, and Merle Kempfert: examination of the animals. Janin Reifenrath, Nina Angrisani, Laura Wassermann, Eva Zentrich, and Merle Kempfert: conduction of surgeries. Annika Krause and Olaf Bellmann: technical and professional support for TI. Eva Zentrich and Laura Wassermann: data gathering and analysis. Marcin Kopaczka, Dorit Merhof, and Eva Zentrich: preparation of images for SGS scoring. Kristin Selke, Manuela Buettner, and Lydia Maria Keubler: scoring of images for SGS. Eva Zentrich and Christine Häger: drafting of manuscript. All of the authors edited and approved the final version of this work.

Data Availability Statement

All data generated or analyzed during this study are included in this article or its supplementary material files. Further inquiries can be directed to the corresponding author.

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