Master Thesis

Stress in alpacas: physiological and behavioral responses during the shearing procedure

submitted by

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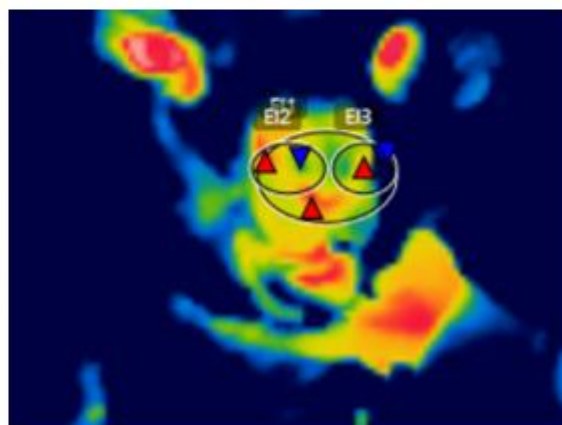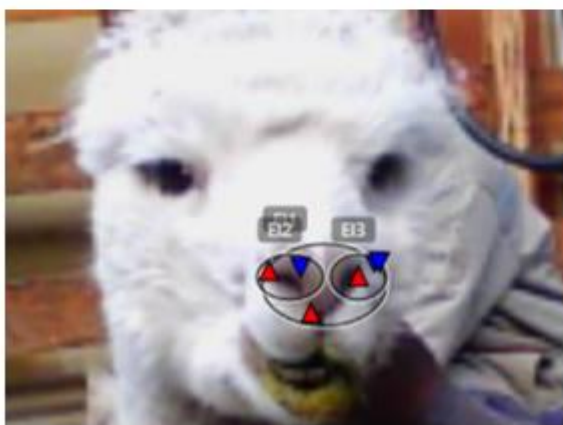## Diplom-Ingenieurin
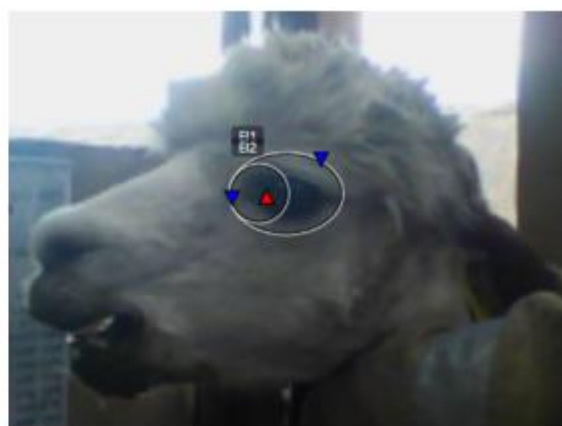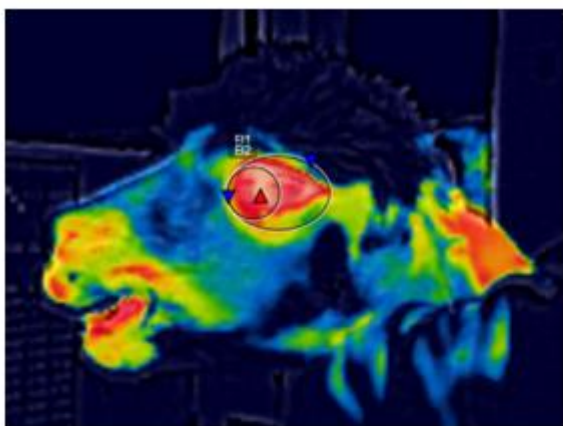
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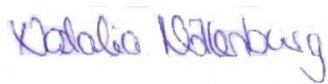Dr. Alan Cruz

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Abstract

Alpacas are primarily raised for fiber production, which involves regular shearing. The shearing process induces stress caused by separation from the herd, handling, restraint and shearing itself. Together with the demand for alpaca fiber also concerns about animal welfare have increased thus requiring an enhanced understanding of the stress reaction. Stress can be measured through physiological as well as behavioral parameters. Therefore, the aim of this thesis was to evaluate the stress reaction of alpacas by assessing core and peripheral body temperature as well as stress-indicating behaviors (e.g., vocalization, defense behavior). For this purpose, in total 42 female pregnant alpacas were subjected to one of two treatments. Animals in the 'Shear' group were handled and shorn (n=32) and 'Sham' animals were handled in the same way but not shorn (n=10). To assess changes in the peripheral body temperature, infrared images were taken non-invasively of the eyes (right and left) and nose (right nostril, left nostril, flew). Additionally, rectal temperature was measured to study how changes in core and peripheral temperature cohere. In both groups, temperatures were taken at five different measurement points: before shearing/sham-shearing ('Pre'), during ('Start', 'Mid', 'End') and after it ('Post'). To capture stress-indicating behaviors during shearing/sham-shearing, videos were recorded and analyzed during five phases (until 'Start', 'Start' to 'Mid', 'Mid' to 'End', 'End' to 'Post' and 'until termination').

Large individual differences were found in the temperature patterns. Nevertheless, mixed effects models revealed a significant increase in right eye temperature followed by a decrease in both groups ($p_{\text{time point}}$=0.018). Nostril and flew temperature consistently decreased as a first reaction to shearing/sham-shearing ($p_{\text{time point}}$<0.07). Rectal temperature stayed rather stable over the whole process, but it was higher in the 'Shear' (LSM=38.0°C) compared to the 'Sham' group (37.8°C, p_{group}=0.04). Frequency of screams ($p_{\text{group*phase}}$<0.001), time spent screaming ($p_{\text{group*phase}}$=0.003) and defense behaviors ($p_{\text{group*phase}}$=0.007) first increased in the 'Shear' group and decreased after the middle of shearing, while the contrary was observed in the 'Sham' group with more stress-indicating behaviors before and after sham-shearing and less during the middle part. Rectal and peripheral temperature correlated only weakly and associations between temperature changes of the right nostril and flew and scream frequency were also weak, indicating a slight increase in temperature with more screams.

In sum, it can be assumed that the shearing/sham-shearing process (capture, handling, restraint) induces stress which is reflected in the overall frequency of stress-indicating behaviors. Core body temperature and peripheral temperature react differently in the course of shearing/sham-shearing and do not correlate well. The unclear pattern of physiological responses indicates that using core or peripheral body temperatures as a measure of stress may not reveal valid conclusions. Nevertheless, an important question to be answered in the future should be how to reduce stress for alpacas during handling and shearing.

Zusammenfassung

Alpakas werden hauptsächlich für die Faserproduktion gezüchtet, welche eine regelmäßige Schur erfordert. Der Scherprozess verursacht Stress durch die Trennung von der Herde, das Handling, das Fixieren und das Scheren selbst. Zusammen mit der Nachfrage nach Alpakafasern haben auch die Bedenken um das Wohlergehen der Tiere zugenommen, welche ein besseres Verständnis der Stressreaktion wünschenswert erscheinen lassen. Stress kann sowohl mittels physiologischer als auch durch Verhaltensparameter gemessen werden. Ziel dieser Arbeit war es daher, die Stressreaktion von Alpakas durch Messungen der Kern- und peripheren Körpertemperatur sowie Stress anzeigenden Verhaltensweisen (z.B. Vokalisation, Abwehrverhalten) zu untersuchen. Zu diesem Zweck wurden insgesamt 42 trächtige weibliche Alpakas einer von zwei Behandlungen unterzogen: 'Schur' (Handling und Scheren, n=32) und 'Scheinschur' (Handling ohne Schur, n=10). Zur Beurteilung der Veränderungen der peripheren Körpertemperatur wurden Infrarotaufnahmen beider Augen und der Nase (beide Nasenlöcher und Lefze) gemacht. Zusätzlich wurde die Rektaltemperatur gemessen, um zu untersuchen, wie Veränderungen der Kern- und der peripheren Temperatur zusammenhängen. In beiden Gruppen wurden die Temperaturen zu fünf verschiedenen Zeitpunkten gemessen: vor dem Scheren/Scheinscheren ('Pre'), während ('Start', 'Mid', 'End') und danach ('Post'). Um stressanzeigende Verhaltensweisen während des Scherens zu erfassen, wurden Videos aufgenommen und entsprechend der Schur-Phasen analysiert.

Es wurden große individuelle Unterschiede in den Temperaturmustern festgestellt. Dennoch zeigten Modelle mit gemischten Effekten einen signifikanten Anstieg der Temperatur des rechten Auges, gefolgt von einem Abfall in beiden Gruppen ($p_{Zeitpunkt}$=0,018). Die Temperatur der Nasenlöcher und der Lefze sank als erste Reaktion auf das (Schein-)/Scheren ($p_{Zeitpunkt}$<0,07). Die Rektaltemperatur blieb während des gesamten Prozesses relativ konstant, war aber in der 'Schur'-Gruppe (LSM=38,0°C) höher als in der 'Schein'-Gruppe (37,8°C, p_{Gruppe}=0,04). Die Häufigkeit der Schreie ($p_{Gruppe*Phase}$<0,001), Dauer der Schreie ($p_{Gruppe*Phase}$=0,003) und das Abwehrverhalten ($p_{Gruppe*Phase}$=0,007) stiegen zunächst in der 'Schur'-Gruppe an und nahmen nach der Mitte der Schur ab, während in der 'Schein'-Gruppe das Gegenteil beobachtet wurde, nämlich mehr Stress anzeigende Verhaltensweisen vor und nach der Scheinschur und weniger während des mittleren Teils. Rektal- und periphere Temperatur korrelierten nur schwach. Korrelationen zwischen Temperaturveränderungen des rechten Nasenlochs und der Schreihäufigkeit waren ebenfalls schwach ausgeprägt und deuteten auf einen leichten Temperaturanstieg bei mehr Schreien hin.

Zusammenfassend kann davon ausgegangen werden, dass der Prozess des Scherens (Einfangen, Handling, Fixieren) Stress auslöst, der sich in der Gesamthäufigkeit von Stress anzeigenden Verhaltensweisen widerspiegelt. Die Körperkerntemperatur und die periphere Temperatur reagierten im Verlauf der Schur unterschiedlich und korrelierten nicht bzw. nur schwach miteinander. Das unklare Muster der physiologischen Reaktionen deutet darauf hin, dass die Verwendung der Körperkerntemperatur oder der peripheren Temperatur als Maß für Stress möglicherweise keine gültigen Schlussfolgerungen zulässt. Eine wichtige Frage, die es in Zukunft zu beantworten gilt, ist, wie der Stress für Alpakas während Handling und Schur reduziert werden kann.

1. Introduction

Out of the four South American camelid species, namely vicuñas, alpacas, guanacos and llamas, alpacas are the most important species with regard to fiber production. With approximately 4 million animals, 75% of the world's alpaca population is located in Peru and it secures the livelihoods of thousands of Andean families and subsistence farmers (Morante et al., 2009). There are two breeds of alpacas, suri and huacaya (Wuliji et al., 2000), the latter with 93% of all bred alpaca representing the most common and economically significant breed in Peru. Huacaya have curly and short fiber which are dense and voluminous.

Alpacas are usually shorn once a year during the warmer wet season between November and April. Shearing practices are often very basic with low management standards and little care for the animal. As experienced fiber buyer all over Peru, INCA Group reported some farmers to shear their alpacas with glass, tins or scissors, often injuring the animal. To ensure better management practices, Pacomarca, a research facility financed by INCA Group, developed a shearing protocol called 'INCA shearing' wherein they describe each step of the process. Nevertheless, their focus remains mostly on fiber management practices during shearing and to a limited extent on animal welfare. Shearing usually starts with brushing the animal to get a cleaner coat, continues with stretching the alpaca on a shearing mattress and tying all its four legs with ropes attached to the walls of the shearing area (Morante et al., 2009). It is a common practice to immobilize alpacas that way as they show a lot more defensive behavior than for example sheep. Their expressive defensive behavior suggests that these animals experience shearing as a stressful and threatening event (Wittek et al., 2017).

Notwithstanding the more recent activities to improve shearing management described above, the Peruvian fiber industry has been criticized for animal cruelty and a lack of animal welfare related to shearing. Through secretly taken video footage, the American animal rights organization PeTA revealed how workers of one of the largest privately owned alpaca farms in Peru mistreat alpacas during the shearing process (https://investigations.peta.org/alpaca-wool-abuse/#video). It is shown how workers pull them by their ears and tail to immobilize or move them, throw them from the shearing table and even step on their necks. The animals were also shown to suffer cutting injuries which were poorly treated.

As animal welfare is increasingly of public concern and in the media, the alpaca fiber industry needs to catch up given that yet, no sustainable or responsible standard for alpaca husbandry has been established internationally. As a first step, the 'Responsible Alpaca Standard' (RAS), a voluntary standard framework for alpaca farmers, was published in 2021 by the global nonprofit organization 'Textile Exchange' which develops industry standards and publishes critical industry data on fiber and materials (https://textileexchange.org/about-us/). The RAS emphasizes the major principles of animal welfare (e.g. the 'Five Freedoms') and presents regulations to minimize pain and stress for the alpaca during handling and shearing amongst others (https://textileexchange.org/standards/responsible-alpaca-standard/). More specifically, also restraining time and conditions during shearing are specified. In general, the RAS aims to create a fiber production that respects and improves animal welfare, the environment and also social aspects of alpaca farming. If an alpaca farmer wants to be RAS certified, he/she needs to comply with all the regulations of the framework. The final product or garment is only certified if all processing steps (from trader to textile producer) comply with the RAS. 'Textile Exchange' already developed similar standards for wool and mohair and put in place an assurance system with regular farm audits and material to final product tracing (https://textileexchange.org/standards/). Nevertheless, this is only a very recent development which still needs to show its effects within the alpaca industry. Besides, little research has

been conducted on the alpaca's stress reaction during handling and shearing which is not only relevant from an animal welfare perspective but also has economic implications as pregnant female alpacas tend to abort their foals when stressed (Cruz et al., 2020). Beside abortions and premature births, injuries and gastrointestinal problems can be other physiological consequences of stress (Fowler, 1994). Therefore, this thesis aims to contribute to stress research in alpacas by evaluating different physiological and behavioral reactions when they are handled and shorn.

1.1 Reasons for stress in camelids

Capture, handling, restraint and shearing induce stress in South American camelids which has been shown in different studies about vicuñas (Bonacic et al., 2006), guanacos (Carmanchahi et al., 2001) and alpacas (Waiblinger et al., 2020). The shearing process comprises all of the above-mentioned actions and therefore seems to be particularly stressful. In the Peruvian Andes, alpacas are mostly kept in extensive, pasture-based production systems with minimal close contact to humans (Gutierrez et al., 2018). Animals do see humans regularly when being maneuvered to different grazing areas, but they are usually not touched or handled, and a distance is being kept between alpacas and stockmen (Kohl, 2019). As 80% of alpacas belong to smallholder farmers with little income, there is a lack of regular medical treatments which would require close animal-human contact (Pizzaro et al., 2019). In the frame of an Austrian study to research alpacas' and llamas' reaction to handling and assess stockman attitudes and practices, it was found that animals that were touched less in early life showed more handling difficulties (e.g., escape attempts). Similarly, more frequently stroked animals cooperated better with humans during physical examination or leading (Windschurer et al., 2020). Although human-animal contact has not been studied much in alpacas, these results suggest that less close contact with humans will lead to more stress-indicating behavior.

During shearing, camelids are not only exposed to the unusual close human contact but are also isolated from their herd and eventually severely restrained by ropes. Therefore, human contact, social isolation (Pollard & Littlejohn, 1995), restraint as well as unusual noises and sounds (Wittek et al., 2017) accumulate to a highly stressful experience for the animals. Stress can be measured via different physiological indicators or behavioral expressions which will be explained in the following chapter.

1.2 Indicators of stress in camelids: An overview

Different physiological parameters such as heart rate, heart rate variability, body temperature, and salivary or blood cortisol as well as behavior can indicate stress responses and have been studied in camelids during situations like handling, restraint or shearing (Bonacic et al. 2003, 2006; Bonacic & MacDonald, 2003; Carmanchahi et al. 2011; Anderson et al., 1999).

Bonacic et al. (2003) studied behavior in combination with physiological indicators in vicuñas. An ACTH-challenge was applied to previously captured vicuñas in order to trigger a physiological stress response by activating the HPA-axis. They found no significant differences in behavior between ACTH-treated and control animals but discovered that animals with higher baseline cortisol levels attempted to escape and vocalized more. Additionally, the animal with the highest blood cortisol level exhibited especially nervous behavior (such as leg kicks, head movements, escape attempts). In a different study, when comparing methods of capturing vicuñas, Bonacic et al. (2006) found plasma cortisol concentrations to be around 41% higher in captured vicuñas compared to baseline levels in similar studies. They also discovered an increase in heart and respiratory rate when obtaining these physiological values after capture and herding and comparing them to baseline levels.

Carmanchahi et al. (2011) conducted a similar study on guanacos, the wild relatives of llamas, to research the effect of capture and shearing on serum cortisol, heart rate and rectal temperature. They found serum cortisol levels to be higher the longer the guanacos were restrained and shorn, concluding that longer handling time increased the physiological stress response. On the contrary, no significant correlations could be found between total handling time and heart rate as well as rectal temperature. When comparing different shearing methods (standing, mattress and table) within two groups ('restraint without shearing' and 'restraint plus shearing'), Wittek et al. (2017) discovered that cortisol concentrations significantly increased for all methods over time for the 'restraint without shearing' group. In the 'restraint plus shearing' group, especially alpacas shorn on the ground showed a significant elevation in saliva cortisol levels. There was also a high individual variation of saliva cortisol in all groups. Heart rate and respiratory rate did not differ significantly between treatments suggesting that the stress level may be quite high for all restraint methods. Nevertheless, Wittek et al. (2017) concluded that shearing while standing might be the least aversive for alpacas as saliva cortisol levels were lower in this group.

Common behavioral stress responses in ungulates include escape attempts, vocalizations and kicking (Grandin, 1997). During shearing, alpacas for example have shown vocalizations, escape attempts and defense reactions such as kicking and laying down on the belly (Waiblinger at al., 2020). Guanacos and vicuñas caught for shearing have been reported to scream loudly during handling and show agonistic behavior, both possible indicators for stress (Arzamendia et al., 2010; Taraborelli et al., 2017).

Various studies have been conducted in guanacos and vicuñas in order to connect physiological stress parameters with stress-indicating behavior and better evaluate animal welfare implications during capture and handling/shearing (Arzamendia et al. 2010; Taraborelli et al. 2017; Marcoppido et al. 2018). Arzamendia et al. (2010) for example showed that the behaviorally most active vicuñas suffered from higher physiological stress responses such as elevated respiratory and heart rate suggesting that behavioral changes might be reflected in physiological alterations. Behavioral observations included vocalizations, abrupt movements like escape attempts and jumping as well as kicking. Blood cortisol levels were also elevated in captured animals compared to baseline values of previous studies. In a different study concerning behavioral stress responses and the examination of serum cortisol levels in guanacos, Taraborelli et al. (2017) recorded behaviors such as vigilance, aggression (including lowered ears, spitting, kicking etc.) and vocalizations (among others). These authors found that the behavioral stress response in male guanacos was negatively correlated with the serum cortisol levels which was also observed in the study of Marcopiddo et al. (2018). Therefore, the authors suggested that it was necessary to continue investigating both stress responses (behavioral and physiological) instead of concluding from one to the other (e.g., frequent stress-indicating behavior necessarily resulting in high blood cortisol levels) and explained that stress-indicating behavior could potentially help alleviate physiological stress responses. This could have led to the relatively low cortisol levels although stress-indicating behavior was high. They nevertheless concluded that agonistic behavior and vocalizations are a suitable behavioral stress indicator in guanacos.

Also, an increase in core body temperature can be a result of stress and is described as stress-induced hyperthermia (Moberg, 2007). According to Fowler (1994), hyperthermia in llamas and alpacas can not only be caused by stressors such as high environmental temperature and humidity, but also by muscular exertion, fighting or prolonged restraint as is the case during shearing. Stress-induced hyperthermia (SIH) is the result of a short rise in core body temperature due to a situation perceived as stressful. This elevation of core body temperature can be compared to the fever reaction of the body but is of shorter duration

(Bouwknecht et al., 2007). The normal core body temperature of adult llamas and alpacas varies between 37.5°C and 38.6°C (Fowler, 1994). Different studies in ungulates have shown that capture and transport (Bonacic and Macdonald, 2003) as well as restraint (Meyer et al., 2008) can cause SIH. In sheep, shearing resulted in stress-induced hyperthermia (Sanger et al., 2011). Core temperature was significantly higher between 4- and 14-minutes post-shearing for shorn animals (with a total increase of 0.58°C) compared to the sham-shorn control group. In vicuñas, Bonacic and Macdonald (2003) found a significant increase in rectal temperature compared to baseline levels after transporting the animals for 10-15 minutes and releasing them back to the wild (39.09 ± 0.10ºC post-transport compared to 38.5 ± 0.12ºC pre-transport). Referring to this obtained 'baseline' rectal temperature of 38.5°C, Bonacic et al. (2006) also found a rise in mean rectal temperature of up to 40.0 (± 0.2 °C) after capturing juvenile vicuñas using different capture methods. Nevertheless, this rise could have also been due to physical activity during the capture event which is not further considered in their study.

In contrast to those results, Wittek et al. (2017) found no significant increase in rectal temperature when comparing three different restraint methods (standing, on the ground, on a table) in two different treatment groups (shearing/sham-shearing). Rectal temperature was measured at start of the procedure and 5, 10, 20, 30, 40 and 60 minutes thereafter and remained more or less constant with little fluctuations, e. g. in the sham-shearing group between 37.9±0.6 and 38.1±0.5°C for the method 'on the ground' (shearing group: 37.9±0.6 and 38.3±0.4°C). A significant increase in rectal temperature was also not found before and after capturing vicuñas with different methods. The capture and restraint event took around 40 minutes and rectal temperature barely changed e.g., from 40.24±0.1°C to 40.20±0.1°C post capture (for males of the mixed capture method). Animals were also shorn at the end of the capture study (Arzamendia et al., 2010). This is also in line with another capture study in vicuñas where body temperature pre and post handling also remained constant (Marcopiddo et al., 2018).

When considering temperature changes in animals to indicate stress, not only core temperature can be taken into account but also peripheral temperature of for example eyes, nose, ears, tails or paws. This change in peripheral temperature can be made visible by infrared thermography, an emerging approach which has been subject to various studies concerning emotional state and stress in different animals but not much yet in alpacas. The following chapter explains the physiological theories behind peripheral temperature changes.

1.3 Changes in peripheral temperature as indicators of stress

The temperature of peripheral body parts (e.g., eye, nose) can be non-invasively and reliably measured using infrared thermography (IRT). IRT has been used in the study of stress responses in a variety of species including ungulates, dogs, horses and pigs (Stewart et al., 2007, 2008; Elias et al., 2021; Bartolomé et al., 2013; Rocha et al., 2019). The advantage of non-invasive methods is that the animal is not stressed additionally when being handled, thus preventing the distortion of study results. There are however other challenges or influencing factors such as ambient temperature/solar radiation, humidity, wind, proximity to the object as well as coat color or thickness (Bartolomé et al., 2013). Nevertheless, especially eye temperature (ET) has been shown to be a reliable anatomical spot to measure changes in peripheral body temperature as the eyes are close to the brain and receive a rich blood supply which reacts immediately to exterior stimuli (Elias et al. 2021). Nose (Nakayama et al., 2005), ear (Ludwig et al., 2007), neck (Rocha et al., 2019), paws and tails (Vianna & Carrive, 2005) as well as wattle and comb (Herborn et al., 2015) of different animal species have also been studied for temperature changes related to negative emotions and stress and shown to be promising spots to measure stress reactions.

The interpretation of eye and nose temperature changes is controversially discussed in literature and still not clear to this day. Especially the changes in eye temperature have led to contradictory results with different explanatory approaches underlying the physiological reactions (Stewart et al., 2008; Elias et al. 2021). On the one hand, the involvement of the two branches of the autonomic nervous system (ANS) – i.e., sympathetic and parasympathetic - have been suggested to play a crucial role in vasoconstriction and vasodilation of peripheral tissues, resulting in a drop (Stewart et al., 2007[a], 2008) or an increase in ET (Bartolomé et al., 2013; Cannas et al. 2018; Rocha et al. 2019; Sutherland et al. 2020). On the other hand, studies also suggest the involvement of the HPA-axis (Cook et al., 2001) or argue that temperature changes react differently according to the perceived valence of the stressor (e.g., pain or excitement) (Elias et al., 2021) or its anticipation (Stewart et al., 2005). In order to better understand the possible physiological mechanisms, the different hypotheses are summarized below.

Hypothesis 1: Stress-induced need to improve visual orientation leads to vasodilation around the eyes and thus a temperature increase
Increases in eye temperature have been explained by vasodilation of the blood vessels around the eye, allowing the animal to enhance its visual orientation in case of 'fight or flight' (Yarnell et al., 2013). A significant increase (0.5-1°C) in eye temperature has been found in competition horses and racing dogs pre to post competition or race (Bartolomé et al, 2013; Redaelli et al., 2019; Elias et al. 2021). Lambs after castration and meat goats after a fear test also reacted with ET increase (Harris et al., 2021; Bartolomé et al., 2019). According to Elias et al. (2021) a rise in eye temperature might be regulated by parasympathetic activity of the ANS and may indicate less acute stress responses (like race anticipation), but the results in castrated lambs (which involved strong pain) contradict this hypothesis. Additionally, Elias at al. attributed the increase in eye temperature to vasodilation caused by the activation of the HPA-axis. Stewart et al. on the contrary, excluded the involvement of the HPA-axis as no correlation between ET increase and HPA-axis activity was found (2007[b], 2008).

Hypothesis 2: Stress-induced centralization of blood circulation leads to vasoconstriction around eyes (and nose) and thus a temperature drop
In contrast to the first hypothesis, it has been suggested that a decrease in eye temperature can be explained by vasoconstriction as a result of sympathetic activity which leads to blood flowing from peripheral body regions towards more centralized areas of the organism (Travain & Valsecchi, 2021). Elias et al. (2021) elaborated that decreases in ET may be caused by an acute stress response of the sympathetic nervous system (like pain or a fright) leading to vasoconstriction which studies of Stewart et al. (2007[b], 2008) seem to confirm.

Stewart et al. extensively studied temperature changes of the eyes in cattle subjected to different stressful stimuli and situations. Their main goal was to measure ET and additionally test for the involvement of the HPA-axis and autonomous nervous system in these temperature changes. They aimed at confirming the results of a previous study by Cook et al. (2001) in horses who measured IRT and cortisol levels in order to determine adrenocortical changes amongst others. Cook et al. found significant correlations between ET and cortisol concentrations in saliva and plasma and concluded that changes in ET may be due to HPA-axis activity, which was partially also confirmed in pigs (Rocha et al., 2019). Nevertheless, Stewart et al. (2005, 2007[b], 2008) drew the opposite conclusions from their studies as they did not find an explicit connection between ET change and cortisol release. For example, when they injected previously disbudded calves with ACTH or saline (control treatment), no differences in eye temperature between the two groups were found. During the previous disbudding (with or without local anesthetic), ET of the calves decreased within

5 min after disbudding without local anesthetic (-0.27°C), followed by an increase above baseline values (>0.6°C). As a consequence, they hypothesized that ET decrease must be regulated by the ANS, more specifically by the activation of the sympathetic branch (2008). To further test this theory and single out the effect of ANS activity, they simultaneously measured ET, HR, HRV, cortisol and plasma catecholamine concentrations in bull calves. On the one hand, pain caused by castration resulted in a first slight decrease of ET followed by a significant increase as well as a rise of catecholamines and HR, pointing at a high involvement of the sympathetic nervous system (2010). In a supplemental experiment with calves, this hypothesis was confirmed during an adrenalin challenge which also led to a drop in ET (-1.4 ± 0.05°C) (2010[b]). However, in order to interpret the significant eye temperature increase of the first experiment (2010), it was argued that the parasympathetic nervous system could have been involved as well leading to vasodilation. These different activations of sympathicus and parasympathicus could be explained by the type of pain, expectation and fear connected to the different procedures.

When Sutherland et al. (2020) wanted to confirm the effect of an adrenalin injection in sheep to examine whether IRT can be used to measure the autonomic nervous system response, they expected decreasing eye temperatures (referring to Stewart et al. in 2010[b]) but found an increase. The scientists attributed this discrepancy to potential differences between data collection times, arguing that the decrease in temperature could have been missed. Comparing their study outcomes to various other ones, they also argued that the types of stressful stimuli as well as the definition of the eye region where IR temperature was obtained may lead to contradictory results.

Besides the eye, also decreases in the temperature of the nose have been reported as indicator of stress and negative emotions. Nakayama et al. (2005) studied the peripheral nasal temperature of rhesus monkeys via IRT. The monkeys were subjected to an aversive situation while nose temperatures were measured before, during and after the situation. Already after 10-30 seconds, nasal temperature declined significantly and continued to drop during the aversive 'stimulation period'. The presumably negative emotional state could further be confirmed by typical facial expressions such as 'silent bared-teeth face', 'staring open-mouth' or 'lip-smacking', all behavioral expressions of monkeys when confronted with a threat. Nakayama et al. (2005) also reported increases in eye temperature (matching hypothesis 1) although those were not consistent for all monkeys. Decreases in nose temperature during the stimulation period ranged between 0.0 and 0.2 °C. In the respective study, they attributed the drop in nasal temperature to the activation of the sympathetic nervous system causing vasoconstriction and thus diminishing blood flow to the peripheral capillary vessels. As a physiological consequence, skin temperature declined. Further studies with rhesus monkeys and chimpanzees confirmed the decrease in nasal temperature as well (Ioannou et al. 2005; Kuraoka and Nakamura, 2011; Kano et al. 2016; Dezecache et al. 2017).

Table 1 summarizes the peripheral temperature reactions of different animal species to various treatments and experimental situations.

Table 1 Overview of studies in different animal species and in different 'stressful' situations using peripheral eye and/or nose temperature as outcome measure

Animal & situation	Anatomical location	Time frame	Temperature reaction
Dairy cows subjected to ACTH challenge & social isolation (Stewart et al., 2005)	Unclear	Unclear	Increase after ACTH & control injection Decrease after social isolation

Dairy cows subjected to 6 different 'stress treatments' (catheterizations & social isolation) (Stewart et al., 2007[a])	Left eye (medial posterior palpebral border of lower eyelid and lacrimal caruncle)	ET assessed every 2 minutes and 30 and 60 minutes after treatments	Increase
Heifers and bulls responding to induced fear (Stewart et al., 2007[b])	Left eye (medial posterior palpebral border of lower eyelid and lacrimal caruncle)	Continuous (video) recording of ET 10/20 minutes pre until 10/20 minutes post treatment	Decrease
Calves disbudded with/without local anesthetic (Stewart et al., 2008)	Left eye (medial posterior palpebral border of lower eyelid and lacrimal caruncle)	ET recorded every 38 seconds on average	Decrease
Bull calves subjected to surgical castration with/without anesthesia (Stewart et al., 2010)	Left eye (medial posterior palpebral border of lower eyelid and lacrimal caruncle)	ET measured continuously for every 20 seconds for 20 minutes pre treatment until 25 minutes post	Not significant small decrease, followed by significant increase
Calves receiving adrenalin infusion (Stewart et al., 2010[b])	Left eye (medial posterior palpebral border of lower eyelid and lacrimal caruncle)	ET recorded every 20 seconds from 15 minutes pre treatment until 10 minutes post	Decrease
Sheep subjected to adrenalin injection (Sutherland et al., 2020)	Left eye (medial posterior palpebral border of lower eyelid and lacrimal caruncle)	Continuous recording of ET 25 minutes before until 20 minutes after treatment	Increase (although a decrease was expected similar to Stewart et al.)
Lambs during castration/sham castration (Harris et al., 2021)	Centre of cornea	Measured for 5-minute intervals starting at 5 min pre procedure, 0-, 1-, 4- & 24-hours post procedure	Increase
Meat goats subjected to 'stressful' person (Bartolomé et al., 2019)	Left eye (max. temperature within oval area around the medial posterior palpebral border of lower eyelid and lacrimal caruncle)	ET recorded just before 'stress test' (at rest) and just after (exact timeframe unclear)	Increase
Horses before and after jumping competition (Bartolomé et al., 2013)	Circular area around the left eye including eyeball & 1cm outside of eyelids	3 hours before, 5 minutes after and 3 hours after competition	Increase
Dogs before and after race (Elias et al., 2021)	7 points in the left/right eye (including lacrimal caruncle)	10 minutes pre-race 15 minutes post-race	Increase (greater in right eye than left & lacrimal caruncle was most sensitive)

Pigs handled and transported (Rocha et al., 2019)	Neck, rump, around eyes and behind ears	4 measurement points (exact time unclear): at rest (pre-handling), loading, unloading, home pen (post-handling)	Increase
Rhesus monkeys confronted with negative stimulus (Nakayama et al., 2005)	Nose temperature	ET recorded every 10 seconds for 9 minutes	Decrease
Rabbits subjected to social stress, noise stimulation & tonic immobility test (Ludwig et al., 2007)	Ears (and eyes)	1 IRT picture recorded/minute for 30 minutes pre stress and 1-4 pictures recorded during stress tests (ranging between 5 seconds and 30 minutes)	Decrease

In conclusion, peripheral temperature changes can be interpreted as the result of physiological alterations before, during and after a stressful event. The physiological changes in temperature are caused by variation of blood flow mediated by vasodilation or vasoconstriction of the capillaries. While vasodilation reflects the activity of the parasympathetic nervous system, vasoconstriction is the result of an active sympathetic nervous system. Especially body regions rich in arteriovenous anastomoses (AVAs) which are linking arterioles and venules by capillary connections are most affected by vasoconstriction and -dilation induced by stress. Ungulate eyes, monkey faces, rat tails or rabbit ears are tissues abundant of AVAs (Travain & Valsecchi, 2021). Nevertheless, the contradicting physiological theories behind the alterations make it hard to predict the direction of peripheral temperature change. Animal intrinsic factors such as the perceived intensity or valence of a 'stressful' stimulus as well as extrinsic factors such as ambient temperature may heavily influence the rise or drop in peripheral temperature.

2. Research questions

This study aimed at investigating the stress experienced by alpacas during the electromechanical shearing procedure through changes in in core and peripheral body temperature as well as behavioral indicators of stress.

Using rectal temperature, infrared temperature measures of the eyes and the nose and behavioral analyses from video footage the following research questions were addressed:

1) What is the response of core body and peripheral (eye, nose) temperature in the course of the handling process (before, during, after) in shorn and sham-shorn alpacas?
2) Do changes in core body and peripheral temperature during shearing/sham-shearing correlate with stress indicating behavior?

3. Animals, materials and methods

3.1 Experimental location and animals

The data was collected at the facilities of Pacomarca in the region of Puno, Peru. Pacomarca is an experimental alpaca ranch founded by one of the main fiber producers in Peru, which is comprised of the two fiber companies INCA TOPS S. A. and INCALPACA TPX. Pacomarca raises about 2050 animals and the facilities are located at 4060 m altitude. The goal of the scientific research facility is to improve alpaca genetics and to spread their expertise and techniques to smaller alpaca farmers and fiber producers in the Andes. They also aim to enhance the economic sustainability of alpaca farming by improving fiber quality in order to enable alpaca famers to reach better market prices (https://pacomarca.com/es/).

For this experiment, 42 pregnant female Huacaya alpacas were used (41 with white coat color (B), 1 with beige coat color (LFX)). They were aged between 2 to 9 years (mean ± SD: 3.9 ± 2.1 years). All females had been shorn at least once in their lives and were thus acquainted with the procedure. They were in good general health condition with no external lesions, lameness or signs of acute physical pain.

3.2 Experimental design and procedures

The 42 alpacas were randomly allocated to a 'Shear' (n = 32 alpacas) and a 'Sham' group (n = 10 alpacas). The 'Shear' group underwent the complete shearing process whereas the 'Sham' group experienced the same handling procedure but not the actual shearing.

The experiment was conducted on the 23rd of April 2021 in a wind still and roofed barn. Excluding wind and solar radiation from the experimental set-up was especially important in order to prevent distortion of the infrared temperature measurements (Church et al., 2013; see 5.3). Data collection took place during one day between 8:20 am and 2:15 pm local time (GMT-5) to ensure a standardized procedure carried out under comparable environmental conditions.

In order to conduct the experiment, the alpacas were gathered in a lairage area next to the shearing barn (*Figure 1*: 1.) from which they were singled out and individually moved to the shearing area (*Figure 1*: 2.) where they were first restrained by humans in order to measure their fiber length and be roughly combed (*Figure 1*: 3.), a usual procedure before shearing. At this point, their first rectal temperature was taken along with the first infrared images (referred to as 'Pre' shearing phase). The alpaca was then lifted by two assistants, laid down on a shearing mattress and tied horizontally with ropes to the two opposite ends of the shearing area (*Figure 1*: 4.). The second temperature measurements (rectal, infrared) were taken right before shearing (in the 'Shear' group) or the supposed start of shearing for the 'Sham' group (further referred to as 'Start' phase). Shearing was carried out using an electromechanical shearing machine which had been installed over the shearing mattress, hanging from the roof. Temperatures were taken again in the middle of shearing/sham-shearing (referred to as 'Mid' phase) and at the end of shearing/sham-shearing (referred to as 'End' phase). After shearing/sham-shearing, the alpaca was again carried back by two assistants to point 3. (*Figure 1*) where the last temperature measurements took place (referred to as 'Post') and then released back to the lairage area (*Figure 1*: 5.). Throughout the experiment, the same six people handled the alpacas.

Aside from the above-mentioned experiment, pictures of 20 non-restrained alpacas in a roofed barn were taken in August 2021 at the facilities of Pacomarca in order to obtain a different baseline situation where animals are not handled and in close contact with humans. This was done because it was hypothesized that IR temperature measurements of non-confined alpacas might present lower baseline temperatures as they are not yet stressed by the capturing and handling process which is part of shearing.

3.3 Data collection

In order to show temperature differences before, during and after the shearing process, infrared temperature and rectal temperature were measured in parallel at 5 different points in time before, during and after the process in order to detect changes in temperature. Table 2 shows at which points in time both (IRT and rectal temperature) were measured. Additionally, video recordings were taken to assess behavior.

Rectal temperature

Rectal temperature was measured with a digital thermometer.

IR measures

A FLIR E6xt Wifi camera was used to take the infrared images of the alpacas with an FOL 7mm objective from approximately 1 meter distance. The camera also measured the ambient temperature and was set to a general emissivity of 0.98.

Video recordings

All animals were filmed with a camera (Nikon D3300) during the entire process from the same perspective in order to assess their behavior. The camera was placed in front of the shearing mattress (*Figure 1*: 6.). This position allowed to observe the entire body of the animal. The camera also recorded sound in order to assess vocalizations.
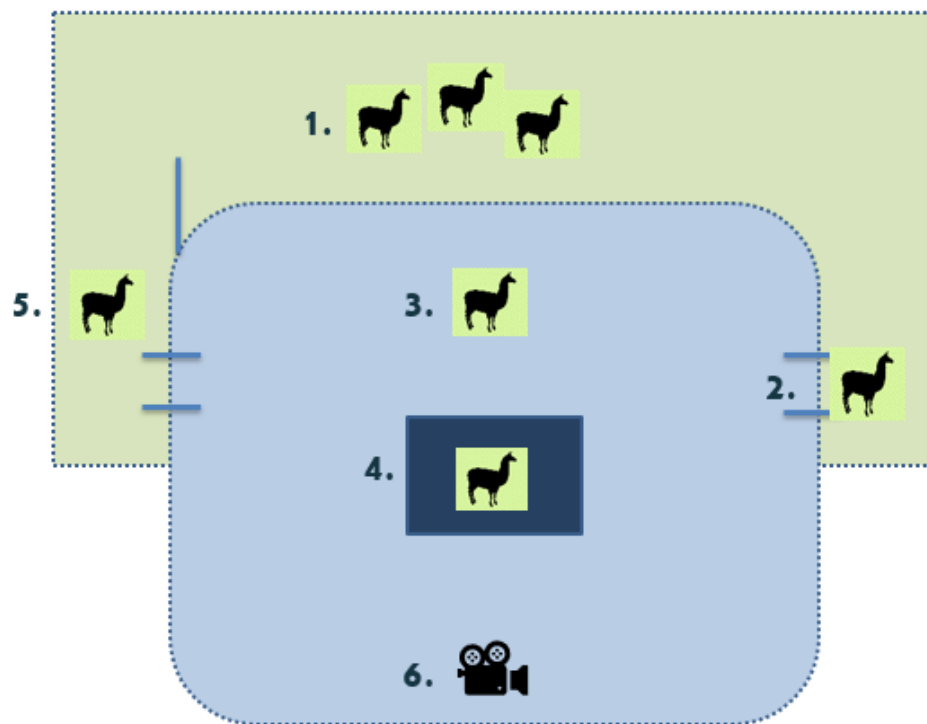
Figure 1 Experimental set-up: 1. Lairage area 2. Entrance to shearing area 3. Handling before/after shearing 4. Shearing mattress and place of shearing/sham-shearing 5. Exit of shearing area 6. Position of video camera

Table 2 Description of the five different measurement points before, during and after shearing and types of measurements taken

Measurement	Name of measurement	Type of action	Type of measurement
1	Pre (baseline)	Before shearing: the alpaca is already captured and standing while being held by a human and handled for about 30-60 seconds (coat combing and rough cleaning, measuring of fiber length)	IR temperature of both eyes (left and right) and nose is measured from approx. 1 m distance; rectal temperature is taken
2	Start	Start of shearing: the alpaca is tied and on the shearing mattress; after the measurements, shearing starts on the right side of the animal	IR temperature of right eye and nose; rectal temperature
3	Mid	Half of the shearing is done/one side of the animal is shorn; measurements are taken before the alpaca is turned to the left side	IR temperature of both eyes (right and left) and nose, rectal temperature
4	End	End of shearing: the entire animal is shorn, still tied and on the shearing mattress	IR temperature of left eye and nose; rectal temperature
5	Post	The alpaca is standing again after being released from its ties, still retained by a human	IR temperature of both eyes and nose; rectal temperature

Pictures of the non-confined alpacas were taken with the same camera and technical settings but from a distance of several meters.

3.4 Processing of temperature data

During the experiment, the rectal temperature and the digital codes of the infrared images were noted in an excel table.

Rectal temperature data could be analyzed without further processing. The infrared temperatures of eyes and nose needed to be determined with FLIR Tools, an application of the infrared camera in order to identify the temperature of a certain body part. An ellipsis was first drawn around the entire eye to capture the location of maximum eye temperature. As it was mostly located in the area of the *Caruncula lacrimalis*[1] (CL), a second ellipsis was drawn from approximately the medial corner of the CL until half of the eye to

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[1] The *Caruncula lacrimalis* is a conjunctiva in the inner corner of the eye and has rich capillary beds which are innervated by the sympathetic system and respond to changes in blood circulation (Stewart et al., 2007)

identify the warmest point in that area *(Figure 2)*. In most cases, the temperature of the *Caruncula lacrimalis* was equal to the maximum temperature of the entire eye. For further statistical analysis, only the maximum temperature of the entire eye was considered which was at or near the *Caruncula lacrimalis* in about 80% of the cases.

In order to systematically determine the temperature of the right and left nostril as well as the triangle of flew and nose (further referred to as flew), an ellipsis was first drawn around both nostrils and slightly under the intersecting triangle (see *Figure 4*). Most of the time, the maximum temperature was within the triangle. To find out the temperature of both nostrils, two smaller elliptic circles were placed within the first ellipsis, so that they touched borders with each other, and the first ellipsis and maximum temperatures were noted. In a later step, changes in temperature between the different measurement time points (Pre, Start, Mid, End, Post) were calculated for all anatomical locations (rectum, eyes, nostrils, flew) by subtracting e.g., the temperature measured at 'Start' from the temperature measured at 'Pre'. This resulted in positive values when there was an increase and negative values when there a decrease in temperature. As the measurement time point 'End' was missing for the right eye, the temperature change could only be calculated between the time points 'Mid' and 'Post', subtracting the temperature measured at 'Post' from the temperature measured at 'Mid'. For the left eye, the same temperature change calculation needed to be done between the measurement time point 'Pre' and 'Mid' as the measurement time point 'Start' was missing. Therefore, the measured temperature at 'Mid' was subtracted from the measured temperature at 'Pre'.
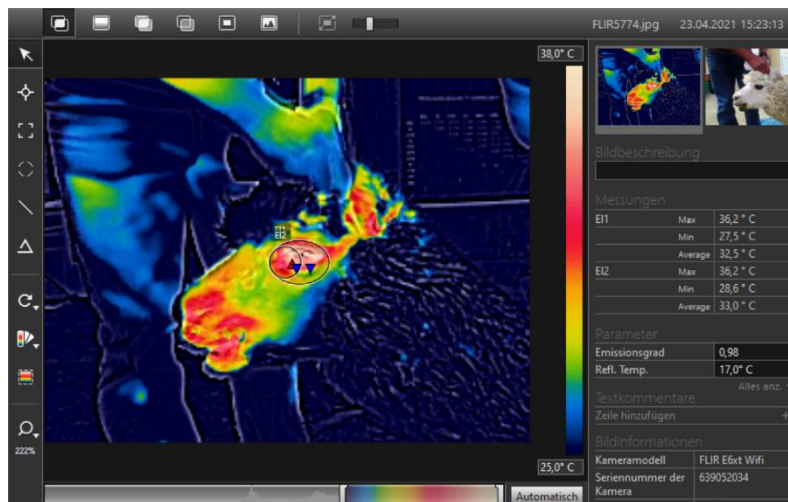

Figure 2 Example of analyzing the maximum eye temperature with FLIR Tools program (alpaca 42, ear tag 16913, left eye, during 'Mid' phase)
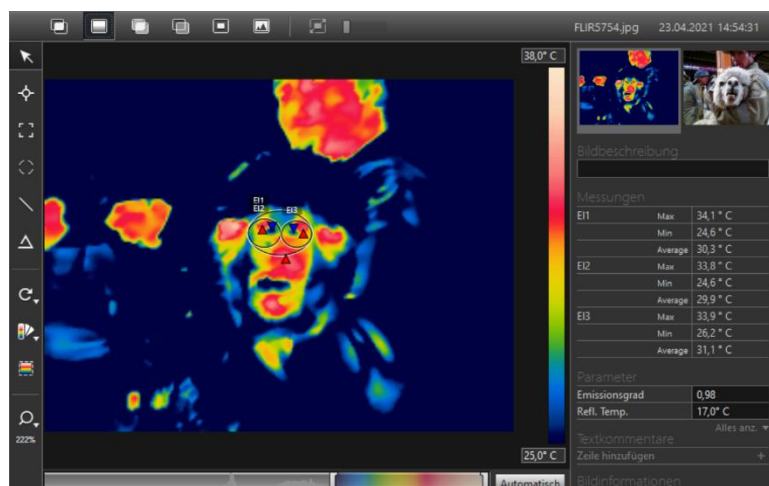
Figure 3 Example of analyzing the maximum nose temperatures; right nostril, left nostril and flews (E1: flew, E2: right nostril, E3: left nostril of alpaca 41, ear tag 08917 during the 'Pre' phase)

Figure 4 The red arrow indicates the location of 'flew'

3.5 Behavioral analysis

Out of 42 videos, only 26 could be saved for analysis due to technical problems during data transfer or battery failure. Three out of the remaining 26 videos could only be partly analyzed because in one video the 'Post' phase and in two other videos the 'End' and 'Post' were missing as the video recordings were interrupted before the end of the experimental procedure.

In order capture stress-indicating behaviors during shearing/sham-shearing, recorded videos were analyzed with the event logging software BORIS according to the following ethogram *(Table 3)*. All video observations were continuous, with total video durations of 4.5 to 10 minutes for the 'Shear' group (mean ± SD: 8.2 ± 1.8 min) and 5.5 to 7 minutes for the 'Sham' group (mean ± SD: 6.5 ± 1.5 min).

Table 3 Ethogram used for the video analysis and phases dividing the entire 'Shear'/'Sham' procedure, for which behavior analysis was carried out

Vocalization	
Behavior	**Definition**
Screaming	Loud, sharp calls, without previous growls
Alarm call	High pitched 'hee haw' (donkey-like) sound
Defense movements	
Escape attempt	Animal lifts both front legs at once or suddenly steps/jumps to the side/back
Reluctance to move	Animal refuses to move even though it is dragged/pulled by a human (e. g. by putting its weight in the opposite direction)
Moving legs (when tied)	Pulling/twitching of the legs when tied by fixating ropes during shearing
Lie down	Animal lies down on its belly/ lets itself fall down before or after shearing
Behavior of the head	
Warning spit	Animal spits air and/or a little saliva towards a human which makes a 'pfff' sound
Vomiting spit	Animal spits regurgitated stomach contents of green-brown

	color
Ears pinned back	Pinned back ears: 90° pinned back compared to 'normal' upright position
Out of sight ears	The ear position is not visible
Phases	
Pre phase (until Start)	Starts from the beginning of the video and ends when shearing machine starts ('Shear' group) or people step away from the tied animal ('Sham' group)
Start phase (Start to Mid)	Starts when the second measurements are taken and ends with animal lying on its belly before being turned
Mid phase (Mid to End)	Starts when animal is turned from left to right side and after temperatures are taken and ends when shearing machine is turned off/temperatures are measured again
End phase (End to Post)	Starts when shearing machine is turned off/temperatures are measured again and ends with animal being released from the ties, standing again
Post phase (remaining time)	Starts when animal is standing again and ends with end of video/when animal is released back to the barn

The behaviors 'screaming', 'alarm call' and 'ears pinned back'/'out of sight ears' were recorded as state events, considering not only their frequency but also duration, whereas all the other behaviors were recorded as point events (only considering frequencies). Due to low occurrence, the behaviors 'Escape attempt', 'Reluctance to move' and 'Lie down' were merged into 'Defense behavior' while 'Warning spit' and 'Vomiting spit' were combined into 'Spit'. The time of the event 'out of sight ears' was subtracted of each phase to obtain the visible amount of 'ears pinned back' in each phase.

All behavior variables were then listed per animal and phase. In order to be able to compare the occurrence of the different behaviors between measurement time points and treatment groups and accounting for the fact that phase durations slightly differed between animals, all results were expressed using a common denominator considering the respective phase duration. For that purpose, the number of a certain behavior observed per phase was divided by the respective phase duration in seconds and then multiplied by 60 to obtain the frequency of behavior per minute. For state events (e.g., scream duration), the average duration per minute of observation was again divided by 60 and multiplied by 100 to obtain the percentage of time performing the behavior.

In a later step, it was the goal to determine associations between the occurrence of certain behaviors in the different phases (e.g., 'Pre' shearing to 'Start' of shearing) and the respective change in temperature which had taken place during a phase. For that purpose, for the right eye, the weighted average for 'screams/minute', 'scream duration' and 'ears pinned back duration' from the 'Mid' to 'Post' phase needed to be calculated as no temperature measurement was taken at the time point 'End' (end of shearing). For the left eye, the same procedure needed to be applied for the 'Pre' to 'Mid' phase as there was no temperature measurement of the left eye for the measurement point 'Start' (start of shearing).

3.6 Inter- and Intra-Observer Reliability
As repeatability of data analysis is important to apply a method like infrared thermography in future studies, an intra-observer as well as an inter-observer reliability test with another person were conducted approximately two weeks after the pictures were analyzed. A set of 54 pictures of infrared images was

randomly selected using the 'random' function in Excel and thus, about 10% of left/right eye and nose pictures were selected for the reliability test. Before the reliability test was conducted, the methods to obtain the temperatures were discussed with the other person (as described in chapter 4.4) and presented in a short manual as well as within the FLIR software. The observer followed the previously discussed instructions independently. The same set of pictures was analyzed again by the author to then correlate the obtained temperature values across observers (inter-observer reliability) and within the author (intra-observer reliability) with Excel's Pearson correlation. For left and right eye, the inter-observer correlation coefficient was r=0.99 whereas for the nose correlations ranged from r=0.94 for the left nostril, over r=0.95 for the flew to r=0.97 for the right nostril. Respective intra-observer correlation coefficients were r=0.99 for both eyes, r=0.96 for the left nostril, r=0.94 for the flew and r=0.96 for the right nostril.

For the assessment of reliability of behavior analysis, 7 videos were randomly selected (again via Excel's random function) and Cohen's kappa coefficient was calculated using the event logging software BORIS with a buffer time frame of one second when comparing the coded events. For inter-observer reliability, kappa values ranged between K=0.38 and 0.79 whereas intra-observer values were between K=0.46 and 0.61. Inter- and intra-observer values can be considered sufficient and of moderate strength if K ≥ 0.4 (Landis and Koch, 1977).

3.7 Data analysis

Due to low occurrence, the behaviors 'spitting' and 'alarm call' were not statistically analyzed. All other statistical calculations were done using the SAS/STAT statistical analysis software version 9.4. For the measures rectal, eye, nose and flew temperature as well as scream frequency and duration and defense behavior a linear mixed model (proc mixed) was used including group (Shear or Sham), measurement time point (further referred to as phase) and the interaction of group and phase as fixed effects and the individual animals as repeated measures. As covariance structure, the autoregressive structure (ar(1)) was chosen as it better reflected the relation between the data. AR(1) considers homogenous variances and correlations which decrease exponentially with distance. Such an observation can be expected for temperatures as consecutive and dependent measures as temperatures measured in shorter time intervals will result in closer values as temperature measurements that lie further apart in time. Alternatively, for rectal temperature and the behavior 'defense', the standard covariance structure (vc) was chosen due to an improved AIC value (Akaike Information Criterion). Additionally, for the core/peripheral temperature and behavior variables (except 'ears pinned back') the ambient temperature was considered as a continuous covariate in the model.

Model residuals were graphically checked for normal distribution. In case normal distribution of residuals was not achieved (behaviors 'moving legs when tied' and 'ears pinned back'), a generalized linear mixed model (proc glimmix) was used considering the same fixed and repeated measures effects as described above (with covariance structure vc). For 'moving legs when tied' the effects of the interaction between group and phase and ambient temperature could not be included in the model due to convergence problems.

Partial correlation coefficients were calculated taking the repeated measures within animals into account to analyze associations between the different temperature measures as well as between behaviors and temperature response variables. For associations between temperature measures, both absolute temperatures and changes in temperatures were considered. For the correlation with behavioral variables, the relative time spent screaming and the frequency of defense behaviors as well as the changes in temperature (e.g., 'Pre' to 'Start', 'Start' to 'Mid' shearing etc.) were considered.

P-values < 0.05 were considered significant and 0.05 < p ≤ 0.1 as tendency.

4. Results

4.1 Core body temperature

As described in Chapter 4.3, rectal temperature was measured for all 42 animals (Shear: n=32; Sham: n=10) at five different points in time before (Pre), during (Start, Mid, End) and after (Post) the shearing/sham-shearing process (see *Figure 5*). Variability was much higher within the 'Shear' group, with rectal temperature ranging between 36.9°C pre-shearing and 38.8 °C post-shearing, while temperature of sham animals ranged between 37.7°C (Pre) and 38.1°C (Post). Mean temperature was significantly higher in the 'Shear' (LSM=38°C, SEM=0.02) than in the 'Sham' group (37.8°C, 0.04) (p<0.001). However, there was no statistically significant effect for neither time point (p=0.880) nor the interaction of time point and group (p=0.872). Numerically, in the 'Shear' group, mean temperature slightly increased from pre- to post-shearing. For the 'Sham' group, mean temperature values rather remained stable. Additionally, an increase in ambient temperature of 1°C resulted in an increase in rectal temperature of 0.08°C which was significant (p<0.001) under the final model.
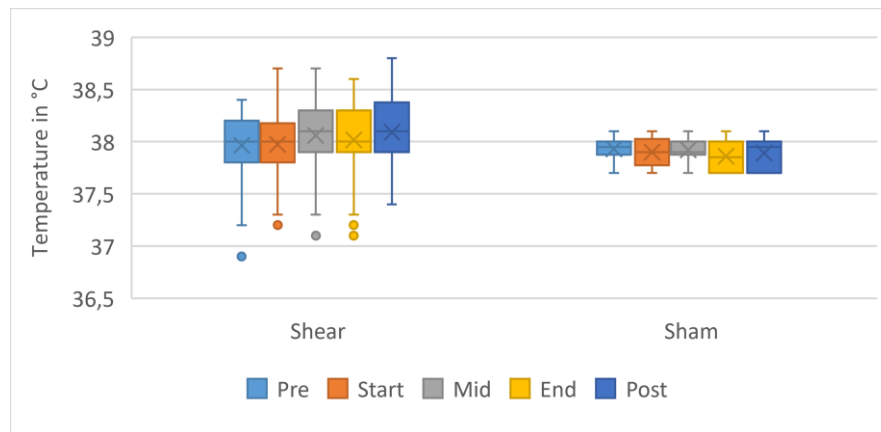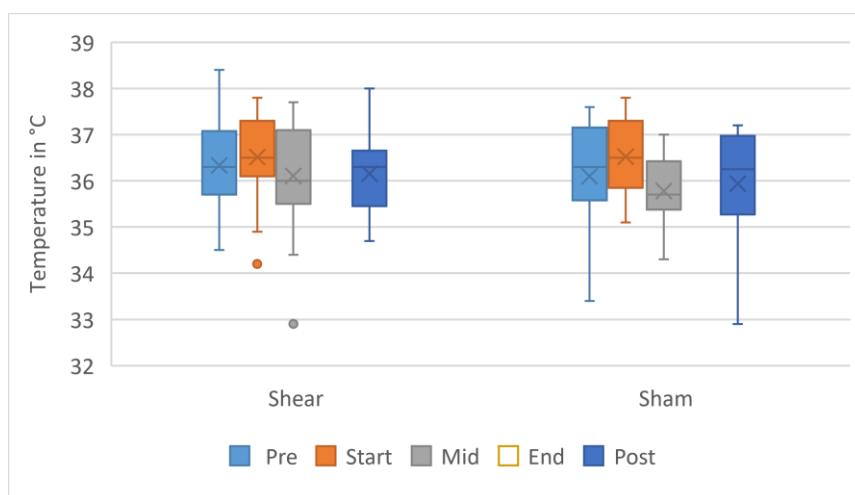

Figure 3 Rectal temperature in °C before (Pre), during (Start, Mid, End) and after (Post) shearing (Shear: n=32) and sham-shearing (Sham: n=10)

When considering the change in temperature between the measurement time points, in both groups rectal temperature remained unchanged, increased or decreased and again variability in these changes was higher in the 'Shear' than in the 'Sham' group, ranging between -0.3 and 0.5 °C ('Sham': -0.2 to 0.3 °C). While overall groups did not differ (p=0.192) and no interaction (p=0.903) or effect of ambient temperature were found (p=0.088), there was an effect of time point (p=0.005). While rectal temperature remained on average largely unchanged during the 'Pre' to 'Start' (*Figure 6*), after the start of shearing/sham-shearing core body temperature increased on average by 0.05°C, followed by a drop of -0.05 and again an increase of 0.05°C.
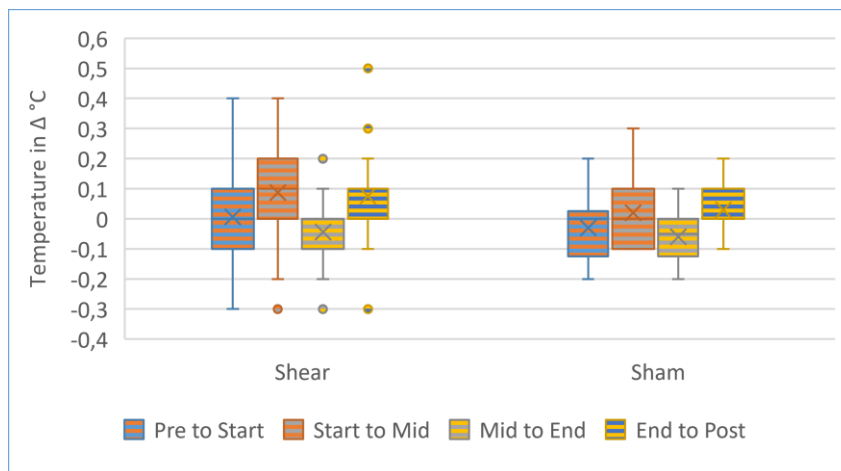
Figure 4 Change in rectal temperature (Δ °C) between phases 'Pre' to' Start', 'Start' to 'Mid', 'Mid' to 'End' and 'End' to 'Post' for 'Shear' (n=32) and 'Sham' (n=10)

4.2 Peripheral temperature

Right eye

For the right eye, temperatures were measured before shearing/sham-shearing (Pre), at the start (Start), in the middle (Mid) and after it (Post) (see *Figure 7*). The temperature variability of the right eye was quite high in both groups (Shear: 32.9°C-38.4°C; Sham: 32.9°C-37.8°C) but the general trend of temperature development between measurement time points was comparable. Hence, no significant difference between groups could be detected (p=0.196) and no significant interaction was found (p=0.818). There was a significant effect of the time point (p=0.018) which was mainly driven by an increase in mean temperature at 'Start' compared to 'Pre', followed by a decrease below 'Pre' values at 'Mid'; mean temperature then remained stable. This trend was similar for both treatment groups. Ambient temperature had a significant effect on right eye temperature (p=0.001) accounting for an increase of 0.15°C per 1°C increase in ambient temperature in the final model.

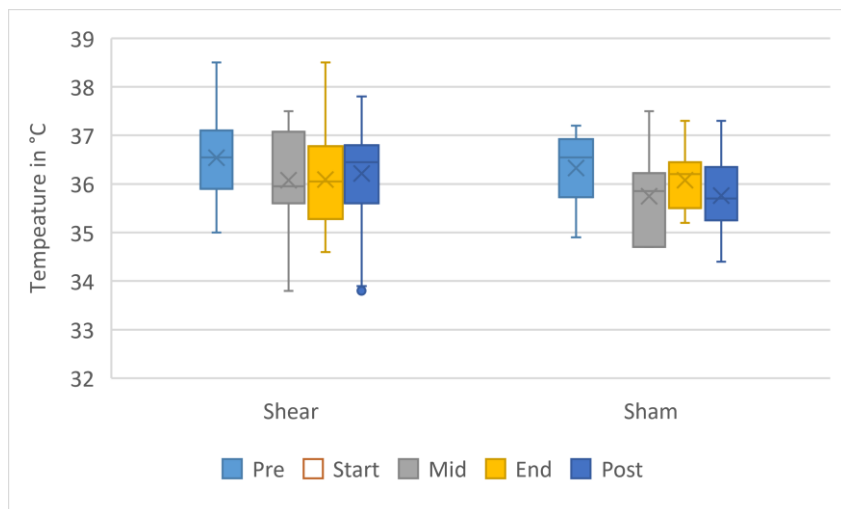*Figure 5 Right eye temperature in °C before (Pre), during (Start, Mid) and after (Post) shearing (Shear: n=32) and sham-shearing (Sham: n=10)*

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When comparing the change in right eye temperature between measurement time points (*Figure 8*), the time point had a significant effect (p=0.006) on the temperature changes. While right eye temperature slightly increased from 'Pre' to 'Start' (0.3°C), this was followed by a more pronounced decrease (-0.56°C) during the phase 'Start' to 'Mid', with almost no further change from 'Mid' to 'Post'. Group (p=0.873), interaction of group and time point (p=0.483) and ambient temperature (p=0.976) had no effect.
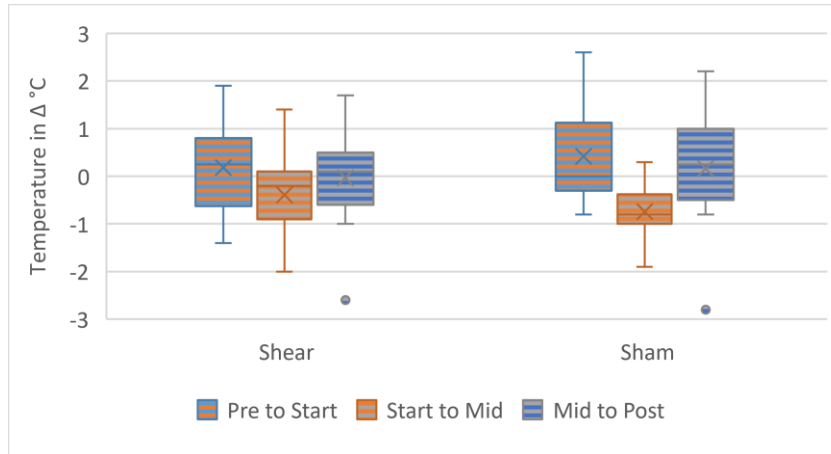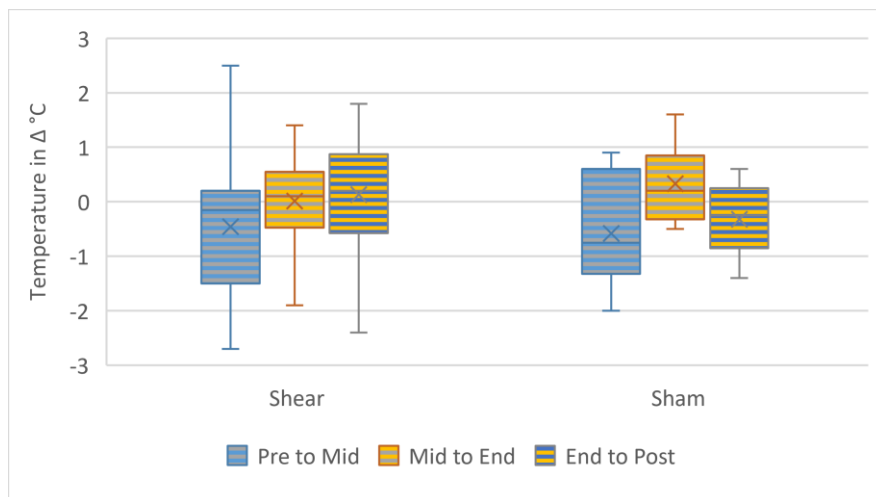

Figure 6 Change in right eye temperature (Δ °C) between phases 'Pre' to 'Start', 'Start' to 'Mid', 'Mid' to 'Post' for 'Shear' (n=32) and 'Sham' (n=10)

Left eye

For the left eye, temperatures were measured before the shearing/sham-shearing process (Pre), in the middle of it (Mid), at the end (End) and after (Post) for both groups (see *Figure 9*). Left eye temperature variability was rather diverse but stronger in the 'Shear' (min=33.8°; max=38.5°C) than 'Sham' group (min=34.4°C; max=37.5°C). The measurement time point tended to affect left eye temperature (p=0.068), with a slight decrease between 'Pre' and 'Mid', but only minor changes in later phases. There was no effect of group (p=0.157), interaction of group and time point (p=0.511) or ambient temperature (p=0.480).

Figure 7 Left eye temperature in °C before (Pre), during (Mid, End) and after (Post) Shearing (Shear: n=32) and sham-shearing (Sham: n=10)

Figure 10 shows that variability in the left eye temperature changes was greater in the 'Shear' (-2.7°C to 2.5°C) than 'Sham' group (-2.0°C to 1.6°C). Again, only the time point had a significant effect on the changes in temperature (p=0.044). There was on average a drop in left eye temperature from 'Pre' to 'Mid' (-0.52°C), and less change was observed from 'Mid' to 'End' and 'End' to 'Post'. Although 'Sham' animals numerically showed a slightly more pronounced average change in temperature during the latter phases, there was neither a significant difference between groups (p=0.543) not an interaction between time point and group (p=0.441). There was also no effect of ambient temperature (p=0.781).

Figure 8 Change in left eye temperature (Δ °C) between phases 'Pre' to 'Mid'', 'Mid' to 'End', 'End' to 'Post' for 'Shear' (n=32) and 'Sham' (n=10)

Right nostril

In general, nostril and flew temperatures were in a lower temperature range than the eyes with minimum values below 29°C. Nevertheless, variability was similarly high within groups. For the right nostril, temperatures ranged between 26.8°C to 35.7°C (Shear) and 27.9°C to 35°C (Sham) (see *Figure 11*). Mean temperature was significantly higher in the 'Shear' (LSM=32.3°C, SEM=0.16) than in the 'Sham' group (31.3°C, 0.30) (p=0.009). The time point tended to affect right nostril temperature (p= 0.068) as in both groups mean temperature constantly decreased until the middle of shearing/sham-shearing, after which

temperature started to increase back towards baseline again. It is noteworthy, that while there is a temperature drop between 'Pre' to 'Start' in the 'Shear' group, temperature remains the same between those points for the 'Sham' group. After that, the pattern is similar between the two groups. However, the interaction between time point and group was not significant (p=0.745). Ambient temperature had a significant effect on right nostril temperature (p=0.011) resulting in an increase of 0.18°C per 1°C ambient temperature.
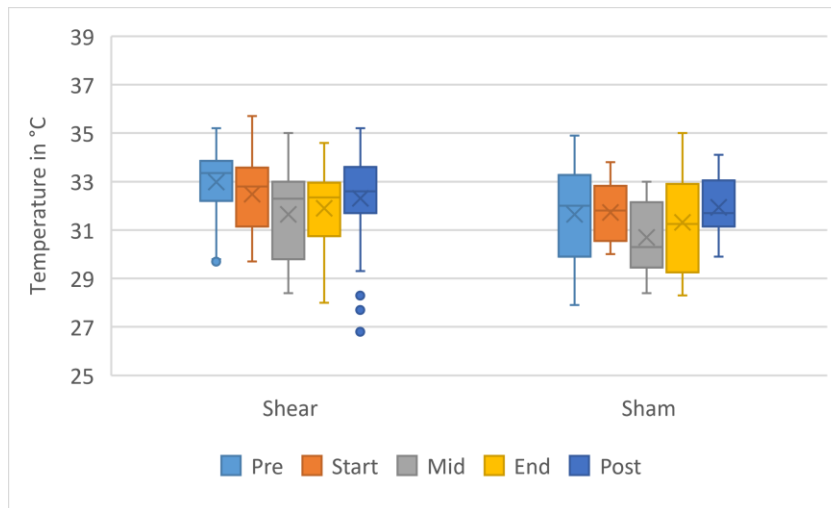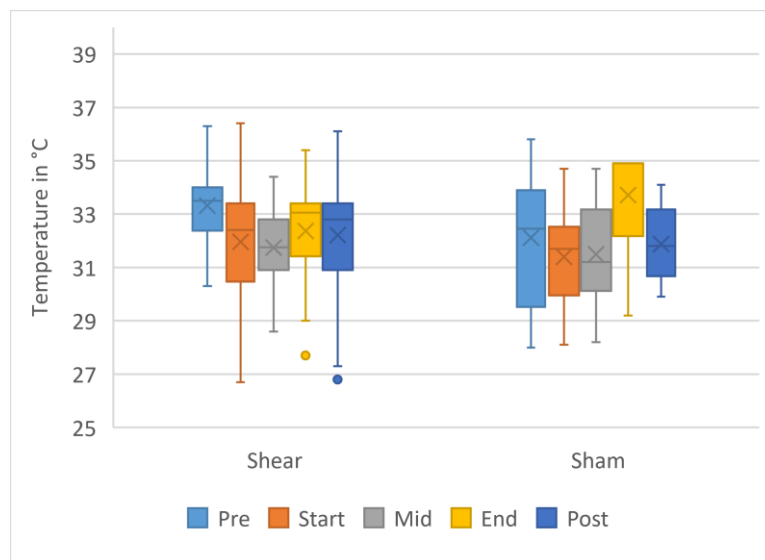

Figure 9 Right nostril temperature in °C before (Pre), during (Start, Mid, End) and after (Post) shearing for 'Shear' (n=32) and 'Sham' (n=10)

Regarding the change in right nostril temperature between time points, variability was higher in the 'Shear' group (-5.1 to 6.2°C) than 'Sham' (-3.2 to 5.4°C) (*Figure 12*). While overall groups did not differ (p=0.559), the time point had a significant effect (p=0.003) with slight decreases in temperature between 'Pre' and 'Start' as well as 'Start' to 'Mid' and slight increases for the phases 'Mid' to 'End' and similarly 'End' to 'Post'. Although those changes appear to be more pronounced in the 'Sham' group for 'Start' to 'Mid' and

End' to 'Post', there was no effect of the interaction of group and time point (p=0.920). Also, the ambient temperature did not affect the change in right nostril temperature (p=0.096).
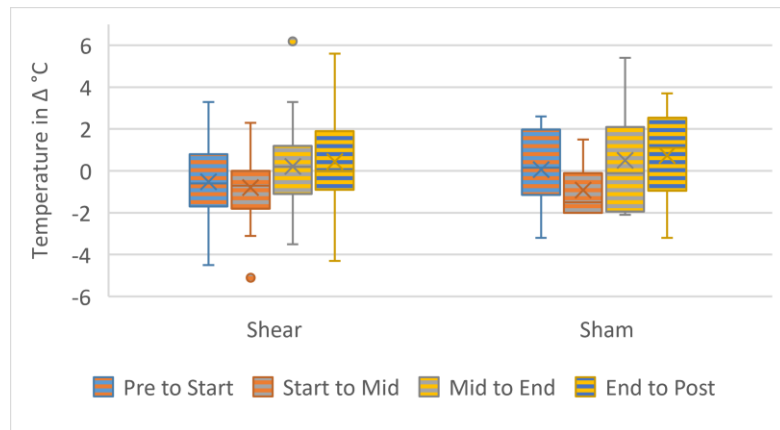
Figure 10 Change in right nostril temperature (Δ °C) between phases 'Pre' to 'Start', 'Start' to 'Mid', 'Mid' to 'End', 'End' to 'Post' for 'Shear' (n=32) and 'Sham' (n=10)

Left nostril

Left nostril mean temperatures behaved comparably to right nostril temperatures except for an exceptionally high temperature rise within the 'Sham' group at the end of sham-shearing (see *Figure 13*). This outlier leads to a significant interaction between group and time point (p=0.050). Temperature variability is again quite high for both groups, but higher for 'Shear' (Shear: 26.7°C to 36.4°C; Sham: 28°C to 35.8°C). Ambient temperature had a significant effect (p=0.005) with a 1°C increase in ambient temperature leading to a 0.22°C increase in left nostril temperature.

Figure 11 Left nostril temperature in °C before (Pre), during (Start, Mid, End) and after (Post) shearing for 'Shear' (n=32) and 'Sham' (n=10)

Patterns of change in left nostril temperature differed between groups (see *Figure 14*), leading to a significant interaction between group and time point (p=0.027). While in both groups temperature on average dropped between 'Pre' and 'Start' and then remained stable, there was with around 2°C a higher increase between 'Mid' and 'End' in the 'Sham' than in the 'Shear' group. For 'End' to 'Post' again groups

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differed as left nostril temperature did not change much for 'Shear' animals, whereas there was a substantial decrease in mean temperature change of around -2°C for sham-shorn alpacas. This was the only peripheral area where ambient temperature had an effect on changes in temperature (p=0.009), resulting in 0.14°C change in left nostril temperature per 1°C increase in ambient temperature.
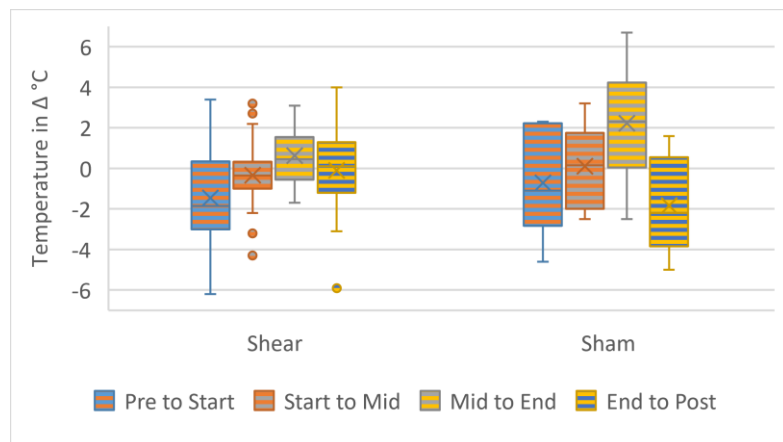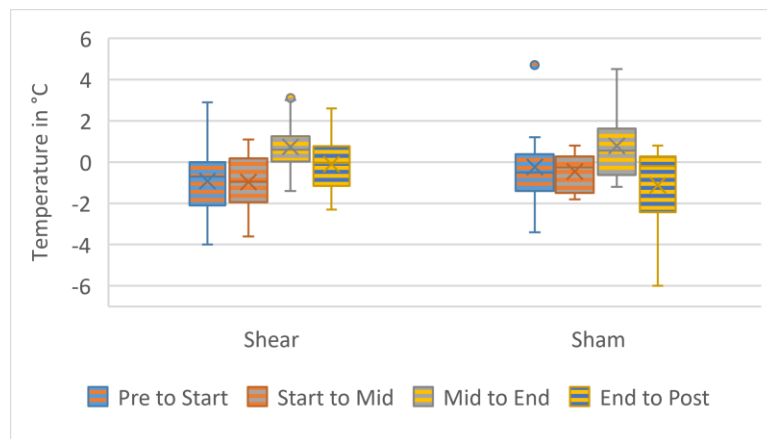

Figure 12 Change in left nostril temperature (Δ °C) between phases 'Pre' to 'Start', 'Start' to 'Mid', 'Mid' to 'End', 'End' to 'Post' for 'Shear' (n=32) and 'Sham' (n=10)

Flew

Variability in flew temperature (*Figure 15*) was comparable to that of the nostrils (Shear: 28.6°C to 37.4°C; Sham: 27.3°C to 35.2°C). The mean temperature tended to be higher in the 'Shear' (LSM=33.1°C, SEM=0.16) than in the 'Sham' group (32.5°C, 0.29) (p=0.082). The temperature curve was similar in shape to the right and left nostril, with mean temperatures dropping until 'Mid' and then increasing again (p=0.002). Temperatures post shearing/sham-shearing were lower than at 'Pre', especially within the
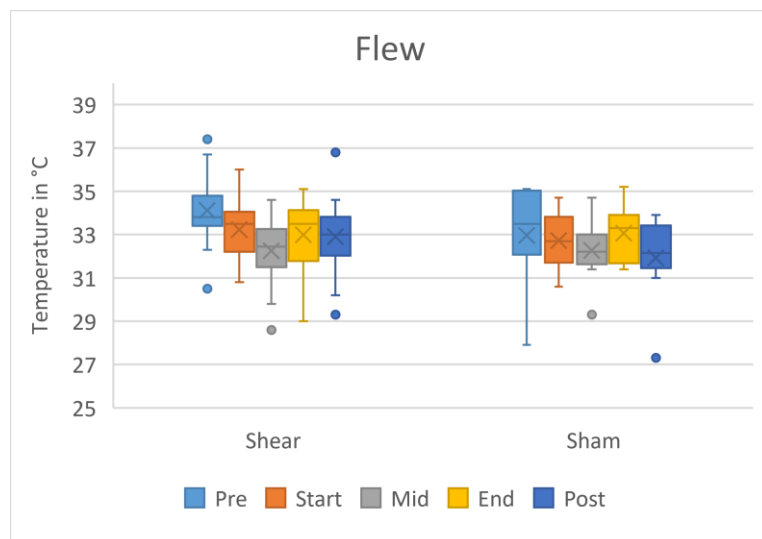
Figure 13 Flew temperature in °C before (Pre), during (Start, Mid, End) and after (Post) shearing for 'Shear' (n=32) and 'Sham' (n=10)

'Sham' group. Ambient temperature accounted for an increase of 0.12°C per 1°C increase in ambient temperature in the final model (p=0.085).

Contrary to the other areas, variability in changes in flew temperature between measuring points was higher in the 'Sham' than in the 'Shear' group, ranging between -6 and 4.7 °C (Shear: -1.4 to 3.1 °C). Patterns of change in flew temperature differed between groups (see *Figure 16*), leading to a significant interaction between group and time point (p=0.027). While in both groups temperature on average dropped between 'Pre' and 'Start' (-0.6) and then remained stable, there was a high increase between 'Mid' and 'End' (0.75°C) and a decrease between 'End' to 'Post' (-0.06). The decrease from 'End' to 'Post' was more pronounced in the 'Sham' group. Ambient temperature was not significant (p=0.281).

Figure 14 Change in flew temperature (Δ °C) between phases 'Pre' to 'Start', 'Start' to 'Mid', 'Mid' to 'End', 'End' to 'Post' for 'Shear' (n=32) and 'Sham' (n=10)

4.3 Baseline measures of non-confined alpacas

Mean eye temperatures of non-restrained alpacas in August (n=20) were 32,2 ± 1,4 °C and mean nose temperatures reached 28,8 ± 2,8 °C.

4.4 Associations between core body and peripheral temperature

Overall correlations between core and peripheral temperatures were inconsistent and rather weak (see Table 4).

Table 4 Correlations between core and peripheral temperatures for absolute temperatures (Abs.) and change in temperatures (Diff.) and respective p-values (p)

		Left eye		Right eye		Left nostril		Right nostril		Flew	
		Abs.	Diff.	Abs.	Diff.	Abs.		Abs.		Abs.	
Rectal temp.	Abs.	0.23		0.13		0.10		0.20		0.02	
	p	0.008		0.114		0.198		0.007		0.817	
	Diff.		-0.27		-0.19		-0.05		-0.04		-0.19
	p		0.079		0.224		0.598		0.691		0.033

Of all peripheral temperatures, only left eye and right nostril temperature weakly correlated with core temperature. The left eye (*Figure 17)* had a stronger positive correlation with rectal values (r=0.23),

indicating that a higher left eye temperature was accompanied by a higher rectal temperature. A positive but even weaker correlations was found for the right nostril (r=0.20). There was almost no association between right eye, left nostril, flew and rectal temperature.
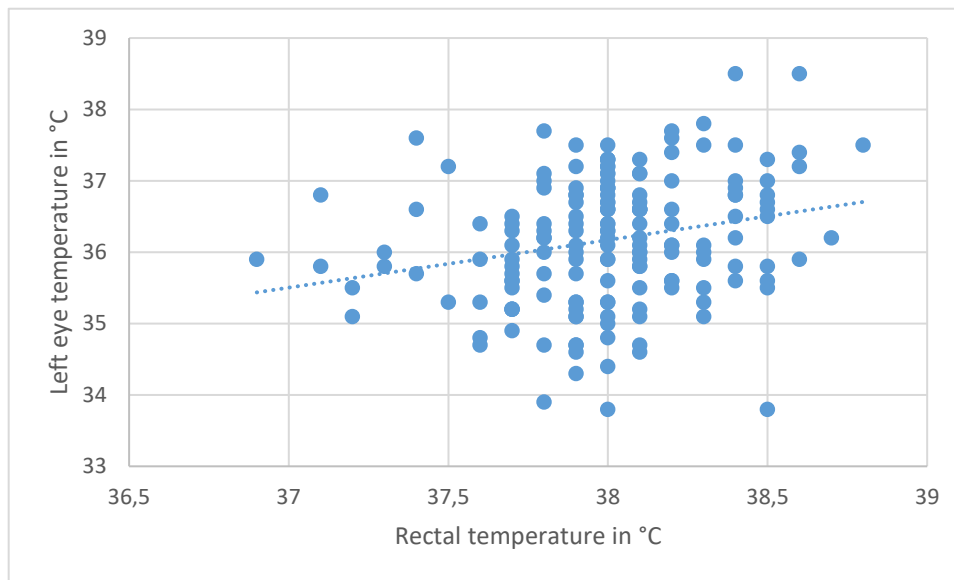

Figure 15 Correlation between rectal and left eye temperature (r=0.23) in °C for both groups (n=42) and all time points (all individual measures depicted)

When considering the changes in rectal and peripheral temperature (Table 4), only one weak negative correlation between rectal and left eye temperature was found (r= -0.27). This correlation indicates that an increase in rectal temperature was accompanied by a decrease in left eye temperature (see *Figure 18*). All other correlations had absolute values of below 0.20.
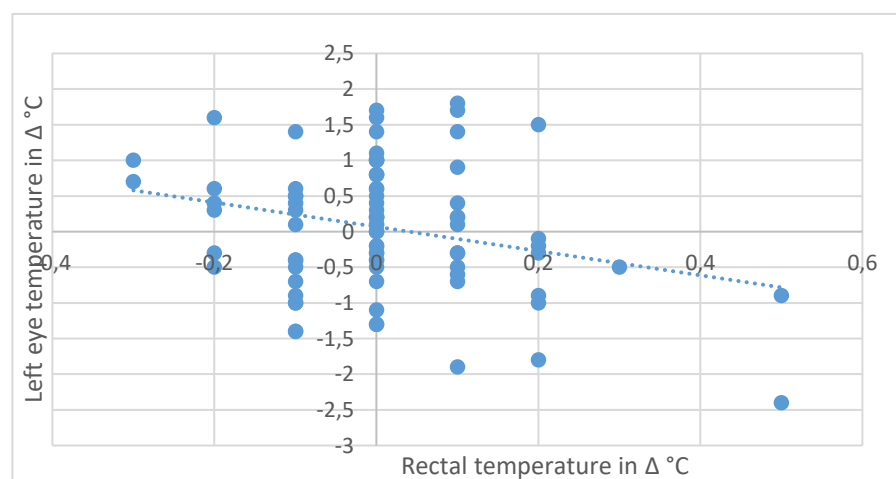
Figure 16 Correlation between changes in rectal and left eye temperature (r=0.27) in Δ °C for both groups (n=42) and all time points (all individual measures depicted)

4.5 Behavior

Screaming and alarm call

The frequency of screams/minute varied between 0 to 31.7 (Shear) and 0 to 36.7 (Sham) (see *Figure 19*). Even though variability between animals was high, most alpacas almost screamed during the entire experiment and only few were quieter. There was a significant interaction between group and measurement time point (p<0.001) due to the opposite trend of scream frequencies in each group: Mean scream frequency in the 'Shear' group increased between time points until the middle of shearing and then dropped again towards the end, whereas the opposite effect could be observed for the 'Sham' group with mean scream frequencies being highest before and after the sham-shearing process and lowest from 'Start' to 'End'. Increasing ambient temperature resulted in increased scream frequency as well (p<0.001).
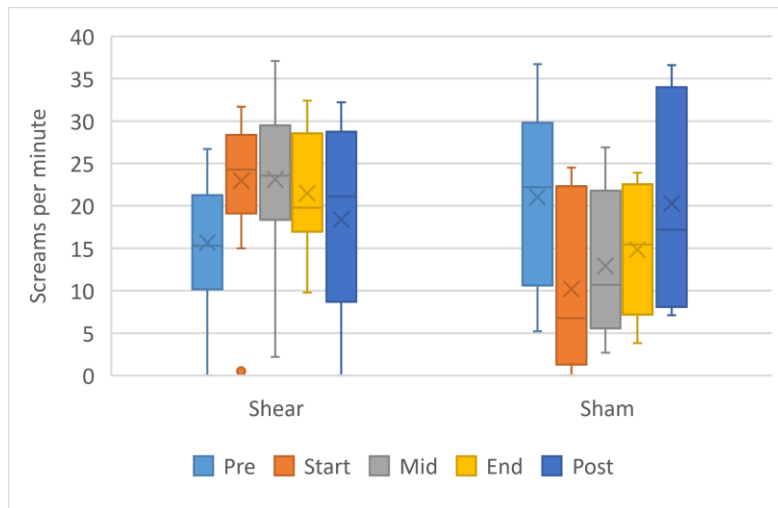
Figure 17 Screams/min before (until Start), during (Start to Mid, Mid to End) and after (End to Post, until termination) shearing/sham-shearing for 'Shear' (n=20) and 'Sham' (n=6)

A comparable pattern could be observed for scream duration (*Figure 20*) expressed as percentage of time between time points: Mean values tended to increase for the 'Shear' group until middle of shearing, followed by a drop to near baseline while the opposite happened in the 'Sham' group with mean values being high before and at the end of the experiment and decreasing during the sham-shearing (Start and

Mid) (interaction between group and time point: p=0.003). Ambient temperature had a significant effect on scream duration (p=0.046) resulting in more time spent screaming as ambient temperature increased.
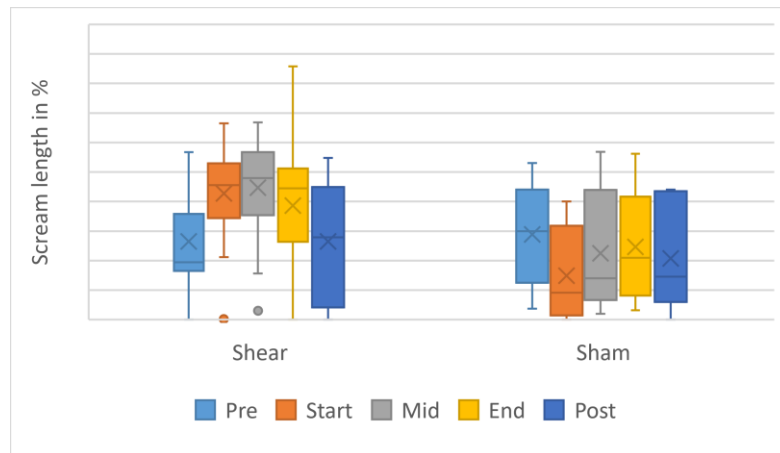

Figure 18 Time spent screaming (% of total duration of phase) before (until Start), during (Start to Mid, Mid to End) and after (End to Post, until termination) shearing/sham-shearing for 'Shear' (n=20) and 'Sham' (n=6)

The behavior 'alarm call' was analyzed separately but occurred in four animals only, which all belonged to the 'Shear' group. In those animals, alarm calls always started in the 'Mid' phase until after shearing (Post), continuously increasing the frequency of the calls, resulting in 5.1 alarm calls/min (Mid), 10.7 calls/min (End) and 16.1 calls/min (Post).

Defense behavior

Defense behavior was the combination of escape attempts, reluctance to move and trying to lie down on the belly when being handled and before being tied over the shearing mattress. Frequency of defense behavior per minute occurred again in a contrary pattern for the two groups (see *Figure 21*): While most defense in the 'Shear' group happened before and after the actual shearing, the 'Sham' group showed most of that behavior at the end of the sham-shearing process (when being handled again) and no defense movements after restraint (interaction between group and time point: p=0.007). There was no effect of ambient temperature (p=0.930).

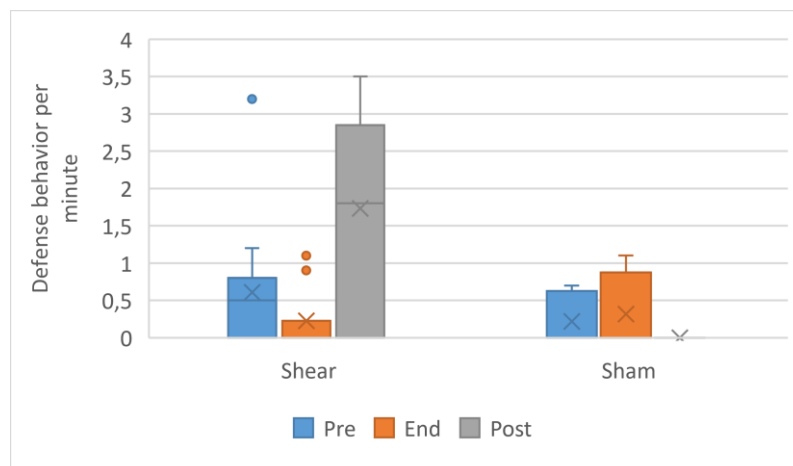*Figure 19 Defense behavior/min before (until Start), and after (End to Post, until termination) shearing/sham-shearing for 'Shear' (n=20) and 'Sham' (n=6)*

Moving legs when tied

The behavior 'moving legs when tied' only occurred during 'Pre' to 'End' as that was the timeframe the animals were tied with ropes (*Figure 22*). It occurred only once in the 'Sham' group (group: p=0.048) and there was no effect of the time point (p=0.112). For lack of convergence reasons, interaction effects between time point and group could not be analyzed.
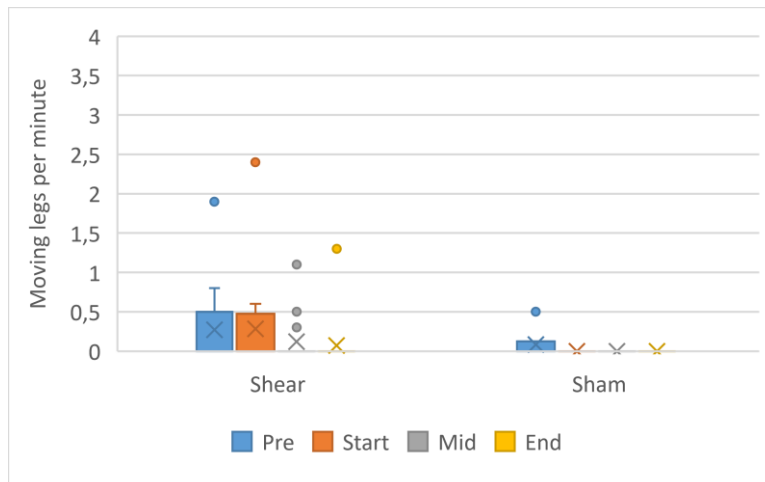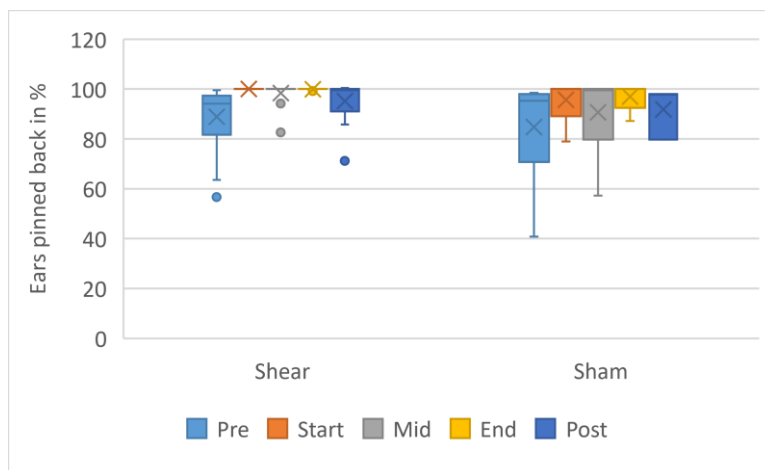

Figure 20 Moving legs/min before (until Start) and during (Start to Mid, Mid to End) shearing/sham-shearing for 'Shear' (n=20) and 'Sham' (n=6)

Ears pinned back

Ears pinned was recorded as duration and calculated in percent of time per phase with no significant differences between groups (p=0.716) and phases (p=0.843) nor the interaction of both (p=0.934) (see *Figure 23*).

Figure 21 Ears pinned back in % before (until Start), during (Start to Mid, Mid to End) and after (End to Post, until termination) shearing/sham-shearing for 'Shear' (n=20) and 'Sham' (n=6)

Spitting

11 out of 26 'Shear' animals but no 'Sham' animal was spitting during the experiment. As this behavior did not occur frequently enough, it was not possible to analyze it statistically. Most of the spitting animals were rather consistently doing so in almost all phases. Spitting was most frequent before (Pre) and at the 'Start' of shearing.

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4.6 Associations between temperature measures and behavioral measures

When correlating changes in temperature with scream frequency and defense behavior, weak positive correlations were found between scream frequency and right nostril (r=0.26), left nostril (r=0.21) and flew (r=0.28), whereas a negative correlation was found between scream frequency and left eye temperature change (r=-0.24). Table 5 shows all associations between behavior and peripheral temperature change.

Table 5 Correlations (r) between screaming/defense behavior and changes in peripheral temperature and respective p-values (p)

	Rectal temp.	Left eye	Right eye	Left nostril	Right nostril	Flew
Screams/min (r)	-0.02	-0.24	-0.01	0.21	0.26	0.28
Screams/min (p)	0.873	0.089	0.918	0.089	0.028	0.014
Defense/min (r)	0.05	-0.38	-0.05	-0.16	-0.05	-0.18
Defense/min (p)	0.270	0.811	0.063	0.835	0.489	0.379

Figure 24 shows the positive correlation for flew temperature and scream frequency (r=0.28). The higher the flew temperature, the more screams per minute occurred.
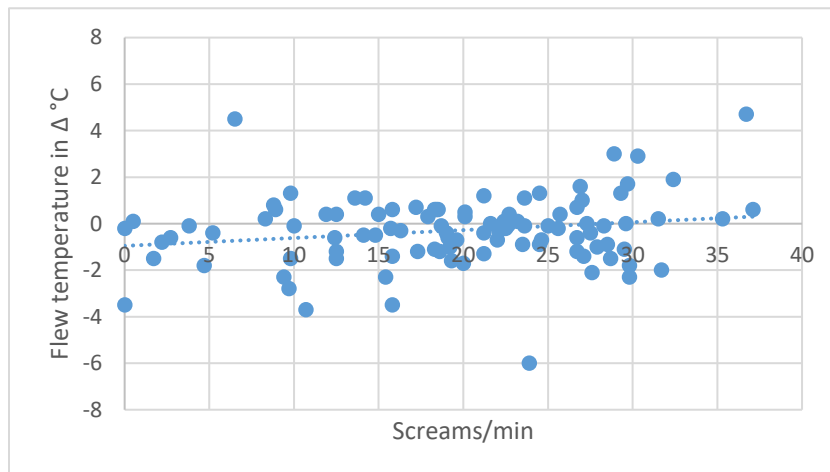

Figure 22 Correlation between changes in flew temperature (Δ °C) and screams/min (r=0.28) for both groups (n=42) and all phases (all individual measures depicted)

The seemingly stronger negative correlation between the left eye temperature change and the frequency of defense behavior was not selected to be graphically illustrated because most animals did not consistently show defense behavior throughout all shearing/sham-shearing phases. Thus, the negative tendency is driven by a few outliers which consistently showed defense behavior.

5. Discussion

5.1 Inconsistent response of core body and peripheral temperature and behavior

When considering the first research question which deals with the response of core body and peripheral temperature in the course of shearing/sham-shearing, it becomes evident that responses vary in intensity and direction as a function of treatment and measurement time point. While rectal temperature seems to be sensitive to the treatment of shearing resulting in slightly higher mean temperatures for the 'Shear' group without an effect of the measurement time point, peripheral temperatures of eyes and nose are affected by the time points (Pre, Start etc.). This shows that eye and nose temperature react to the temporal progression of the shearing/sham-shearing procedure, irrespective of the animals being shorn

or not. As a consequence, this suggests that capture, handling and restraint are already so stressful that shearing seems to have little additional effect. At the same time, rectal and peripheral temperatures only correlated very weakly (correlation coefficient between 0.20 and 0.23), thus confirming findings of Jansson et al. (2021) in horses. Nevertheless, right nostril and flew temperature reacted to the treatment effect of shearing (the 'Shear' group had a higher temperature), but not the left nostril and eyes. This could mean that nose temperature is more sensitive to stressful stimuli. However, even though the group effect was not significant for eyes and right nostril, LSM for temperature was always higher for the 'Shear' group than 'Sham', which could cautiously be interpreted as a general pattern towards higher temperature in the 'Shear' treatment.

Further, it also needs to be considered that the experimental pre-shearing conditions in April did not depict 'natural' baseline temperatures of eyes and nose. The previously mentioned IR pictures of non-confined alpacas taken in August show that the baseline situation of the first experiment did not truly reflect the situation of a non-stressed alpaca. Pre-shearing/sham-shearing temperature values of eyes and nose seem to already be quite high at the beginning of the experiment, possibly making it difficult to see a group effect and also showing a less intense temperature increase of the right eye (of around 0.2°C) or less pronounced decrease in the nose (of around 0.5-2°C). When taking IR pictures of the non-restrained alpacas, baseline values were much lower (approx. 3.8°C less for the eyes; approx. 4.2°C less for the nose). Of course, it needs to be considered that experimental conditions are not comparable, as animals, day and weather differed. Considering ambient temperature, it also needs to be taken into account that August is rather dry and cold, possibly lowering peripheral temperatures too. Therefore, the two situations should be compared cautiously. Nevertheless, the latter results are also in line with temperatures found in alpaca eyes before (35.77°C), during (37.13°C) and after (36.04°C) mechanical shearing (Cruz et al., 2020). In that experiment, temperature differences between pre-shearing conditions compared to shearing and post-shearing were higher as well (around 1°C). Pre-shearing IR pictures were taken before capturing and closely handling the animals, providing a more natural baseline value.

Moreover, the initial increase in right eye temperature matches study results found in race dogs (Elias et al., 2021), competition horses (Bartolomé et al., 2013), pigs (Rocha et al., 2019) and sheep (Sutherland et al., 2020) and could be interpreted as a stress response to the 'Start' point of shearing/sham-shearing. However, a later decrease in eye temperature resembles studies in cattle subjected to fear-tests, ACTH challenges or castration (Stewart et al., 2007, 2008, 2010). For practical reasons (side of the head visible in the tied animal), measurements of the left eye are lacking for this time point and it is therefore unclear whether a similar increase also took place in the left eye. The initial increase in right eye temperature could be interpreted as a sign of sympathetic ANS activation. When preparing for the 'fight or flight' reaction, the eyes need sufficient blood supply for enhanced vision. As the pupil dilator muscle is connected to sympathetic neurons, those are most likely activated to dilate the pupil. At the same time, as pupil size is regulated by two muscles (dilator and sphincter), it could also be that the activity of the sphincter muscle, which is innervated by parasympathetic neurons, is inhibited (Liu et al., 2017).

The initial decrease in nose temperature (Pre to Mid) could be explained by vasoconstriction during which blood circulation is rather centralized towards more important inner organs (like heart and lungs) to prepare for 'fight or flight'. Vasoconstriction in the nasal area is likely a result of stress in the form of handling and shearing/sham-shearing as the drop in temperature was consistent for all three nasal locations (both nostrils and flew) and is also in line with several studies in rhesus monkeys (Nakayama et al., 2005; Kuraoka & Nakamura, 2011). There are no comparable studies in ungulates, yet.

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Concerning the behavioral indicators of stress, there was an opposite trend in 'Shear' and 'Sham' animals across the different phases which can be attributed to a differential reaction to the actual wool cutting which only 'Shear' animals experienced. It is noteworthy, that while pre- and post-shearing behavioral expressions are comparable for both groups, start of shearing/sham-shearing until the end develops differently and in opposite directions: stress-indicating behaviors substantially increase in the 'Shear' group whereas the contrary happens for the 'Sham' group resulting in a pronounced decrease once they have been tied to the mattress. This suggests that shearing itself does irritate or additionally stress the animal, an observation in line with Wittek et al. (2017) and Waiblinger et al. (2020). When they subjected alpacas to different restraint methods in sham-shearing and shearing conditions, vocalizations and defense behavior also appeared to be higher in the shorn group of animals. However, it was not possible to quantify this group difference as they did not directly compare the two groups. In the videos prepared for this thesis, it is apparent that alpacas increased their vocalizations and defense behavior as soon as they were touched, manipulated or shorn by humans. Therefore, the sham-shearing group showed significantly less defensive behavior between start to end of the sham-shearing procedure compared to the 'Shear' group as nobody touched or handled them during that period. Behaviors such as alarm calls or spitting exclusively occurred within the 'Shear' group but never in 'Sham'. As spitting (McGee Bennett, 2014) and alarm calls (Cebra & Gemensky-Metzler, 2014) seem to be signs of fear or agitation, it can be hypothesized that shorn animals were even more stressed. Nevertheless, this effect was not consistently reflected in the peripheral temperature measurements but only in rectal temperature.

Rectal temperature was influenced by the treatment shearing/sham-shearing and not by the measurement time point. Even though mean differences between groups were smaller than suggested in literature for vicuñas and sheep (Bonacic et al., 2003; Sanger et al., 2011), shearing seemed to induce additional stress resulting in a higher mean rectal temperature in the 'Shear' group. This is in line with conclusions of Wittek et al. (2017) that the additional intense handling and the loud noises of the shearing machine amplify the stress-level of shorn animals as was the case in this thesis. This hypothesis is further strengthened by the more frequent expression of stress-indicating behavior in the 'Shear' group such as vocalizations, alarm calls, spitting and defensive behavior. In the frame of the same experiment of Wittek et al., Waiblinger et al. (2020) also found more vocalizations in animals restrained and shorn than in alpacas only restrained which suggests that shearing does have an additional stressful effect. Nevertheless, Wittek et al. did not find significant changes in rectal temperature over the time of the experiment which is also in line with the results of this thesis as no significant effect of 'time point' was found. In captured vicuñas, no significant difference in rectal temperature pre and post capture was found either (Arzamendia et al., 2010; Marcopiddo et al., 2018) further underlining the results of this thesis. This lacking effect could be interpreted as the organism's successful attempt to maintain homeostasis and keep core body temperature more or less stable over a relatively short period of time as shearing took around 8 and sham-shearing around 6.5 minutes on average. It could also be that a significant rise in rectal temperature was missed due to the fact that temperature recordings stopped after the animals had been released from the shearing area while for example in a study by Sanger et al. (2011), core body temperature in shorn sheep increased significantly 4 and 14 minutes after treatment compared to sham-shorn animals. Nonetheless, they used vaginally inserted temperature loggers which may have been more exact in assessing temperature development. Significant increases in rectal temperature of around 0.5°C in vicuñas pre- and post-transport were also recorded within a 10–15 minutes time interval (Bonacic et al., 2003) which may additionally suggest that significant increases in rectal temperature over time were missed within the experiment of this thesis. When comparing all the different results of the reactivity of rectal temperature

(Bonacic and Macdonald, 2003; Sanger et al., 2011; Wittek et al., 2017; Arzamendia et al., 2010), it becomes apparent that rectal temperature alone might not be the most suitable indicator to measure stress in camelids and should probably only be considered in combination with other physiological measures and/or behavior. Elevations in core temperature of course also depend on the experimental conditions, environment and ambient temperature. For example, vicuñas during capture (Bonacic et al., 2006) might have exerted more energy than alpacas during shearing and therefore have higher body temperatures which increased more significantly over time (Bonacic et al., 2006).

5.2 Other influencing factors: Individuality and ambient temperature

When considering the distribution of rectal temperature as well as the changes of it (*Figure 5* and *6*), the high variability in absolute temperature and changes in temperature suggest substantial individual differences between the alpacas. This is even more pronounced in peripheral temperature fluctuations with some animals first dropping in temperature and then rising or the other way around. Hence, temperature development seems to be very individual for each animal and thus, patterns are rather difficult to determine. This was also suggested by Wittek et al. (2017) who found high individual variability between alpacas for other physiological parameters. Concerning eye temperature measurements via IRT, Jansson et al. (2021) also found a high variability between the maximum eye temperature of individual horses (between 29.4 and 37.6°C). In their model, 37.4% of total temperature variation was attributable to the individual maximum eye temperature. Therefore, they reasoned that the individual animal had a bigger impact on IR eye temperature than environmental factors. Bonacic et al. (2003), comparing cortisol levels of captured vicuñas immediately after capture and some days later, also argued that there may be differences in individual animals with some being more nervous than others. Considering these individual differences in temper which might not only be reflected in differing cortisol levels but also in peripheral temperature developments, it is challenging to classify what an increase or decrease in peripheral temperature might mean for the individual alpaca. If a peripheral temperature decrease indicates stress or a negative emotional reaction, a remaining question would be if an increase suggested a lack thereof (or the other way around). As the physiology of an animal seems to reflect emotions based on individual experience and temper, it may be difficult to potentially monitor alpaca stress by generalized peripheral temperature threshold values. Possibly, there might be no clear physiological pattern in peripheral temperature changes.

Furthermore, it also needs to be considered that ambient temperature had an influence on peripheral and core temperature as well as on frequency and duration of vocalizations. This resulted in temperature increases of 0.08-0.22°C per 1°C of ambient temperature increase and more and longer screams the warmer the environment. As shearing/sham-shearing started in the morning with 10°C and gradually increased until 19°C around noon, this could have partially distorted temperature values of animals that were shorn towards the end of the experiment. However, this effect was at least consistent for all anatomical spots and treatment groups. In literature, it is argued that local climate might have even a bigger effect on peripheral temperature than core body temperature (McFarland et al., 2020). Therefore, this effect should not be underestimated for future studies. However, individual temperature patterns between animals seem to be the bigger influencing factor as peripheral and core temperature fluctuate more substantially compared to the numerically much lower impact of ambient temperature.

5.3 Correlations: Changes in temperature and behavior

Concerning the second research question, it was assumed that the intensity or changes in stress-indicating behavior might be reflected in changes of core body and peripheral temperature. However, correlations

were non-existent or very weak (maximum 0.28). Core body temperature changes were not reflected in behavioral changes whereas right/left nostril and flew temperature changes weakly correlated with scream frequency and scream duration. This correlation could be explained by a more intense use of the mouth and nose region with consequently more air passing through the nasal cavities as a result of heavy breathing to be able to scream. As an outcome, it could be suggested that the more and longer the alpacas screamed, the higher the temperature changes around the mouth and nose region. Nevertheless, no comparable literature exists to further discuss correlations between behavior and peripheral temperature changes in more detail.

5.4 Methodological considerations

Further influencing factors to impact study results could have been relative humidity of the outside air which was not evaluated for the purpose of this experiment. A study in race dogs showed, that humidity had a significant effect on eye temperature resulting in an increase with rising humidity (Elias et al., 2021). Moreover, six different people handled and 'treated' the animals during the experiment which might have caused additional stress and could be reduced for future studies. Another distorting factor concerning the effect of treatment was the relatively small number of sham-animals which could have led to the missing group effect within peripheral temperatures. For future experiments, 'Sham' and 'Shear' group are suggested to be of comparable size to avoid this unbalanced effect.

Initially, the idea to measure right and left eye temperature was inspired by the wish to potentially predict emotional lateralization. This concept suggests that negative emotions (e.g., elicited by fear) are mostly processed in the right-hemisphere of the brain whereas positive emotions (e.g., induced by reward) are processed in the left. A similar emotional lateralization pattern has been described for most vertebrates (Leliveld et al., 2013). In connection to the experiment of this thesis, it was decided to measure both eyes and nostrils. Nevertheless, the analysis of side related effects was discarded as measurement points were partly different for both eyes (e.g., 'Start' missing for the left eye) and therefore temperature developments were only partly comparable. However, the concept of emotional lateralization by studying left and right eye or left and right nostril temperature could be of interest to determine emotional valence in alpacas, as it could be hypothesized that a more pronounced temperature change in the left eye could mean an activation of the right-hemisphere of the brain, suggesting processing of negative emotions such as anxiety or fear.

6. Conclusions

The present results indicate that the shearing/sham-shearing process (capture, handling, restraint, shearing) induces stress which is reflected in the overall occurrence of stress-indicating behaviors (frequency, time spent performing). However, core body temperature and peripheral temperature react differently to different effects (group, time point) in the course of shearing/sham-shearing and do not correlate well. Eye temperature seems to first rise, and nose temperature seems to first drop as a reaction to handling and shearing/sham-shearing whereas rectal temperature remains stable throughout the process. Therefore, within the timeframe considered, core body temperature in alpacas may not be taken as a validation method for stress in terms of using peripheral temperature.

More research is needed to conclusively prove the present patterns and it is suggested to create alternative stressful situations in order to see if alpacas' eye and nose temperatures respond differently to alternative scenarios. In order to prove sympathetic and parasympathetic involvement or research the possible activation of the HPA-axis, animals could be subjected to adrenalin or ACTH challenges. If IRT is

to be validated as a reliable tool to indicate stress, HR and HRV should also be considered to be added to subsequent studies. To complete the picture, salivary or blood cortisol could also be measured to know if and how it would change or if it is correlated at all as different studies suggest a different or even contrary interrelation between cortisol level and peripheral temperature. An additional recommendation for future studies would also be to choose a different baseline situation with animals that are not handled yet. This would ensure that baseline temperature values correspond to the 'natural' ones and changes in temperature could be detected more easily.

It is indeed questionable if infrared thermography is an appropriate tool to quickly and non-invasively measure stress response in alpacas as their behavioral expressiveness can already reveal a lot about their psychological or mental state. Constant high-pitched vocalizations (screams), alarm calls, strong defense movements and spitting are all very clear signs of the alpaca's unwillingness to participate in an aversive process like shearing. Other ungulates like sheep, goats or cattle for example, are not as easy to read and therefore non-invasive measures to detect pain or stress seem to make more sense.

Concerning alpaca welfare, an even more important question to be answered but which was not the primary topic of this thesis should rather be how to reduce stress for alpacas during handling and shearing. One reasonable approach could be a better human-animal relationship, as the videos of the shearing process showed that alpacas especially reacted very aversively when touched, lifted, shorn or pushed/pulled by humans in order to force them into the shearing/sham-shearing process. As reported by Pacomarca, all their animals are handled by humans maximum twice per month (including medical procedures) which they already consider to be a lot in a facility with a herd size of around 2050 alpacas. Therefore, there is possibly not enough time to habituate alpacas to such aversive procedures. Capture, restraint and handling represent an unusual situation for them and put them under stress. According to Grandin (1997), animals which are used to "frequent handling and close contact with people are usually less stressed by restraint and handling than animals that seldom see people". Ideally and in line with recommendations (Waiblinger et al., 2020) it would thus be necessary to train the alpacas by positive reinforcement to willingly participate in different husbandry procedures so that restraint and rough handling would become superfluous. If habituation and training is not possible, an alternative solution could at least be to shear the alpacas standing and without ropes as that seemed the least stress-inducing procedure in the shearing experiment of Waiblinger et al. (2020). There are unfortunately only few studies on the impact of a good human-alpaca interaction, but Windschnurer et al. (2020) found significant associations between alpaca behavior and caretaker attitude (in Europe). For example, alpaca owners who rated touching as pleasant or touched their animals more frequently had less escape attempts of alpacas during leading and handling or owners who found stroking and talking to be important had fewer animals moaning during a physical examination or rise or freeze during handling. This indicates that alpacas used to close human contact are likely to be easier to handle and would hence be less stressed. Additional research is needed to further investigate stress reducing measures during shearing.

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Abbreviations

ACTH: Adrenocorticotropic hormone – released by the anterior pituitary as part of the HPA-axis

ANS: Autonomic nervous system

AVA: arteriovenous anastomoses - linking arterioles and venules by capillary connections

CL: *Caruncula lacrimalis* – small nodule at the inner corner of the eye

ET: Eye temperature

HPA-axis: Hypothalamic-pituitary-adrenal axis – 'stress' axis releasing cortisol at the end of the cascade

IRT: Infrared thermography

LSM: Least Square Means

RAS: Responsible Alpaca Standard

SIH: Stress-induced hyperthermia

SEM: Standard error of the mean

List of figures and tables