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Stress responses relating to vaginal examinations in dairy cows

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Only when we understand can we care,
only when we care will we help.
Jane Goodall

Meiner Schwester
in Dankbarkeit, Verbundenheit und Liebe

Table of contents

1. INTRODUCTION

Modern farm management has to ensure reproductive efficiency on commercial dairy farms (Roche et al. 2000; Lucy 2001). Thus, vaginal examinations are routine procedures to detect fertility disorders like endometritis (LeBlanc et al. 2002) or silent heat (López-Gatius et al. 1996). While working in a veterinary practice for cattle in Brandenburg, Germany, the veterinarians noticed that cows showed different behavior when being confronted with veterinary personnel compared to the farm personnel the cows were used to. Cows were more restless and more often tried to flee from their cubicles when veterinarians entered the stable. Thus, we wondered whether this behavior might be caused by negative experiences resulting from past vaginal examinations conducted by veterinarians due to puerperal protocols of the farm.

Vaginal and other veterinary examinations or treatments were considered to cause discomfort and stress in cattle (Minton 1994; Petyim et al. 2007). Cows coping with stress show various behavioural reactions depending on the individual, species and breed (Koolhaas et al. 1999). Despite domestication – which limited the range of behavioural reactions – dairy cows are still capable of some feral behavioural reactions (Price 1999). While assessing animal behaviour, etiologists usually describe the mere occurrence of different reactions (Marti et al. 2010), the extent of reactions (Flower and Weary 2006) or use a combination of both (Sprecher et al. 1997). Stress responses in animals were classified into active (fight and flight) and passive (freeze and fiddle about) coping styles (Van Reenen et al. 2005). The reduction of stress during veterinary procedures is assumed to affect animal welfare on farms positively (Waiblinger et al. 2004). Nonetheless, the behaviour of cows relating to vaginal examinations has not been investigated yet.

Therefore, the present thesis "*Stress responses relating to vaginal examinations in dairy cows"* was conducted to assess behavioural reactions of dairy cows before and during vaginal examination. It includes two corresponding studies. The overall objective was to evaluate cow behaviour during vaginal examination and whether stress and discomfort in cows during vaginal examinations can be eventually reduced by an adequate examination technique.

In the first study, I determined specific behavioural reactions which cows express their discomfort during vaginal examination with. The goal was to create a tool allowing the assessment of stress responses directly in the stable. I hypothesized that stress responses during vaginal examination can be semi-quantitatively scored with an avoidance reactivity score (ARS) and that the examination with the Metricheck device® is less invasive than the examination with the gloved hand.

Regardless of the assessment method, the interpretation of behavioural reactions is always affected by the individual observer (Sambraus 1998). Physiological parameters such as heart rate measurements were suggested as suitable to improve analyses and interpretations of behaviour more objectively (Koolhaas et al. 1997). Moreover, behavioural reactions can be classified either in states or events, and the registration of presence or absence of a certain stress response can be useful for scoring behaviour (Altmann 1974). Including measurements of the time which is spent in certain behaviour takes additional information for the interpretation of stress responses into account (Altmann 1974). All behavioural reactions of the ARS were frequently shown by cows depending on their chosen scheme of active (fight or flight) or passive (freeze or fiddle about) behaviour. One signal of discomfort – the arched back – was expressed by nearly all cows during vaginal examination. Additionally, the detection of painful procedures in cows can be supported by the use of non-steroidal anti-inflammatory drugs (Earley and Crowe 2002; Banting et al. 2008) or epidural anaesthesia which provide both, analgesia and blocked reflexes (Skarda 1996). Thus, we suggested, it might be possible to distinguish between a pain- or reflex-associated origin of the arched back during vaginal examination.

The general objective of the second study was to determine the background intention of the arched back during vaginal examination. Specifically, I hypothesized that avoidance reactions and the duration of the arched back can be influenced by epidural anaesthesia or analgesic treatment.

The results of the first study were published in the journal Applied Animal Behaviour Science (impact factor 2012: 1.918): M. Pilz, C. Fischer-Tenhagen, G. Thiele, H. Tinge, F. Lotz, W. Heuwieser (2012). *Behavioural reactions before and during vaginal exa mination in dairy cows.* Appl. Anim. Behav. Sci. 138: 18-27.

The manuscript with the results of the second study has been recently accepted by the Journal Tierärztliche Praxis Großtiere (impact factor 2012: 0.283): M. Pilz, C. Fischer-Tenhagen, M. Grau, W. Heuwieser (2014). *Behavioural und physiological assessment of stress reactions during vaginal examination in dairy cows.* Tierärztl. Prax. (G) 42: 88-94.

The outline of both articles is adapted to the guidelines for authors of the corresponding journals.

2. RESEARCH PAPERS

2.1. Behavioural reactions before and during vaginal examination in dairy cows. Applied Animal Behaviour Science 138 (2012): 18-27.

2.2. Behavioural and physiological assessment of stress reactions during vaginal examination in dairy cows. Tierärztliche Praxis Großtiere 42 (2014): 88-94.

The manuscripts of the papers are formatted according to the guidelines for authors of the journal Applied Animal Behaviour Science and Tierärztliche Praxis Großtiere, respectively.

2.1. Behavioural reactions before and during vaginal examination in dairy cows

Behavioural reactions before and during vaginal examination in dairy cows

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Abbreviations

ARS: Avoidance reactivity score; DIM: Days in milk; GH: Gloved Hand; MD: Metricheck device; SD: Standard deviation; VDS: Vaginal discharge score

Abstract

Vaginal examinations are routine procedures on dairy farms to diagnose puerperal diseases. Cows express their discomfort in certain situations with discrete behavioural reactions. These reactions can be described in their occurrence and extent. Although there is evidence that human contact is potentially stressful for cows, the impact of vaginal examinations on animal welfare has not been evaluated. Therefore, we hypothesized that 1) cows show discomfort before and during vaginal examination with different behavioural reactions, 2) these reactions can be semi-quantitatively scored and 3) the examination with a Metricheck device is less invasive than an examination with the gloved hand. In experiment 1, the behaviour of 10 cows during vaginal examination was videotaped and analysed. In total, 15 different behavioural reactions were identified. Based on these observations, a numerical rating system was created. The avoidance reactivity score (ARS) includes evasive reactions, which are scored on a 4-point scale, and signals of discomfort, scored with 1 point each. In Experiment 2, evasive reactions of cows were videotaped and scored by two observers and three times by the examiner. The inter- and intra-repeatability was 0.70 and 0.87, respectively. In experiment 3, vaginal examinations of 30 cows were videotaped and scored with the ARS by four observers and three times by one observer. The inter- and intra-observer repeatability during examination was 0.71 and 0.87, respectively. In experiment 4, 435 vaginal examinations were conducted either with the gloved hand (group GH) or the Metricheck device (group MD). Behaviour before and during examination was scored. The median ARS increased from 1 before (IQR: 1 – 2) to 3 during examination (IQR: 2 – 4) and cows in the group MD showed less avoidance reactions compared to cows in the group GH ($P < 0.05$). Parity, days in milk, vaginal discharge or repeated examinations did not influence the ARS. Our study provides evidence that vaginal examinations cause stress in cows. Furthermore, we demonstrated that behavioural reactions can be assessed with a score. Substantial inter-observer and substantial intra-observer repeatability proves that the ARS can be applied in practice. The ARS –although imperfect- might be a useful tool in the field and in research to estimate a cow's stress level.

Keywords

Behaviour, Cattle, Examination, Avoidance reactivity score, Animal welfare, Metricheck

1. Introduction

Veterinary examinations and treatments have the potential of causing discomfort, stress and pain in cattle (Minton, 1994; Petyim et al., 2007). Transrectal or vaginal examinations to diagnose pregnancy or endometritis, respectively, are routine practice for most commercial dairy herds (LeBlanc et al., 2002; Romano et al., 2007). Interestingly, the impact of such examinations on animal behaviour has not been evaluated.

Stress behaviour of mammals can be categorized into the patterns fight, flight, freeze, and fiddle about (Bowen and Heath, 2005), which help an animal to cope with stress. While fight and flight are part of an active coping style, freeze and fiddle about are indicative of a passive coping style (Van Reenen et al., 2005). The choice of stress responses depends on the specific situation and varies between individuals, species and breeds (Koolhaas et al., 1999). Individual variation in stress behaviour is a function for the animal's adaptability to changes in its environment (Koolhaas et al., 2007). Some behavioural reactions of feral animals still can be observed in dairy cows. However, the process of domestication limited the range of behavioural reactions (Price, 1999).

On commercial dairy farms, stressors are prevalent in a wide variety and intensities. It has been demonstrated that the mere human contact might cause discomfort in dairy cows (Hagen et al., 2004). Most kicking followed human handling (i.e. teat cleaning, teat cup attachment) and significantly less kicking was observed in automatic milking systems compared to milking in a 2 x 6 herring-bone parlour (Hagen et al., 2004). Vocalisation (Grandin, 2001), tail flicking, flinching or lifting a leg (Waiblinger et al., 2004) were described as signals of discomfort in cows.

Various scientific approaches to assess behaviour of cows can be found in the recent literature. The mere occurrence of behaviour describes the presence of a reaction, i.e. head turning (Marti et al., 2010) or arching the back (Walker et al., 2008). However, the extent of certain behavioural reactions characterizes a progression. Especially, reactions of the locomotor system vary in their extent, i.e. the inclusion of one or more legs into a gait pattern (Sprecher et al., 1997) or the stiffness of joints (Flower and Weary, 2006). The combination of occurrence and extent of different reactions has been used to analyse and score animal behaviour. Specifically, Sprecher et al. (1997) modified a lameness score, which consists of the presence of an arched back in combination with different gait patterns.

Vaginal and transrectal examinations are conducted to identify cows at risk of postpartum infections, and they are a part of protocols for reproductive management in bovine practice (LeBlanc et al., 2002). Transrectal palpation of the reproductive tract is performed multiple times during lactation to monitor involution of the uterus for diagnosing puerperal diseases (Tsousis et al., 2010) or pregnancy (Dunne et al., 2000).

Vaginal examination in cows can be conducted with a speculum, the Metricheck device (Zimcro, Hamilton, New Zealand), or the gloved hand (LeBlanc et al., 2002; Pleticha et al., 2009; Runciman et al., 2009). An endometritis scoring system has been described (Sheldon et al., 2006) to determine the severity of postpartum infections based on the quality of the vaginal discharge (LeBlanc et al., 2002).

Direct observations of spontaneous behaviour have been used widely in the literature. As suggested, behaviour may be regarded as events or states (Altmann, 1974). She explained that event behaviour describes all behaviours in the moment they occur, while state behaviour lasts over a certain time. Furthermore, she established different sampling methods for interpretation of behavioural observations.

Several numerical scoring systems were created to help farmers and veterinary practitioners to assess a cow's health. Zaaijer and Noordhuizen (2003) proved that the scores for body condition, rumen fill and faecal consistency are useful to indicate fertility risks in a herd due to nutritional problems. Another study showed the association between a scoring system of the cows' gait and sole ulcers (Chapinal et al., 2009). Referring to behavioural reactions of cows, this score has been demonstrated to be a good predictor of hoof lesions. Streyl et al. (2011) created a parturition score which is also based on behavioural observations. This score helps to predict calving within the next 12 hours, allowing a better monitoring of calving and management of individual cows (Streyl et al., 2011), which affects cow welfare on farms.

In the past, several issues related to animal welfare have been studied intensively (Rushen, 2003; Whay et al., 2003). Both, physiology and behaviour can be used to investigate the mental health of animals (Dawkins, 2006). Von Keyserlingk et al. (2009) reviewed different concepts of animal housing and husbandry and concluded that animal welfare consists of three important issues relating to affective states; i.e. pain, pleasure, and naturalness of animals. Repeated vaginal examinations, as conducted on commercial dairy farms due to fertility management, are interference to a cow's daily environment. Hence, the impact of vaginal examinations on cow's behaviour may be part of an impact on animal welfare on farms.

Animal welfare per se is difficult to measure, because the complexity of behaviour relies on subjective interpretations of different observers (Mason and Mendl, 1993). Moreover, there are many various stimuli on farms which may affect animal behaviour, and different species show different behaviours to a similar pain stimulus (Livingston, 2010). Although, there is an ongoing discussion whether cows have a sensation of discomfort or pain, the impact of reproductive examinations on animal behaviour has not been examined. Therefore, the overall objective of this study was to determine the effect of vaginal examinations on the behaviour of dairy cows. Specifically, we hypothesized that 1) cows show discomfort before and during vaginal examination with different behavioural reactions,

2) these reactions can be semi-quantitatively scored and 3) the vaginal examination with the Metricheck device is less invasive than an examination with the gloved hand.

2. Material and methods

Four experiments were conducted between April 2010 and November 2011.

2.1. Experiment 1

In a preliminary study, 10 dairy cows were examined by manual vaginal examination 30 ± 7 (mean ± standard deviation, SD) days in milk (DIM). The examination was videotaped (Handycam®, Sony, Berlin, Germany) from behind and the behavioural reactions were described by three independent investigators who are quite experienced in dairy cows and their behaviour. They trained observing and assessing cow behaviour in various studies before.

Cows were driven into a free cubicle and restrained by one assistant, who was holding the cow's tail on the left side. A second assistant touched the cow's crupper on the right side. The examiner cleaned the perivaginal region with dry paper towels. Afterwards, the investigator covered her gloved hand or the Metricheck Device with lubricants (Vet-Gel®, cp-pharma, Burgdorf, Germany). This preparation period took between 45 to 60 seconds (mean ± standard deviation). Following, the examiner conducted a vaginal examination with the gloved hand or the Metricheck device. This period took between 5 to 10 seconds (mean ± standard deviation). All reactions before and during this examination period were videotaped and analysed.

All three investigators consistently identified 15 behavioural reactions, which occurred regularly before or during examination. These reactions were used to generate a scoring system to describe avoidance behaviour. The rationale for developing an avoidance reactivity score (ARS) was the semi-quantitative evaluation of avoidance behaviour in dairy cows during reproductive examinations. Three behavioural reactions were indicative of discomfort (i. e. arched back, stretched neck, vocalisation) and documented when expressed by the animal. Their occurrence was scored with one point each. These reactions were considered as signals of discomfort and include movements of body parts which do not belong to the locomotor system. Standing still, tripping, sideways movements, leaning against divider, kicking out and escape were scored on a 4-point scale with 0, 1, 2, 3, 3, and 3 points, respectively. This behaviour includes reactions of the locomotor system only and was considered as evasive reactions. Evasive reactions were scored according to their extent, whereas signals of discomfort were scored according to their occurrence. The total ARS was calculated for each examination by adding the points for the reactions observed considering

occurrence (signals of discomfort) and extent (evasive reactions). For example, a cow which kicked out, arched the back and stretched the neck received 3 points for evasive reactions and 2 points for signals of discomfort. Thus, the ARS represents cumulative points given for the highest evasive reaction added with all points for shown signals of discomfort.

Completing, avoidance reactions were defined as all behavioural reactions which are shown by a cow when it comes in contact with humans and which can be observed from a view behind the cow. The ARS is a tool which allows an examiner to assess a cow's behaviour directly during vaginal (or transrectal) examination.

2.2. Experiment 2

In the second preliminary study we wanted to prove the reliability of distinction between evasive reactions. Therefore, we gave the observers a concrete definition of the possible reactions: Tripping was defined as the movement of one leg in all three dimensional directions (i.e. back and forth, up and down, left and right). In contrast to stepping sideways, one leg is steadily on the floor and does not change place during tripping. The tripping leg rests on the same place on the floor after tripping compared to the place before tripping. When stepping sideways, a cow moved both legs and rests both legs in a different place on the floor. Kicking out was defined as sudden movement backwards of one hind limb in the direction of the examiner. Leaning against the divider was defined as a movement sideways with the cow coming to rest its body on or in close position to the divider. Escape was defined as successful attempt to back out of the cubicle.

For inter-observer repeatability, two veterinarians, who were acquainted with cows, watched the video of 10 lactating dairy cows (43.8 ± 18.6 days in milk, DIM; mean ± standard deviation, SD) which were vaginal examined with a gloved hand for signs of chronic endometritis, independently. For intra-observer repeatability, the first author analysed the movements in a random order three times in intervals of two weeks. Cohen's kappa coefficient was calculated.

2.3. Experiment 3

A total of 30 lactating dairy cows (43.8 ± 18.6 DIM; mean ± SD) were used to validate the ARS. The vaginal examinations conducted with the gloved hand were videotaped (Handycam®, Sony, Berlin, Germany). To determine inter-observer repeatability, the video was evaluated by two experienced veterinarians and two people not acquainted with farm animals, independently. All behavioural reactions before and during vaginal examination were documented and the ARS was calculated. To study intra-observer repeatability the first author analysed the videos presented in a random order three times. The first evaluation was

conducted one day after the vaginal examination. The following evaluation was conducted with an interval of two weeks.

2.4. Experiment 4

The study was conducted between May and September 2010 on a commercial dairy farm, milking approximately 1,100 cows, in Brandenburg, Germany. Cows were housed in a free-stall barn with concrete, slotted floors. Cubicles were equipped with rubber mats and covered with dried manure from a biogas plant. Cows were fed corn and grass silage mixed with distiller grain and shredded grain which was delivered by an automatic feeding system twice a day. They were fed concentrates delivered by an automatic feeder and milked two times daily in a 2 x 33 side-by-side milking parlour. All cows were regularly exposed to human contact by different personnel.

Holstein-Friesian dairy cows (n = 435) were vaginally examined, behavioural reactions determined, and was the ARS calculated. Every Wednesday, all cows between 14 and 21 DIM were randomly assigned to one of two examination groups using their 4 digit collar numbers and examined for signs of chronic endometritis. Cows with odd collar numbers were examined with a gloved hand (group GH) and cows with even collar numbers were examined with the Metricheck device (group MD). In the group GH and MD 219 (50.4 %) and 216 (49.6%) vaginal examinations were conducted, respectively. Parity of group GH and MD was 1.9 ± 1.1 and 2.0 ± 1.2, respectively ($P > 0.05$). Cows of group GH and MD were 19.8 ± 4.7 DIM and 19.9 ± 4.7 DIM, respectively ($P > 0.05$). Cows were compared according to their lactation period. In total, 196 cows were first calf heifers and 237 cows were in their second lactation or higher. Among the older cows, 123 were in their second lactation, 66 in their third, 36 in their fourth, 11 in their fifth, one in its sixth and two in their seventh lactation. The age of primiparous and multiparous cows was 2.4 ± 0.3 and 4.3 ± 1.1 years, respectively ($P < 0.05$). Overall, 251 cows were examined once, and – due to purulent vaginal discharge diagnosed at the first examination – 63 and 18 cows were examined twice and three times, respectively. One cow was examined four times. Additionally, cows were compared considering days in milk (DIM). In total, 311 cows were up to 21 DIM (group 1), 93 cows were 22-27 DIM (group 2), 28 cows were 28-34 DIM (group 3) and 3 cows were more than 34 DIM (group 4). Based on the clinical findings (LeBlanc et al., 2002) and the vaginal discharge score (Sheldon et al., 2006), 160 cows were classified as healthy (clear mucus) while 94, 77 and 104 had a vaginal discharge score (VDS) of 1 (clear mucus with flecks of pus), 2 (mucopurulent discharge) and 3 (purulent discharge), respectively.

The study started with selecting the cow in the barn by the farm manager. Cows were driven into a free cubicle. Now, observations referring to the period ‘before examination’ started. The herd manager took hold of the cow’s tail from the left, whereas a second assistant

touched the cow on its right crupper. The examiner began cleaning the perianal region with dry paper towels. Following, in the group GH a disposable glove was lubricated (Vet-Gel®, cp-pharma, Burgdorf, Germany) and the examination performed manually, while in the group MD the Metricheck device was covered with lubricant. This period lasted 45 to 60 seconds depending on the degree of perivaginal contamination with dirt or faeces. The handling was identical for both groups. Then the period 'during examination' started. All vaginal examinations, which lasted 5 to 10 seconds, were conducted by the first author, a licensed female veterinarian. The circumference of the examiner's hand was 20.7 cm while the circumference of the Metricheck device was 9.7 cm. The manual examination included insertion of the hand into the cow's vagina up to the caudal end (cervical os). Then it was twisted carefully on the cervical bottom and pulled out again. As formerly described, the Metricheck device was advanced to the anterior end of the vagina, the handle end slightly elevated and the device was retracted caudally (McDougall et al., 2007).

The behaviour of the cows was observed and all reactions before and during vaginal examination were documented and analysed by the examiner.

Cows with a VDS of 1 to 3 were re-examined on a weekly schedule until discharge was clear mucus or absent.

After each vaginal examination all observed reactions were documented and the ARS was calculated.

The examiner was used to conduct postpartum examinations for more than one year. The frequency of examinations was adapted according to her constitution. Every week 25.6 ± 4.2 vaginal examinations were conducted (minimum 19, maximum 35 examinations) based on the calving frequency and puerperal protocol of the farm. This number is even lower than conducted during a normal herd visit on large commercial dairy farms. Therefore, we are convinced that tiredness of the examiner was not an issue.

All cows were housed in the same barn, independently from their degree of endometritis. The vaginal examinations were all conducted to the following time design. They were fed at 6.00 a.m. One hour later, the examiner and two assistants entered the barn and started vaginal examinations. Examinations of the cows lasted one hour every Wednesday. When the examiner and the assistants left the barn, the cows were able to continue feeding, walk or rest. They were directed to the milking parlour at 1.00 p.m.

2.5. Statistical analysis

All statistical analyses were conducted with PASW Statistics (Version 18.0, SPSS Inc. Munich, Germany). Chi-Square tests were carried out. The ARS before and during examination were the independent variables, and parity, DIM and VDS were the category variables. For the comparison of the ARS between the groups GH and MD the Mann-

Whitney-Wilcoxon test was used. Examination methods (gloved hand or Metricheck device) were the independent group variables and the ARS before and during examination, vaginal discharge score, parity, and DIM were the test variables. The significance level was set at $P < 0.05$. The association between ARS and number of examination was determined by calculating the Spearman correlation coefficient (ρ).

Reliability of agreement between different observers (inter-observer) and repeated observations (intra-observer) were analysed with Cohen's kappa coefficient (Hoehler, 2000). Cohen's kappa (κ) coefficient can result in values between 0 and 1. Values near 1.00 illustrate almost perfect agreement, whereas values around 0.00 demonstrate poor agreement (Landis and Koch, 1977). The confidence limit was set at 95 % and the significance level at $P < 0.05$.

3. Results

3.1. Experiment 1

The observers identified 15 different behavioural reactions and assigned them to different body regions. The locomotor system was associated with the following behavioural reactions: standing still, tripping, sideways movements, escape, kicking out and leaning against the divider. The tail was associated with shaking or stretching. The head was assigned to shaking, rubbing, movements towards the examiner or ear movements. The cow's vocal tract was associated with vocalisation, its neck with stretching and its back with kyphosis.

The objective of developing the ARS was to semi-quantitatively assess the behavioural reactions of cows to vaginal examination by the investigator from behind. Therefore, ear movements, head shaking and rubbing were excluded from the score. Head movements towards the examiner were considered as explorative behaviour and also not used.

During vaginal examination, an assistant took hold of the cow's tail to prevent the cleaned perivaginal region from re-contamination with dirt and faeces. Therefore, tail stretching and shaking were excluded from the score. All other signals could be detected from behind and were included in the score.

3.2. Experiment 2

A total of 30 examinations were conducted and videotaped (Handycam®, Sony, Berlin, Germany). 105 evasive reactions were used for analysis. For inter-observer repeatability, Cohen's κ was 0.70 ($P < 0.05$). For intra-observer repeatability, Cohen's κ was 0.87 ($P < 0.05$).

3.3. Experiment 3

Veterinarians and people not acquainted with farm animals analysed the videos separately. For veterinarians and non-veterinarians, minimum and maximum ARS scored for the 30 cows was 1 and 4, respectively. Therefore, the Cohen's κ coefficient was calculated with four categorical variables (i.e. ARS 1 to 4). Among veterinarians, Cohen's κ was 0.60 for the period before examination and 0.61 for the period during examination. Among non-veterinarians, Cohen's κ was 0.69 for the period before examination and 0.71 for the period during examination. Intra-observer repeatability was 0.74 and 0.84 for the period before and during examination, respectively.

3.4. Experiment 4

All possible behavioural reactions (n = 9) of the ARS were observed before and during examination and all examinations (n = 435) were used for statistical analyses (Table 1 and Table 2).

The proportion of cows that arched their back increased from 2.5 % before examination to 93.1 % during the vaginal examination. The proportion of cows stretching their neck increased from 60.5 % to 82.5 %. Vocalisation was observed in 1.1 % of the cows before examination and in 0.7 % of the cows during examination.

Proportion of cows standing still decreased from 45.1 % before examination to 35.2 % during examination. Cows which were standing still before examination started to trip (n = 28) or to move sideways (n = 12) during examination. Evasive reactions such as leaning against the divider, kicking out, and escape were observed in 6.3 % of the cows before and in 6.9 % during the examination.

The median ARS increased from 1 before examination (interquartile range, IQR: 1 - 2) to 3 during examination (IQR: 2 - 4). A stretched neck and an arched back had the main influence on the increase of the ARS. Only 4.4 % of all cows had an ARS of 3 or more before examination (Table 3). This proportion increased to 26.9 % during examination (Table 4). An ARS of 0 was observed in 17.0 % of cows before and in 0.5% during examination. No cow was scored with an ARS of 5 or 6 before examination or score 6 during examination, but 5.1 % of the cows had an ARS of 5 during examination.

3.5. Repetitive examinations and methods

An association between the first and repeated vaginal examinations (n = 101) did not exist regardless of the number of the repetition. Spearman correlation coefficient (ρ) was -0.05 before examination and -0.08 during examination. There was no difference between the ARS of the first examination and the first re-examination (n = 82) before (ρ = -0.03) and

during examination (ρ = -0.08). Some cows were examined three times (n = 19). The median ARS before and during second re-examination did not differ from the ARS before (ρ = -0.05) and during the first re-examination (ρ = -0.02). The number of the repetition did not influence the ARS.

The ARS in cows examined with a gloved hand was significantly higher ($P < 0.05$) compared to cows of the group MD during the examination (Figure 1).

3.6. Other factors

None of the cofactors (i.e. parity, DIM, VDS) had a significant effect on the ARS before or during examinations.

The median ARS of 1 before examination was the same for first-calf heifers and multiparous cows ($P > 0.05$). The ARS increased to 3 in both groups ($P > 0.05$).

Regardless of DIM, the ARS increased from 1 before to 3 during examination for cows in the groups 1, 2 and 3. The ARS in cows of group 4 (n = 3) was 3 before and during examination. Differences in the ARS between the two categories were small ($P > 0.05$ before and during examination).

The ARS increased from 1 before examination ($P > 0.05$) to 3 during examination ($P > 0.05$) in both, healthy cows (VDS 0) and cows with chronic endometritis (VDS 1, 2 or 3).

4. Discussion

4.1. Experiment 1

The objective of developing the ARS was to create a practical tool for semi-quantitatively assessing the influence of vaginal examinations on behavioural reactions of cows, which are conducted routinely on dairy farms. It has been demonstrated that human contact and veterinary examinations have the potential to cause stress (Breuer et al., 2000; Hagen et al., 2004). Therefore, it was reasonable to assume that vaginal examinations with the gloved hand or Metricheck device also cause discomfort or stress.

Initially, 15 behavioural reactions were identified in experiment 1, i.e. standing still, tripping, stepping sideways, kicking out, leaning against the divider, tail stretching or shaking, stretching the neck, head movements towards the examiner, head shaking or rubbing, ear movements, eye expression, arching the back and vocalization. Reproductive examinations in cows are performed from the back end. Due to feasibility reasons and the objective to develop a practical tool directly applicable cowside, the investigator should be able to both, conduct the examination and assess the behavioural reactions. Thus, the ARS includes reactions, which could be evaluated from the back end, only. Reactions, which have to be observed from

the front end (i.e. head shaking or rubbing, ear movements, eye expression) were not included in the ARS. Since an assistant retained the cow's tail to prevent re-contamination of the cleaned perivaginal region, two behavioural reactions encompassing unrestrained tail movements (i.e. tail stretching or shaking) were not included in the ARS. Head movements towards the examiner were considered as explorative behaviour (Welp et al., 2004) and also not used. All reactions of the locomotor system (i.e. standing still, tripping, stepping sideways, kicking out and leaning against the divider) and the reactions stretching the neck, arching the back and vocalisation could be detected from the back end of the cow, and, consequently, were included in the ARS. As a result, the ARS included nine of the originally detected possible reactions of dairy cows during vaginal examination. Evasive reactions include all movements of the locomotor system. The motivation of movements increases with the length of confinement (Jensen, 2001). Therefore, we rated evasive reactions on an increasing scale and considered the highest reaction.

Since an assistant retained the cow's tail to prevent re-contamination of the cleaned perivaginal region during vaginal examination, two behavioural reactions encompassing unrestrained tail movements (i.e. tail stretching or shaking) were not included in the ARS. All remaining 9 signals could be detected from the back end of the cow before and during the vaginal examination, so they were included in the ARS.

Behavioural reactions can be evaluated by their occurrence and extent (Sprecher et al., 1997). The avoidance reactivity score uses a combination of both.

An animal's mental state is an important issue in animal welfare (Von Keyserlingk et al., 2009). Koolhaas (1999) established the terms proactive (fight and flight) and reactive (conservation and withdrawal) coping. Evasive reactions of the ARS suggested herein are also comprised of proactive (i.e. tripping, sideways movement, escape, kicking) and reactive (i.e. leaning against the divider) behaviour. All three signals of discomfort (arched back, stretched neck, vocalisation) are reactive behaviour. The nine utilized reactions could also be categorized into the 'four F pattern' including the behaviours of fight (kicking out), flight (sideways movements, escape), freeze (standing still, leaning against the divider), and fiddle about (tripping) as described by Bowen and Heath (2005).

The ARS includes five reactions which could be classified as state behaviour, i.e. standing still, leaning against the divider, vocalisation, arching the back and stretching the neck. The reactions tripping, stepping sideways, escape and kicking out could be classified as event behaviour. In our study, only occurrence and extent of reactions were counted. The duration was not measured.

A similar approach was utilized to identify foot diseases in lame cows by means of a numerical rating system, most recently. This system included 7 different gait attributes in 5 intensities (Chapinal et al., 2009). Flower and Weary (2006) assumed, that a numerical rating

system provide a better estimate of hoof pathologies, because they used defined points. In our study, evasive reactions were scored on a 4-point scale to estimate the intensity of movement.

4.2. Experiment 2

In this experiment we wanted to show the reliability of the distinction between evasive reactions, for they are rated with increasing points. In contrast to signals of discomfort, they are not only evaluated by their occurrence but also by their extent. So, evasive reactions of the locomotor system assess the quantity of behavioural reactions. We based these evasive reactions, as well as the ARS itself, on mere observations. By giving concrete definitions of the reactions, we tried to make the assessment of reactions more comparable. Cohen's kappa coefficient (κ) is a measure for the strength of agreement between different observers (Landis and Koch, 1977) and can be used for assessment of agreement between more than two observers (Fleiss, 1971). In our study, the inter-observer repeatability can be considered as substantial agreement, whereas intra-observer repeatability can be considered as almost perfect agreement (Landis and Koch, 1977). Kappa values higher than 0.40 represent an 'acceptable' agreement between clinicians evaluating clinical judgements on an ordinal scale (Sim and Wright, 2005). Our data provide evidence that the observational differentiation between evasive reactions is reliable which warrants different assessment, i.e. by using increasing points.

4.3. Experiment 3

It seems difficult to measure cows' sensations directly, but there are indirect ethological methods which can be useful (Sambraus, 1998). During the observation of behaviour in experiment 1, a total variety of 15 behavioural reactions, were observed before and during vaginal examination. This number of behavioural reactions was reduced to create a simple and practical tool for the evaluation of stress in cows on commercial dairy farms. The objective of experiment 3 was to validate the ARS by determining inter- and intra-observer repeatability. Cohen's kappa coefficient (κ) is a measure for the strength of agreement between different observers (Landis and Koch, 1977) and can be used for assessment of agreement between more than two observers (Fleiss, 1971). In our study, the inter-observer repeatability referring to the reactions before and during examination can be considered as substantial agreement among both, veterinarians and non-veterinarians (Landis and Koch, 1977). Intra-observer repeatability can be considered as substantial and almost perfect agreement for the reactions before and during examination, respectively (Landis and Koch, 1977). Kappa values of inter- and intra-observer agreement before examination were lower compared to those during examination. We suspect that the increase in repeatability was due

to the increase in median ARS and the larger range observed. Intra-observer repeatability was higher compared to inter-observer values, which is common with other studies (O'Callaghan et al., 2003; Thomsen et al., 2008). The examiner watched the video three times (intra-observer), whereas the inter-observers watched the video one time, only. Our data provide evidence that the ARS can provide a reasonable measure to assess the behavioural reactions during vaginal examinations and, therefore, be indicative of stress in dairy cows.

4.4. Experiment 4

The objective of this experiment was to compare behavioural reactions caused by two different methods of vaginal examination (gloved hand vs. Metricheck device). Both examination methods are routine on commercial dairy farms. The duration of the periods before and during examination might have varied only to a small extent from cow to cow and, thus, might have biased the behavioural reactions. However, we speculate that due to the large number of animals per group the effects were balanced off.

The first author conducted vaginal examinations and analysed the behaviour. Experiments 2 and 3 demonstrated her reliability and ability to use the ARS properly. Due to practical reasons, no video recordings were made during the 435 vaginal examinations of experiment 4.

During the months the experiment lasted, no changes in time or personnel were made. Although the experiment lasted five months we tried to ensure a similar environment to the cows by repeating the examinations in always the same manner and time.

The most obvious changes in behavioural reactions during examination were related to signals of discomfort (i.e. arched back, a stretched neck) as part of a passive coping style. The proportion of cows which arched their back increased from 2.5 % before examination to 93.1 % during examination and cows which stretched their neck increased from 60.5 to 82.5 %.

Data from our trial provide evidence that vaginal examinations cause discomfort in cows. Cows expressed their discomfort with discrete signals of discomfort. Most consistently an arched back was observed, followed by a stretched neck, but hardly any vocalisation.

A physiologically arched back can be observed in defecating or urinating cows (Sambraus, 1978; Süss and Andreae, 1984), during parturition (Houpt, 2011) or, pathologically, in lame cows (Berry et al., 2008; Flower and Weary, 2006; Sprecher et al., 1997). During vaginal examination, we consider an arched back as a reflective urge of emptying the vagina from the examiner's hand or the Metricheck device. Cows may try to diminish physical resistance to reduce discomfort. Further studies are needed to differentiate whether the arched back is a reflex or a conscious reaction to evade a painful examination.

Interestingly, 82.5 % of the cows stretched their necks during examination. A low neck position has been described as a sign for submission towards other cows when being confronted with a possible fight or a higher-ranked cow (Hall, 2002; Sambraus, 1978). We assume that the stretched neck is part of the 'freeze' pattern of behaviour which is expressed to make the situation more endurable.

It is noteworthy that 263 cows (60.5 %) already stretched the neck before the vaginal examination started. We speculate that these reactions were based on former experiences and might be an expression of fear. Dawkins (2006) pointed out that animals remember their experience with humans and adapt their behaviour accordingly. Many routines on farms are associated with stress or pain for the cow (Raussi, 2003), especially veterinary treatments or examinations. A stretched neck before the examination might show the expectance of a previous painful experience. For veterinary activities such as transrectal palpation, insemination, treatment and blood sampling, the cows are often driven in the cubicle and retained.

Vocalisation was also described as an indicator for pain or discomfort (Rushen et al., 2008; Schwartzkopf-Genswein et al., 1997), hunger (Watts and Stookey, 2000), heat (Schön et al., 2007) or social separation (Müller and Schrader, 2005; Rushen et al., 2001). In our study only few cows showed vocalisation before (n = 5) or during examination (n = 3). This is in line with the observation that concise characteristics like vocalisation usually occur in long-term stressful situations (George, 2003). We assume that the duration of the entire process (i.e. cleaning and vaginal examination) was too short (maximum of 1 min) for the induction of long-term stress related reactions.

In our study, we observed an increase of avoidance reactions from the period before examination to the period during examination and fewer cows were standing still, when vaginal examination had started. They started to trip or to step sideways. The increase in avoidance reactions may be an indicator for increased stress. Vaginal examination is more painful than mere restraining and cleaning, because a certain physical resistance makes the examination more invasive. This assumption is supported by the result that the median ARS increases during examination in both groups.

4.5. Repetitive examinations and methods

'Freeze' pattern in behaviour, such as leaning against the divider, arching the back or stretching the neck, can be observed often in cows facing a potential danger (Rushen et al., 2001). Interactions between humans and cows are a part of the cow's mental well-being and play an important role on commercial dairy farms. Some studies provide evidence that fearful behaviour towards humans strongly depends on the intensity of the environmental interference (Munksgaard et al., 2001; Rushen et al., 2001; Waiblinger et al., 2004). We could

not observe a noteworthy increase in ARS from the first to repeated vaginal examinations. Therefore we assume that the experience – even though stressful per se – was not so intense that an increased stress behaviour was transferred to the next examination as has been described previously for handling experiences (Rushen et al., 1999). Cows with a high ARS at the first examination also had a high ARS during following examinations, and cows with a low ARS did not respond with more intense avoidance reactions to following examinations.

Stress behaviour can, also, be transferred easily from former handling experiences to routine inspections or veterinary treatments that require restraining the cows (Hemsworth, 2003). Furthermore, various factors influence the cow's mental state and beneficial experiences might decrease the averseness of a stimulus (Mason and Mendl, 1993).

To learn about behavioural reactions to a certain feeling, it is important to analyse farm animals' mental states and emotions (Désiré et al., 2002). Observations of cow's reactions in different environments can help to create husbandry facilities which increase animal welfare on farms (Grandin, 2003). We speculate that the procedure of vaginal examination per se affects the cow's behaviour.

The examiner's hand with a circumference of 20.7 cm exceeded the circumference of the Metricheck device of 9.7 cm. Therefore, we had hypothesized that the manual examination would be more invasive compared to the examination with the Metricheck device, and cows of the group MD showed less avoidance reactions compared to cows in the group GH. The Metricheck device is more suitable for the detection of purulent vaginal discharge than the gloved hand (Pleticha et al., 2009). The examiner, however, can react faster to the cow's avoidance reactions and, thus, decrease the risk of injuries during examination. We assume that many examiners' hands exceed the circumference of 20.7 cm of the investigator's hand of this study. Therefore, future research should elucidate whether the circumference of the examiner's hand might influence the intensity of discomfort.

4.6. Other factors

An association between VDS and ARS did not exist. Obviously, pain due to a local inflammation of the vaginal mucosa does not increase the cow's sensitivity in regard to tactile contact of the vagina and the perivaginal region, despite the observation that pain perception increases in an inflamed area (Fitzpatrick et al., 1998).

During the dry-off period, which lasted six to eight weeks, cows did not have had much human contact. The calving marks the beginning of a period with increased human contact. Our results show, that the state of lactation as determined by DIM did not influence the ARS. Considering DIM, cows showed the same increase in the ARS before compared to during examination in all four groups.

The ARS was compared between primiparous (first calf heifers) and multiparous cows (cows in second lactation or higher), to obtain a similar number of animals in both groups.

Raussi (2003) stated that interactions of older cows with humans are more stable compared to heifers. Surprisingly, our data did not confirm an effect of parity on behavioural reactions. Primiparous cows (n = 196) were not used to the procedure of vaginal examination. They had only experienced a transrectal palpation at artificial insemination (AI) and pregnancy diagnosis. Harding et al. (2004) showed that an animal's behaviour is affected by its previous experiences or environmental conditions. Our results confirm these findings, because first calf heifers in our study were used to the barn at least four month before examination. Moreover, housing conditions were the same for heifers and older cows. They were all used to human contact to various persons. The ARS of primiparous cows was not higher compared to multiparous cows. This finding underlines the individuality of behaviour.

Additional research including physiological parameters such as heart rate measurement as suggested (Koolhaas et al., 1997) is warranted to further elucidate the effect of potentially stressful or painful routine procedures on dairy farms.

5. Conclusions

Modern animal husbandry faces the need to reconcile necessary procedures with animal welfare (Weary et al., 2006). We demonstrated that behavioural reactions caused by potentially stressful examination procedures can be assessed with a score. Our data provide evidence that the ARS – although based on subjective observations– does provide a reasonable measure of behavioural reactions and can be a practical and useful tool in the field and in research to estimate a cow's level of discomfort or stress. Moderate repeatability proves that the ARS can be applied easily.

Our results demonstrated that vaginal examinations provoke more avoidance reactions in cows than mere restraining in the cubicle, so it can be considered a challenging and stressful situation. However, vaginal examinations are an important mean to diagnose uterine diseases and cows benefit from early treatment. Our results show that the examination with the Metricheck device is less invasive than the manual examination.

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Tables

Table 1. Frequency distribution of behavioural reactions before vaginal examination conducted with the gloved hand (n = 219) or the Metricheck device (n = 216)

Table 2. Frequency distribution of behavioural reactions during vaginal examination conducted with the gloved hand (n = 219) or the Metricheck device (n = 216)

Table 3. Frequency distribution of the avoidance reactivity score before vaginal examination conducted with the gloved hand (n = 219) or the Metricheck device (n = 216)

Table 4. Frequency distribution of the avoidance reactivity score during vaginal examination conducted with the gloved hand (n = 219) or the Metricheck device (n = 216)

Figures

Figure 1. Frequency distribution of the ARS before and during examination considering the method of vaginal examination

Table 1. Frequency distribution of behavioural reactions before vaginal examination conducted with the gloved hand (n = 219) or the Metricheck device (n = 216)

	GH[1]		MD[2]		Cows in total	
	absolute	%	absolute	%	absolute	%
Evasive Reactions						
Standing still	89	40,6	107	49,5	196	45,1
Tripping	55	25,1	52	24,1	107	24,6
Moving sideways	60	27,4	45	20,8	105	24,1
Leaning against divider	8	3,7	4	1,9	12	2,8
Kicking out	2	0,9	2	0,9	4	0,9
Escape	5	2,3	6	2,8	11	2,5
Signals of discomfort[3]						
Arching back	6	2,7	5	2,3	11	2,5
Stretching neck	137	62,6	126	58,3	263	60,5
Vocalisation	2	0,9	3	1,4	5	1,1

[1]GH: Gloved hand

[2]MD: Metricheck® device

[3]Note that percentage values of signals of discomfort do not equal 100 %, because cows can show up to all three signals of discomfort together or no signals at all.

Table 2. Frequency distribution of behavioural reactions during vaginal examination conducted with the gloved hand (n = 219) or the Metricheck device (n = 216)

	GH[1]		MD[2]		Cows in total	
	absolute	%	absolute	%	absolute	%
Evasive Reactions						
Standing still	69	31,5	84	38,9	153	35,2
Tripping	73	33,3	62	28,7	135	31
Moving sideways	63	28,8	54	25	117	26,9
Leaning against divider	12	5,4	12	5,5	24	5,5
Kicking out	1	0,5	1	0,5	2	0,5
Escape	1	0,5	3	1,4	4	0,9
Signals of discomfort[3]						
Arching back	213	97,3	192	88,9	405	93,1
Stretching neck	193	88,1	166	76,9	359	82,5
Vocalisation	1	0,5	2	0,9	3	0,7

[1]GH: Gloved hand

[2]MD: Metricheck® device

[3]Note that percentage values of signals of discomfort do not equal 100 %, because cows can show up to all three signals of discomfort together or no signals at all.

Table 3. Frequency distribution of the avoidance reactivity score before vaginal examination conducted with the gloved hand (n = 219) or the Metricheck device (n = 216)

	GH[2]		MD[3]		Cows in total	
ARS[1]	absolute	%	absolute	%	absolute	%
0	32	14.6	42	19.4	74	17.0
1	75	34.2	84	38.	159	36.6
2	58	26.5	49	22.7	107	24.6
3	42	19.2	34	15.7	76	17.5
4	12	5.5	7	3.2	19	4.4
5	0	0.0	0	0.0	0	0.0
6	0	0.0	0	0.0	0	0.0

[1]Avoidance reactivity score

[2]Gloved hand

[3]Metricheck® device

Table 4. Frequency distribution of the avoidance reactivity score during vaginal examination conducted with the gloved hand (n = 219) or the Metricheck device (n = 216)

	GH[2]		MD[3]		Cows in total	
ARS[1]	absolute	%	absolute	%	absolute	%
0	0	0	2	0,9	2	0,5
1	12	5,5	23	10,6	35	8
2	66	30,1	80	37	146	33,6
3	74	33,8	61	28,2	135	31
4	57	26	38	17,6	95	21,8
5	10	4,6	12	5,6	22	5,1
6	0	0	0	0	0	0

[1]Avoidance reactivity score

[2]Gloved hand

[3]Metricheck® device

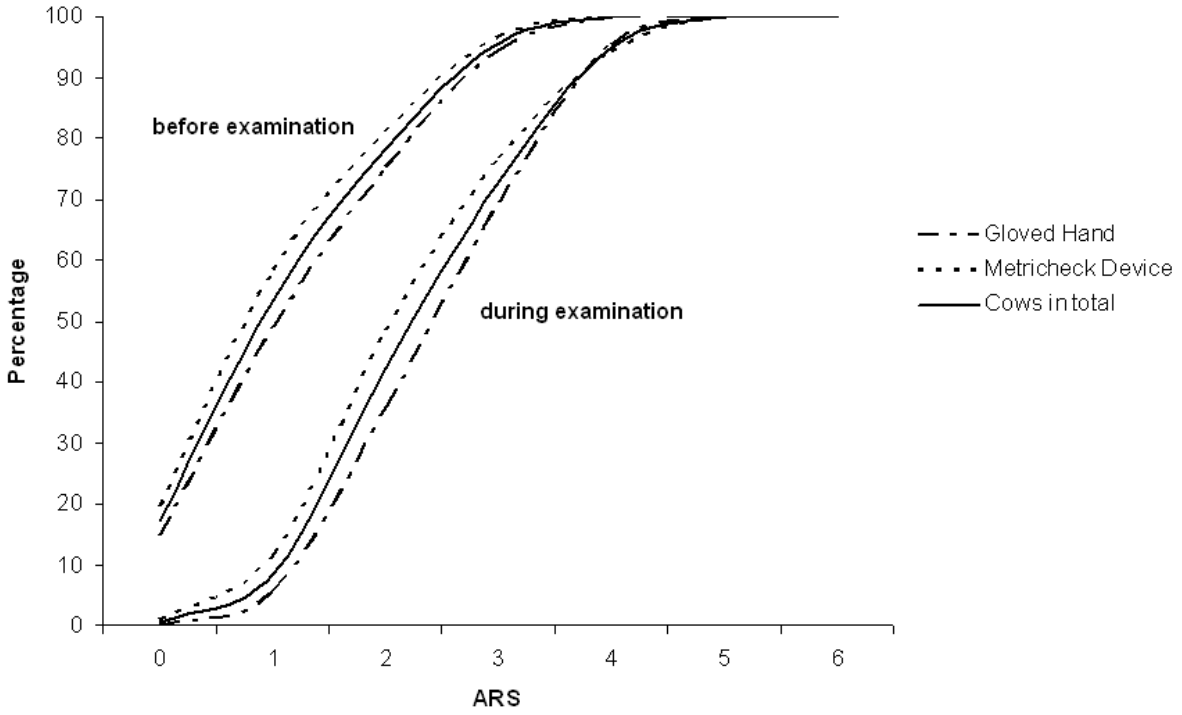

Figure 1. Frequency distribution of the ARS before and during examination considering the method of vaginal examination

2.2. Behavioural and physiological assessment of stress reactions during vaginal examination in dairy cows

Behavioural and physiological assessment of stress reactions during vaginal examination in dairy cows

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Summary

Objectives and aim: The objective of this study was to determine the origin of an arched back during vaginal examination. Moreover, we tested the hypothesis whether the duration of an arched back and avoidance reactions during vaginal examination can be decreased by epidural anaesthesia or analgesic treatment. Material and methods: Behaviour during cleaning the perivaginal region and vaginal examination was scored using the avoidance reactivity score (ARS). Heart rate (HR) was recorded in 10 dairy cows considering four experimental phases, i.e. baseline, cleaning the perivaginal region, vaginal examination and post-examination. Each cow was examined three times and received no treatment (CON), an epidural anaesthesia (EPID) or an analgesic treatment (NSAID). The duration of an arched back during and post-examination was measured. Results: The expression of the arched back was shortest in cows of group EPID and longest in cows of group CON. Avoidance reactions did not differ between the cleaning phase and vaginal examination in cows of group EPID. Cows of group CON showed the strongest avoidance reactions during examination, whereas cows of group EPID showed least avoidance reactions. Mean HR increased during cleaning and vaginal examination and decreased post-examination. Mean HR during vaginal examination did not differ between treatment groups. Conclusion: The results show that cows express discomfort during vaginal examination with an increase in avoidance reactions and HR. Although epidural anaesthesia could reduce sensitivity in the perivaginal region, cows still felt the urge to empty the vagina from the examiner's hand and, thus, were arching their back. Clinical relevance: In practice, routine vaginal examinations in dairy cows have not been considered as invasive examinations. Our results show that vaginal examinations indeed do cause discomfort. We do not suggest the application of any anaesthetic treatment as appropriate before routine vaginal examinations. Nonetheless, the examiner should be aware of the stress potential of vaginal examinations and conduct such examinations most carefully.

Key words

Cattle, Vaginal examination, Behaviour, Stress, Animal Welfare

Zusammenfassung

Ziel der Studie war Ursachen von Abwehrverhalten von Kühen bei der vaginalen Untersuchung beurteilen zu können. Es wurde untersucht, ob das Aufkrümmen des Rückens eine reflexartige Handlung durch Reizung des dorsalen Scheidendaches oder eine schmerzvermittelte Ausweichreaktion ist.

Wir stellten die Hypothese auf, dass Dauer der Rückenkrümmung und Stärke der Abwehrreaktionen während der vaginalen Untersuchung durch eine Epiduralanästhesie oder Schmerzmittel beeinflusst werden kann. Material und Methoden: Das Verhalten von Kühen wurde während des Säuberns der Scham und während der vaginalen Untersuchung mittels Avoidance reactivity score (ARS) bewertet. Die Herzfrequenz (HF) von 10 Milchkühen wurde über vier Phasen (Ruhephase, vor, während und nach der vaginalen Untersuchung) gemessen. Jede Kuh wurde dreimal untersucht: ohne Behandlung (CON), mit Epiduralanästhesie (EPID) und mit Schmerzmittel (NSAID). Die Dauer der Rückenkrümmung wurde während und nach der Untersuchung wurde gemessen. Ergebnisse: Die Rückenkrümmung dauerte bei Gruppe EPID am kürzesten und in Gruppe CON am längsten. Kühe der Gruppe EPID zeigten vor und während der Untersuchung gleichbleibende Abwehrreaktionen. Kühe der Gruppe CON zeigten die Stärkste, Kühe der Gruppe EPID die geringste Abwehr. Die mittlere HF stieg beim Säubern der Scham und während der vaginalen Untersuchung an, nach der Untersuchung fiel sie ab. Die mittlere HF unterschied sich während der Untersuchung zwischen den Behandlungsgruppen nicht. Schlussfolgerung: Unsere Ergebnisse zeigen, dass Kühe ein Unwohlsein während der vaginalen Untersuchung durch Abwehrreaktionen zeigen und es zu einem Anstieg der HF kommt. In der Gruppe EPID und NSAID waren die Abwehrreaktionen vermindert. Die Herzfrequenz war in allen Behandlungsgruppen gleich. Wir schlussfolgern, dass schon das Handling vor der vaginalen Untersuchung zu stressassoziiertem Verhalten führt. Wir vermuten, dass die Epiduralanästhesie zwar zur Schmerzausschaltung führte. Diese Tiere zeigten jedoch trotz medikamentöser Beeinflussung einen, wenn auch verminderten, Entleerungsreflex. Klinische Relevanz: In der Praxis werden vaginale Routineuntersuchungen von Kühen im Rahmen des Reproduktionsmanagementes nicht als invasiv angesehen. Unsere Ergebnisse zeigen jedoch, dass Kühe während dieser Untersuchungen Unwohlsein spüren und ausdrücken. Wir haben keine Hinweise auf eine Schmerzreaktion gefunden. Dennoch sollte sich der Untersucher im Vorfeld der Untersuchung bewusst machen, dass die Untersuchungen für die Kuh unangenehm sind und diese daher mit größtmöglicher Sorgfalt durchführen.

Schlüsselwörter

Rind, Vaginale Untersuchung, Verhalten, Stress, Animal Welfare

Introduction

Vaginal examinations in cows are routine procedures on dairy farms for detecting fertility reducing diseases such as endometritis (19). Handling cows is a potentially stressful intervention (25, 29). Stress behaviour of cows includes various reactions of cows, such as locomotion or vocalisation (37). In a previous study, we assessed stress responses in cows during vaginal examination and validated a 6-point scoring system (avoidance reactivity score, ARS) based on behavioural observations (30). The ARS consists of evasive reactions (i.e., standing still, tripping, stepping sideways, kicking, leaning against the divider and escape) and signals of discomfort (i.e., arching the back, stretching the neck, vocalisation). Using the ARS to evaluate avoidance reactions, behaviour of cows can be scored with 0 (no avoidance reactivity) up to 6 points (strong avoidance reactivity). The most prominent reaction in this study was the arched back (Fig. 1), shown by nearly all of the cows during examination. To our knowledge, information on the aetiology of an arched back during vaginal examination (i.e. mere reflex or pain-associated behaviour) is not available.

Cows physiologically arch their spine during emiction and defecation (35), parturition (17) and pathologically during lameness (3, 8). The arched back is anatomically described as dorsoflexion caused by contraction of the abdominal and internal lumbar muscles, which are innervated by the corresponding ventral branches of the intercostal and lumbar nerves (4, 28). Tactile contact to the vagina stimulates these nerves, the abdominal muscles are activated and the cow arches its back (28).

Epidural anaesthesia or analgesic treatment can affect the transfer of tactile stimuli via the lumbar nerves and might provide insights into the physiological principles of the arched back. It reduces pain related to obstetrical or surgical procedures in the pelvic and perianal area in cows (34). Non-steroidal anti-inflammatory drugs (NSAID) are assumed to affect neural pain associated pathways and, thus, can reduce stress and pain in cattle during human handling and treatment (7). Physiological and behavioural adaptations enable the cow to cope with challenging situations (16) and, thus, changes in heart rate (HR) can be an indicator for stress (13). The objective of this study was to determine the origin of an arched back during vaginal examination. Specifically, we set out to investigate whether the duration of an arched back during vaginal examination and avoidance reactions can be decreased by epidural anaesthesia or analgesic treatment.

Material and Methods

The study was conducted on a commercial dairy farm in Brandenburg, Germany, housing 450 Holstein-Friesian dairies in free stall barns with concrete, slotted floors and cubicles equipped with straw. Vaginal examinations were conducted three times in a total of

ten cows: five cows were examined in November 2010 (replicate 1) and another five cows in December 2010 (replicate 2). The cows had an average age of 4.0 ± 2.0 years (mean ± standard deviation), were 43.8 ± 18.6 days in milk, clinically healthy, not bred and not vaginally examined for postpartum diseases after the last calving. Cows were fixed in the head locks of the feeding panel with two metres space between each cow. During vaginal examination cows either received no treatment (CON), an epidural anaesthesia (EPID) or NSAID. A 10 cm area between the last sacral and the first coccygeal vertebrae on the cow's back was shaved and desinfected with 90 % ethanol. Epidural anaesthesia was conducted using a procaine solution (100 mg, Procasel 2 %®, Selectavet, Weyarn-Holzolling, Germany) and applied 25 min before vaginal examination began. Efficacy was evaluated in every cow and considered as successful when the cow did not show any tension of its tail or the ability to move the tail actively after manual manipulation. Analgesic treatment consisted of 1,500 mg ketoprofen (15.0 ml Romefen 10 %®, Merial, Hallbergmoos, Germany; group NSAID) and was administered intravenously 20 min before vaginal examination began. The sequence of treatments was CON (day 0), EPID (day 2), NSAID (day 9) and NSAID (day 0), CON (day 2), EPID (day 9) for replicate 1 and 2, respectively.

An elastic girth was attached to each cow's chest and a HR belt with two electrodes (Polar Equine RS800CX®, Polar, Kempele, Finland) was connected with the girth. Heart rate was measured in beats per minute (bpm). A baseline HR was recorded for 5 min after the cows were habituated to the equipment (20 min). Following, the cows' perivaginal region was cleaned with dry paper towels (cleaning; 1 min) and a vaginal examination was conducted with the gloved and lubricated hand (10 s). Heart rate recordings continued after examination for 5 min. The experiment was videotaped and behavioural reactions of the cows were analysed on the same day by the examiner. There was one female examiner who conducted all vaginal examinations during this study. She was used to these examinations in dairy cows for two years. The frequency of examinations was adapted to her constitution. The circumference of her hand was 20.7 cm. Behavioural reactions during cleaning and vaginal examination were classified with the ARS (30) and the median ARS was calculated for the groups CON, EPID and NSAID. The duration of the arched back during vaginal examination was measured with a stop watch. Cows were managed according to the guidelines set by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicine Products (11). The experimental procedures reported herein were conducted with the approval of the Institutional Animal Care and Use Committee.

Data from the heart rate receiver was downloaded via an infrared port into the software Polar Pro Trainer and converted into Excel spread sheets (Version 2003, Microsoft Corporation, Redmont, USA). Durations of the cleaning phase, vaginal examination and expression of the arched back were documented in the same Excel spread sheets. Data was analysed using PASW Statistics (Version 18.0, SPSS Inc. Munich, Germany). HR data and

durations of the phases (i.e. baseline, cleaning, examination, post-examination) and expression of the arched back were tested for normal distribution using the Kolmogorov-Smirnov test. The effects of vaginal examination on stress responses were analysed using a linear mixed model ANOVA. Cow within groups was included as random effect. Phases were considered as the repeated measure. First model was built using HR as dependent variable, treatment (CON, EPID and NSAID) and phases were included as fixed factors. Second and third model were built, in order to evaluate the effect of treatment on expression of arched back and ARS, respectively. Post hoc comparison was carried out applying LSD test. The significance level was set at 0.05. The post hoc power analysis for the effect of heart rate and duration of the arched back in the three treatment groups was performed using the software G*Power 3 (Version 3.1.4, Heinrich-Heine-Universität Düsseldorf, Germany). Interpretation of statistical power ($P = 1 - \beta$) and effect size (f) was carried out as described by Prajapati et al. (31).

Results

In total, 30 vaginal examinations of 10 cows were conducted. The durations of baseline and post-examination were 300 sec in all treatments (CON, EPID, NSAID). Due to different degrees of dirtiness of the perivaginal region, the cleaning phase lasted 57.3 ± 5.6, 53.8 ± 10.8 and 56.4 ± 10.1 sec for the cows in groups CON, EPID and NSAID, respectively ($p > 0.05$). The vaginal examination phase lasted 10.8 ± 2.8, 9.7 ± 1.8 and 10.4 ± 1.5 sec for the groups CON, EPID and NSAID, respectively ($p > 0.05$).

Behavioural parameters

Power analysis and effect size considering the duration of arched back and cow numbers of three treatment groups were $P = 1 - \beta = 0.92$ and $f = 0.71$, respectively. In the groups CON (n = 10) and NSAID (n = 10) ten cows and in group EPID (n = 10) seven cows arched their back during vaginal examination. Expression of the arched back lasted 51.0 ± 44.4, 7.4 ± 11.8 and 28.4 ± 19.6 sec in groups CON, EPID and NSAID respectively, (Fig. 2; $p < 0.05$). Cows continued arching their back after the vaginal examination had ended (i.e. post-examination) for another 43.3 ± 43.2, 4.9 ± 9.9 and 22.5 ± 18.2 sec in groups CON, EPID and NSAID respectively (Fig. 2; $p < 0.05$).

The median ARS during examination differed between all treatment groups (Fig. 3; $p < 0.05$). The median ARS increased from 2 during cleaning phase to 4 and 3 during vaginal examination in group CON and NSAID, respectively ($p < 0.05$). The median ARS of 1 did not change during cleaning and vaginal examination in group EPID.

Heart rate measurements

Power analysis and effect size considering heart rate and cow number in the treatment groups were $P = 1 - \beta = 1.00$ and $f = 0.71$, respectively.

Heart rate data was normally distributed ($p > 0.05$). Heart rate was recorded in 10 cows of groups EPID and NSAID and - due to an error during heart rate recordings – in nine cows of group CON. Mean HR differed among cows in different treatment groups and within the phases (Tab. 1). Within the treatment groups, mean HR during cleaning increased by 4.8, 4.9 and 5.6 bpm compared to the baseline HR in cows of groups CON, EPID and NSAID, respectively ($p < 0.05$). Mean HR during vaginal examination increased by 5.1, 4.9 and 7.1 bpm compared to the baseline HR in groups CON, EPID and NSAID, respectively ($p < 0.05$). Mean HR post-examination decreased compared to mean HR during examination in all treatment groups. Mean baseline HR and mean HR during vaginal examination did not differ between treatment groups ($p > 0.05$), but mean HR during cleaning did differ between groups CON and EPID or EPID and NSAID ($p < 0.05$). Mean HR post-examination also differed between groups CON and EPID or EPID and NSAID ($p < 0.05$). In group EPID and NSAID mean HR post-examination decreased compared to mean HR during cleaning ($p < 0.05$).

Discussion

The durations of the cleaning phase and vaginal examination correspond well with the durations of these manipulations conducted during routine examinations by experienced practitioners of the cooperating veterinary practice.

The effect size of cows on the basis of duration of arched back is large according to Prajapati et al. (31). The chance of rejecting the null hypothesis in error is 92 %, which demonstrates excellent statistical power for the arched back data (31). The effect size of cows on the basis of heart rate is large according to Prajapati et al. (31). The chance of accepting the null hypothesis in error is 0.0 or 0 %, which demonstrates excellent statistical power for the heart rate data (31).

The experiment was conducted on a commercial dairy farm and integrated into its protocol for monitoring postpartum cows. Behaviour of cows is strongly influenced by human interactions such as feeding, leading the cows to the milking parlour or cleaning the cubicles (12). Therefore potentially confounding activities were excluded during the observation period.

To avoid such confounders our study required complete absence of activities and quietness during the experiments. The weekly work routine of the farm had dictated the 2 and 7 day intervals for replicate 1 (day 0: CON, day 2: EPID, day 9: NSAID and replicate 2

(day 0: NSAID, day 2: CON, day 9: EPID). We are aware of the possibility that the treatments might have affected each other. An epidural anaesthesia, however, is only efficacious for 1 to 2 hours and long term effects have not been described (23, 36). Therefore, a carryover effect from the epidural anaesthesia administered on day 2 onto day 9 at which the cows were treated with NSAID in replicate 1 is highly unlikely. The effect of ketoprofen lasts only 6 to 10 hours (2, 7, 22). Pain can be reduced but not completely eliminated by a single injection of ketoprofen and pharmacological effects 24 hours after application are not described (7, 22). Thus, we do not assume that there were any relevant effects of the first medication at the time of the following examination 2 and 7 days later, respectively.

It is well known that scoring behavioural observations is influenced by the individual observer (1, 32). The arched back is a signal of discomfort and the mere occurrence of that signal is validated in the ARS (30). In the present study we rated the behaviour using the ARS. Additionally, we measured the precise duration of the arched back. The combination of different sampling techniques can increase the informative contents of behavioural observations (1).

Behavioural parameters

To ensure an efficacious anaesthesia, the epidural anaesthetic was administered 25 min before vaginal examination, which is even earlier than the 10 to 15 min time which is at least required (34). Ketoprofen was administered 20 min before the vaginal examination started, an interval chosen due to descriptions in the literature (7, 22). The cited studies provide evidence that epidural anaesthesia and analgesia were efficacious after the appropriate interval described above. Thus, we assume efficacy of epidural anaesthesia or ketoprofen at the beginning of our experiment.

Cows in group NSAID arched their back for a longer time than cows in group EPID ($p < 0.05$), but considerably shorter than cows in group CON ($p < 0.05$). Cows in group CON arched their back for nearly 1 min although vaginal examination only lasted 10 seconds. Reference data for the duration of an arched back or other physiological measures during vaginal examination are not available in the recent literature. We assume that due to pharmacological effects of epidural anaesthesia or analgesic treatment cows were able to relieve the discomfort of manual manipulation before and during examination faster than untreated cows. We conclude that the application of ketoprofen reduced the pain sensation of the caudal part of the vagina, but did not influence the urge to empty the vagina from the examiner's hand due to experienced discomfort.

Avoidance reactions during vaginal examination compared to the cleaning phase increased in groups CON and NSAID. The increase of the ARS in these two groups is equivalent to the increase of mean HR and agrees with previous findings, which proved that

vaginal examinations cause increased avoidance reactions in cows (30). Interestingly, avoidance reactions were the same during cleaning and vaginal examination in cows receiving an epidural anaesthesia. Thus, we speculate that the epidural anaesthesia blocked the transfer of stimuli to the pain receptors of the sacral nerves in the spinal cord. This reduced sensitivity of the vagina could have led to the shorter duration of an arched the back in groups EPID or NSAID and the missing increase in avoidance reactions in cows of group EPID.

The extension of epidural anaesthesia varies depending on the amount of epidural fat (20). An epidural solution of 5 ml can spread out across 3 spinal segments in maximum (21). Thus, we assume that a volume of 5 ml anaesthetic as administered between the last sacral and first coccygeal vertebra in our study was sufficient for perivaginal anaesthesia, which also includes the vagina. Nevertheless, seven cows in group EPID arched their back demonstrating the ability to activate their muscles despite anaesthesia. Although those cows also received a sufficient dose of anaesthetics we assume that these cows experienced discomfort, but not pain during vaginal examination and tried to ease it by arching their backs.

Similar to HR, the avoidance reactivity score varied between individual cows. This observation has been considered previously as an indicator for cows reacting individually to stress (30).

In the present study, we included heart rate measurements to confirm the validity of the ARS. Also, the duration of specific reactions (i.e., the arched back) was timed. Nonetheless, the interpretation of sensations of pain or discomfort by physiological parameters like heart rate – although an objective measure – remains difficult, because they also reflect autonomous responses (6).

Heart rate measurements

The baseline heart rate was comparable to the average heart rates described for lactating cows by Laister et al. (18).

The increase in HR during cleaning was slightly higher than those detected in cows which experienced sudden human contact in form of stroking different body regions (33). The increase of mean HR during vaginal examination is comparable to the increase in HR of up to 7 bpm in cows during a test period including transrectal palpation and sham insemination conducted by an unknown person (38). A perception of stress might increase the sympathetic outflow of the central nervous system (24), which can be reflected by changes in HR (9). We assume that the increase in HR during cleaning or vaginal examination in both groups might be an indication of stress due to discomfort caused by the manual manipulation. In the present study, mean HR during cleaning und vaginal examination was also comparable to the mean HR found in cows during venepuncture for blood collection (15).

Since mean HR decreased post-examination, we speculate that the impact of the stressor abated within a 5 min period in all cows independent from treatment. Data on the duration of the impact of human contact are not described in the studies cited above.

Heart rate also reflects physical activity (10) and cows in group EPID arched their back post-examination for shorter time than cows in group CON or NSAID ($p < 0.05$). Thus, continuous elevated HR during cleaning might also be caused by longer lasting muscle activation for keeping the back arched.

Epidural anaesthesia blocks the tactile sensitivity of the vagina and perivaginal region (34), whereas NSAID affect the pain transmission (36). The tactile sensitivity was not affected pharmacologically in cows of group CON. Therefore, we assume that cows, which received an epidural anaesthesia or analgesic treatment, experienced the human contact as less disturbing.

The heart rate monitor used in this study has been validated for the use in cows (14). It has been demonstrated that HR measurements seem to be an effective and suitable tool to analyse stress in cows (13, 38). Furthermore, cows did not show signs of being hampered by the equipment (14).

Heart rate variability (HRV), which describes cardiac activity more precisely than mere HR (26), requires a recording interval of at least 30 seconds for time and frequency and 20 minutes for nonlinear parameters (5). Due to the short durations of the cleaning phase and the vaginal examination (adapted to routine examinations in the field), HRV could not be analysed in the present study.

Conclusions

The results show that cows experience discomfort during vaginal examination. They react with an increase in avoidance reactions and heart rate. Both could be alleviated by epidural anaesthesia or analgesic treatment. We consider the arched back as a signal of discomfort. Our results indicate that arching the back is neither clear pain behaviour nor a mere reflex. We speculate that cows have the urge to empty the vagina from the examiner's hand independently from pharmacological treatment. In practice, vaginal examinations are predictive for reproductive performance in dairy herds (19). Although vaginal examinations with a speculum (i.e., vaginoscopy) have already been considered as stressful for cows (27), such reproductive examinations traditionally are not considered as invasive. In the present study we demonstrated that vaginal examinations conducted with a gloved hand also have the potential to cause discomfort in cows.

In the present study, all examinations were conducted by an examiner whose circumference of the hand was 20.7 cm. Compared to other practitioners' hands of the cooperating veterinary practice this circumference is relatively small. Thus, further research is

warranted to examine how discomfort during vaginal examinations can be reduced by modifying the invasiveness of such examinations (e.g. circumference of examination tools, speed of penetration).

Clinical relevance

In practice, routine vaginal examinations to manage reproductive health in dairy cows have not been considered as invasive examinations yet, but our results show that vaginal examinations do cause discomfort in dairy cows. We do not claim the application of such treatment as appropriate before routine vaginal examinations. Nonetheless, the examiner should be aware of the stress potential of vaginal examinations and conduct such examinations most carefully.

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Conflict of interest

None of the authors have any conflict of interest to declare.

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List of figures

List of tables

Figures

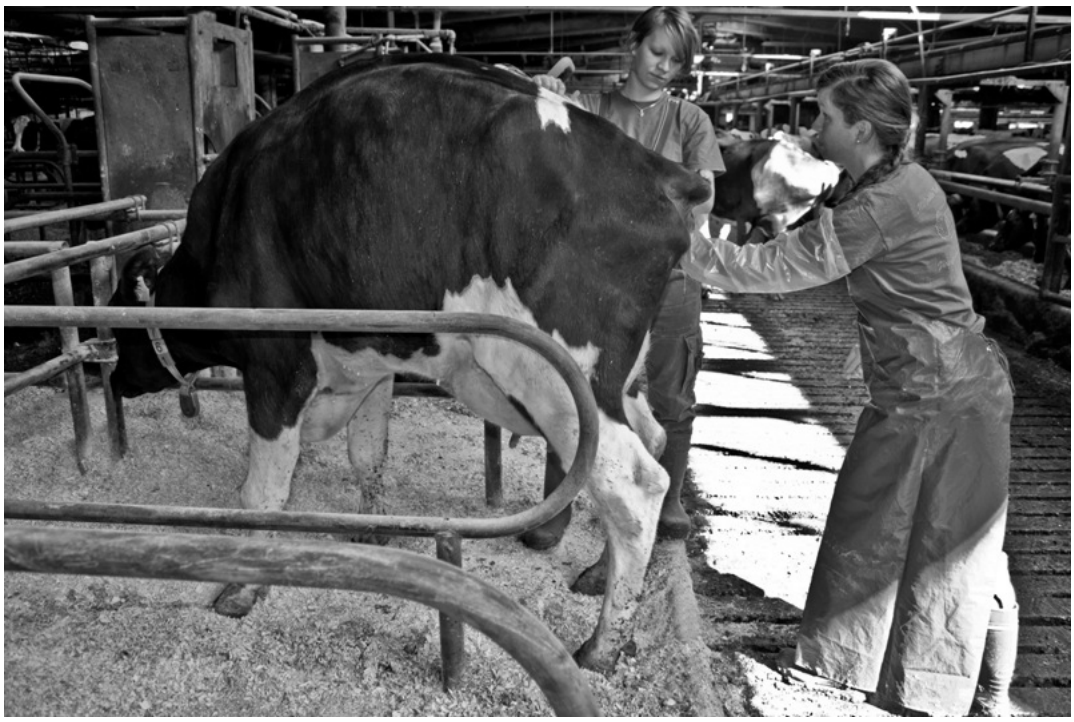

Figure 1. Cow with an arched back during vaginal examination.

Fig. 1. Kuh, die während der vaginalen Untersuchung den Rücken aufkrümmt.

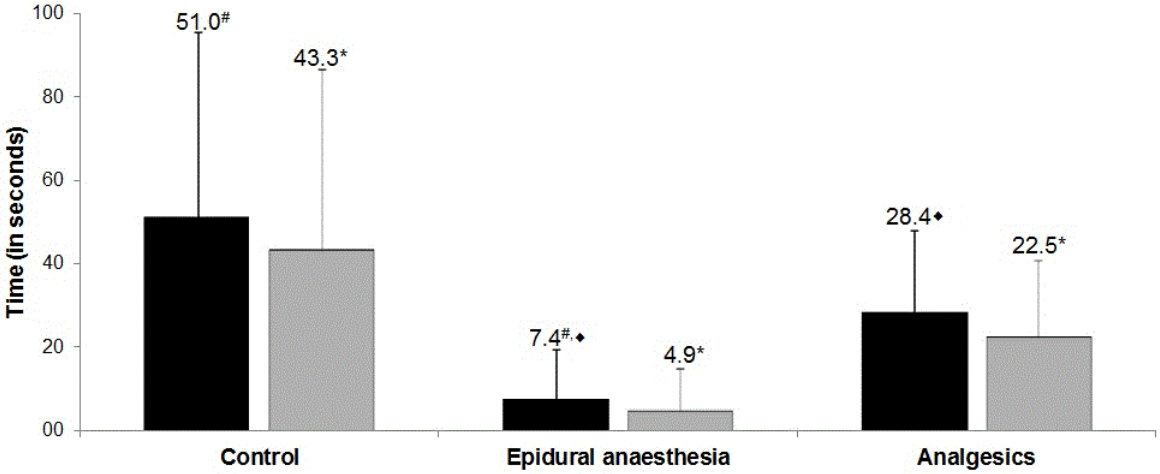

Figure 2. Durations of arched back in total (■ black bars) and after vaginal examination had ended (■ grey bars) in three treatment groups (n = 30; p-Values with the same icons (#,*,◆) differ significantly; $p < 0.05$).

Fig. 2. Gesamtdauer der Rückenkrümmung (■ schwarze Balken) und Dauer der Rückenkrümmung nach Beendigung der vaginalen Untersuchung (■ graue Balken) in den drei Behandlungsgruppen (n = 30; p-Werte mit den gleichen Symbolen (#,*,◆) unterscheiden sich signifikant; $p < 0,05$).

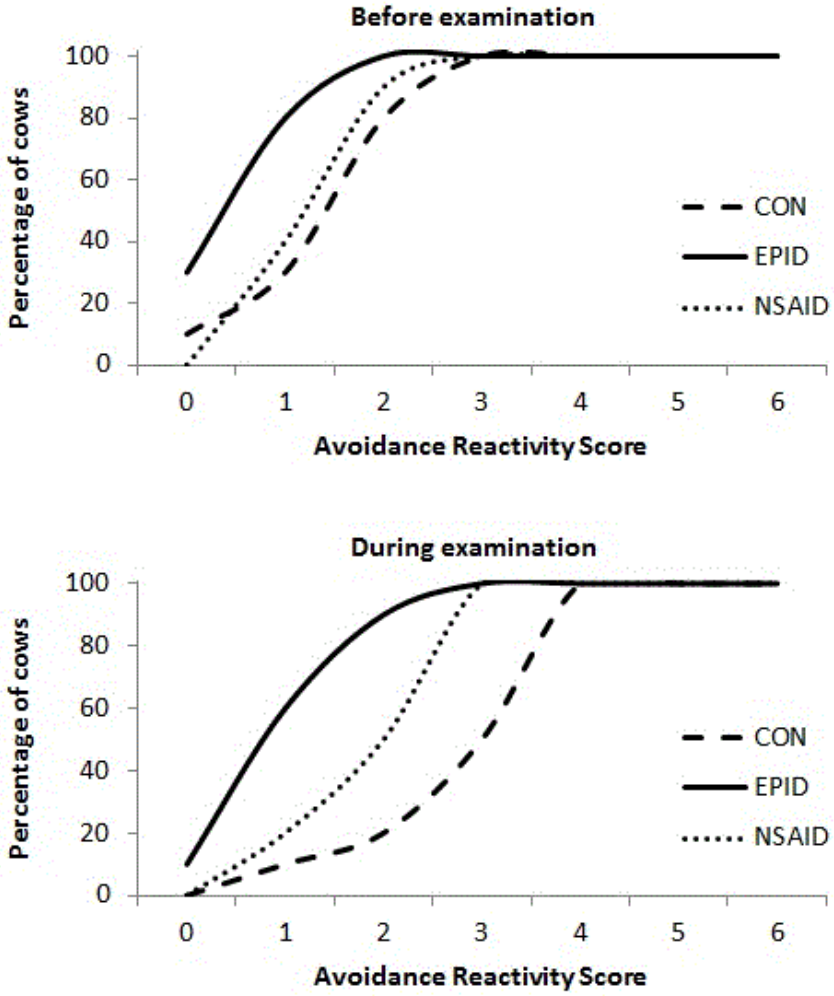

Figure 3. Cumulative percentage distribution of the avoidance reactivity score (ARS) before and during examination considering three treatments (n = 30; CON = no treatment; EPID = epidural anaesthesia; NSAID = analgesic treatment).

Fig. 3. Prozentuale Verteilung des Avoidance Reactivity Score (ARS) vor und während der vaginalen Untersuchung in den drei Behandlungsgruppen (n = 30; CON = keine Behandlung; EPID = Epiduralanästhesie; NSAID = Schmerzmittel).

Tables

Table 1. Heart rate (beats per minute; mean ± standard deviation) of 29 (CON) and 30 (EPID; NSAID) cows before, during and after vaginal examination.

Tab. 1. Herzfrequenz (Schläge pro Minute; Mittelwert ± Standardabweichung) von 29 (CON) bzw. 30 (EPID; NSAID) Kühen vor, während und nach der vaginalen Untersuchung.

Group	Baseline	Cleaning	Vaginal examination	Post-examination	*P*-value[5]
CON[1]	91.1 ± 4.6^{a,b}	96.2 ± 5.6$^{a\ *}$	96.4 ± 7.4^{b,c}	92.7 ± 4.0$^{c\ *}$	0.026
EPID[2]	86.5 ± 6.7^{a,b}	91.3 ± 8.6$^{a,c\ *\#}$	91.4 ± 7.5^{b,d}	87.4 ± 6.9$^{c,d\ *,\#}$	0.002
NSAID[3]	90.4 ± 6.4^{a,b}	96.0 ± 5.3$^{a,c\ \#}$	97.5 ± 5.3^{b,d}	93.8 ± 6.2$^{c,d\ \#}$	0.000
P-value[4]	0.173	0.037	0.058	0.030	

[1]CON: Cows without treatment (n = 9)

[2]EPID: Cows with epidural anaesthesia (n = 10; 100 mg procaine hydrochloride)

[3]NSAID: Cows with analgesic treatment (n = 10; 1,500 mg ketoprofen)

[4]within phases between treatments effects

[5]within treatment between phases effects

[a,b,c,d]: values with same superscripts within lines differ ($p < 0.05$)

*, [#]: values with same superscripts within rows differ ($p < 0.05$)

3. DISCUSSION

Physiological and behavioural reactions can be regarded as adaptive mechanisms to stressful situations (Wechsler 1995), and human contact (e.g. veterinary treatment) was considered as potentially stressful to cows (Breuer et al. 2000; Hagen et al. 2004). Thus, the objective of the first study was to create a tool, which allows the assessment of stress responses during vaginal examination directly on the farm. The examiner should be able to both, conduct the examination and assess the cow's behaviour during the procedure and ideally critically reflect the stressfulness of a given procedure. Therefore, we created the avoidance reactivity score (ARS) which included behavioural reactions observable from standing behind the cow. The ARS is a 4-point-scale and consists of evasive reactions and signals of discomfort. Whereas evasive reactions describe the extent of movements of the locomotor system, signals of discomfort contain the occurrence of behavioural reactions of different body regions (i.e. back, neck and vocalisation). The combination of occurrence and extent has already been established in other numerical rating systems (Flower and Weary 2006; Chapinal et al. 2009; Streyl et al. 2011). The reliability of the ARS was proved to be substantial for inter-observer agreement between veterinarians and people not acquainted with farm animals (Landis and Koch 1977). The intra-observer agreement was proved to be almost perfect (Landis and Koch 1977). These results indicate that the ARS can be applied reliably in the stable.

Furthermore, we utilized the ARS on a commercial dairy farm to compare two vaginal examination methods: the gloved hand and the Metricheck® device. Both methods are suitable to detect reproductive performance decreasing diseases (Pleticha et al. 2009). We demonstrated that cows examined with the Metricheck® showed less avoidance reactions compared to cows examined with the gloved hand. We assume that the Metricheck® is less invasive to cows, as the diameter is about half the size smaller than the diameter of the hand of the examiner in our study.

As expected, cows in both examination groups showed higher avoidance reactions during vaginal examination compared to the cleaning period before examination. An increase of behavioural reactions can be regarded as an increased in stress in cows (Wehrend et al. 2006; Von Keyserlingk and Weary 2007). Accordingly, we assume that the vaginal examination is more stressful than mere touching to dairy cows.

Surprisingly, the age of the cows (lactation period) did not influence avoidance reactions. Although interactions of older cows with humans might be more stable (Raussi 2003), in our study, first-calf heifers did not have a different ARS from cows in second

lactation or higher. Housing conditions might have affected the cows' behaviour (Harding et al. 2004). Our first-calf heifers were housed together with older dairies in the same stables for several months before the study started. They were used to daily human contact to various persons. Thus, we assume that their relationship to humans is primarily influenced by their individuality as it is also in older cows.

Some avoidance reactions were already expressed before vaginal examination even started, i.e. the stretched neck (freeze behaviour). Munksgaard et al. (2001) detected fewer movements of legs and tail in the presence of a familiar aversive handler. Such freeze behaviour can be observed more often in cows facing a potential danger (Rushen et al. 2001). Both studies give evidence that fearful behaviour towards humans strongly depends on the situation and the intensity of the environmental interference. Moreover, social learning – the observer cow imitating the behaviour from the demonstrator cow – can affect avoidance reactions, as well (Munksgaard et al. 2001).

Another interesting fact is that repetitive examinations also had no influence on avoidance reactions. This might be evidence for an individual variation in stress behaviour, which is considered as functional for the animal's adaptability to changes in its environment (Koolhaas et al. 2007). Cows are able to transfer behaviour due to previous negative experiences to repeated handling (Rushen et al. 1999) or other routine inspections (Hemsworth 2003). Though, vaginal examinations were – although stressful per se – not that intense that increased avoidance reactions could be observed.

It is well known that scoring behavioural observations is influenced by the individual observer (Altmann 1974; Sambraus 1998). Thus, we used heart rate measurements to validate the ARS in a second study. Additionally, we measured the precise duration of the arched back, because the combination of different sampling techniques can increase the informative contents of behavioural observations (Altmann 1974).

Untreated cows still arched their back for nearly one minute although vaginal examination had already ended. The duration of the arched back decreased in cows treated with epidural anaesthesia (procaine) or with analgesics (ketoprofen). Unfortunately, no reference data considering the durations of an arched back could be found in the recent literature. We speculate that both, procaine and ketoprofen, reduced the pain sensation of the caudal part of the vagina, but not the urge to empty the vagina from the examiner's hand. Our results indicate that cows do experience discomfort during vaginal examination independently from treatment. But cows treated with epidural anaesthesia or analgesics seemed to be able to relieve the discomfort of vaginal examination faster than untreated cows.

Avoidance reactions during vaginal examination were stronger compared to the cleaning period before examination in untreated cows and cows treated with analgesics. Demonstrating that vaginal examinations are more stressful than mere touching, these results agree with the results of the first study. Interestingly, cows which have received an epidural anaesthesia did not show stronger avoidance reactions during vaginal examination. The fact that seven of ten cows were still capable of arching their back shows that cows could activate their muscles despite anaesthesia. We assume that these cows also experienced discomfort during vaginal examination and tried to ease it by arching their back.

An increase in heart rate during vaginal examination in all cows supports the previous results. Nonetheless, heart rate also reflects autonomous responses (Dawkins 2006). In our study, interpretation of heart rate data remains difficult, because increased muscle activation during an arched back might lead to an increase in heart rate due to lasting physical exercise. The increase in heart rate during vaginal examination is comparable to heart rates found in cows during venipuncture for blood collection (Hopster et al. 1995). Thus, we consider that the increase in heart rate during vaginal examination might be an indication of increased discomfort during this procedure.

Heart rate decreased and nearly reached baseline values within five minutes after vaginal examination had ended. Since data on the duration of the impact of human contact is not described in the recent literature, we expect that five minutes are sufficient to abate the stressor of vaginal examinations in dairy cows.

Concluding, we demonstrated that vaginal examinations are stressful to cows. However, they are necessary to detect postpartum diseases and cows benefit from early treatment. Modern animal husbandry faces the need to reconcile necessary procedures with animal welfare (Weary et al. 2006). A positive attitude towards cows can enhance human-cattle interactions (Raussi 2003) and positive experiences affect behaviour during following procedures (Schmied et al. 2008). Although avoidance reactions and heart rate could be alleviated by epidural anaesthesia or analgesic treatment, we do not suggest the application of any pharmacological treatment as appropriate before routine vaginal examinations. Nonetheless, the examiner should always be aware of the stress potential of every vaginal examination and carefully choose an adequate examination method, also considering the impact on avoidance reactions.

4. SUMMARY

The present thesis assesses the stress responses relating to vaginal examinations in dairy cows. Therefore, two consecutive studies were conducted on two commercial dairy farms in Brandenburg, between 2010 and 2011.

Although there is evidence that human contact is potentially stressful for cows, the impact of vaginal examinations on a cow's stress level has not been evaluated. Therefore, we hypothesized that (1) cows show discomfort before and during vaginal examination with different behavioural reactions, (2) these reactions can be semi-quantitatively scored, (3) the heart rate increases during vaginal examination and decreases during the duration of the arched back and (4) the examination with a Metricheck® device is less invasive than the examination with the gloved hand.

In the first study, the behaviour of 10 cows during vaginal examination was videotaped and analysed. In total, 15 different behavioural reactions were identified. Based on these observations, a numerical rating system was created. The avoidance reactivity score (ARS) includes six evasive reactions (standing still, tripping, stepping sideways, kicking, escape, leaning against the divider), which are scored on a 4-point scale. The ARS also includes signals of discomfort (arching the back, stretching the neck, vocalisation), scored with 1 point each. Evasive reactions are counted according to the strength of movement on an alternate scale. Signals of discomfort are counted with 1 point each on a cumulative scale. We also checked the reliability of the ARS by calculating the kappa (κ) coefficients for inter- and intra-observer repeatability. Values near 0 can be interpreted as poor and values near 1 as almost perfect agreement between observers. For inter-observer agreement the coefficients were $\kappa = 0.61$ and $\kappa = 0.71$ between veterinarians and non-veterinarians, respectively. For intra-observer agreement, the coefficient was $\kappa = 0.84$. Moreover, we compared two different examination methods, the Metricheck® device (MD) and the gloved hand (GH). The median ARS increased from 1 before (interquartile range, IQR: 1–2) to 3 during examination (IQR: 2–4) and cows in the group MD showed less avoidance reactions compared to cows in the group GH ($P < 0.05$). Parity, days in milk, vaginal discharge or repeated examinations did not influence the ARS.

In the second study, behaviour during cleaning the perivaginal region and vaginal examination was scored using the avoidance reactivity score (ARS). Heart rate (HR) was recorded in 10 dairy cows considering four experimental phases (i.e. baseline, cleaning the perivaginal region, vaginal examination and post-examination). Each cow was examined three times and received no treatment (CON), an epidural anaesthesia (EPID) or an analgesic

treatment (NSAID). The duration of an arched back during and post-examination was measured. The expression of the arched back was shortest in cows of group EPID and longest in cows of group CON. Avoidance reactions did not differ between the cleaning phase and vaginal examination in cows of group EPID. Cows of group CON showed the strongest avoidance reactions during examination, whereas cows of group EPID showed least avoidance reactions. Mean HR increased during cleaning and vaginal examination and decreased post-examination. Mean HR during vaginal examination did not differ between treatment groups.

The results of the present thesis demonstrate that behavioural reactions can be assessed with a score. Substantial inter-observer and almost perfect intra-observer repeatability proved that the ARS can be applied easily in practice. The ARS – although imperfect – might be a useful tool in the field and in research to estimate a cow's stress level.

Moreover, the results show that vaginal examinations provoke stronger avoidance reactions in cows than mere restraining in the cubicle, so it can be considered a challenging and stressful situation. Cows cope with that challenge with various behavioural reactions and an increase in heart rate. In practice, routine vaginal examinations in dairy cows have not been considered as invasive examinations. However, vaginal examinations are an important mean to diagnose uterine diseases and cows benefit from early treatment. We do not suggest the application of any anaesthetic or analgesic treatment as appropriate before routine vaginal examinations. Nonetheless, the examiner should be aware of the stress potential of vaginal examinations and conduct such examinations most carefully. The examination with the Metricheck® device is less invasive than the manual examination.

5. ZUSAMMENFASSUNG

Stressreaktionen im Zusammenhang mit der vaginalen Untersuchung bei Milchkühen

Die vorliegende Dissertation beschäftigt sich mit der Bewertung von Stressreaktionen von Milchkühen vor und während der vaginalen Untersuchung. Dazu wurden 2010 bis 2011 zwei aufeinander aufbauende Studien auf zwei kommerziellen Milchviehbetrieben in Brandenburg durchgeführt.

Obwohl es in der aktuellen Literatur Hinweise darauf gibt, dass der Kontakt zu Personen für Kühe häufig mit Stress verbunden ist, wurde die Auswirkung vaginaler Untersuchungen auf das Wohlbefinden der Kühe bisher nicht untersucht. Deshalb stellten wir die Hypothesen auf, dass (1) Kühe das Unwohlsein vor und während der vaginalen Untersuchung mittels verschiedener Verhaltensreaktionen ausdrücken, dass (2) die Reaktionen semi-quantitativ bewertet werden können, dass (3) die Herzfrequenz während der vaginalen Untersuchung ansteigt und während der Dauer der Rückenkrümmung abfällt und dass (4) die Untersuchung mit dem Metricheck® weniger invasiv ist als die Untersuchung mit der Hand.

In der ersten Studie wurde das Verhalten von 10 Kühen während der vaginalen Untersuchung auf Video aufgezeichnet und analysiert. Insgesamt wurden 15 verschiedene Verhaltensreaktionen identifiziert, auf deren Grundlage ein numerisches Bewertungssystem erstellt wurde. Dieser Avoidance Reactivity Score (ARS) besteht zum einen aus sechs Ausweichreaktionen (still stehen, trippeln, beiseite gehen, ausschlagen, gegen das Geländer anlehnen und flüchten), welche auf einer Skala von 0 bis 3 Punkten entsprechend der stärksten gezeigten Reaktion alternativ bewertet werden. Zum anderen wurden Signale des Unwohlseins (Rückenkrümmung, Halsstrecken, Lautäußerung) erfasst und kumulativ mit jeweils einem Punkt bewertet. Außerdem wurde die Wiederholbarkeit des ARS mittels Kappa-Koeffizient errechnet (Inter- und Intra-Beobachter-Variabilität) geprüft. Dabei sind Werte nahe 0 als schlechte Übereinstimmung zwischen den Beobachtern, Werte nahe 1 als fast perfekte Übereinstimmung zu werten. Bei der Bewertung der Wiederholbarkeit des ARS betrug die Variabilität zwischen verschiedenen Beobachtern $\kappa = 0{,}61$ bzw. $\kappa = 0{,}71$ (Veterinärmediziner bzw. Nicht-Veterinärmediziner). Die Variabilität zwischen den einzelnen Beobachtungen eines Beobachters lag bei $\kappa = 0{,}84$. Des Weiteren wurden die Abwehrreaktionen bei zwei verschiedenen Methoden – die Untersuchung mittels Metricheck® und mittels Hand – verglichen. Der Median-Wert des ARS betrug vor der Untersuchung 1 (Quartilsabstand: 1–2) und stieg während der Untersuchung auf 3 (Quartilsabstand: 2–4).

Kühe, die mit dem Metricheck® untersucht wurden, zeigten schwächere Abwehrreaktionen als Kühe, die mit der Hand untersucht wurden ($P < 0.05$). Das Alter der Kühe (Anzahl der Laktationsperioden), Qualität des vaginalen Ausflusses, Anzahl der Tage postpartum und wiederholte Untersuchungen hatten keinen Einfluss auf die Stärke der Abwehrreaktionen.

In der zweiten Studie wurde das Verhalten vor und während der vaginalen Untersuchung bei Milchkühen mittels ARS bewertet. Bei 10 Kühen wurde über die vier Phasen Baseline, Säubern der Scham, während und nach der vaginalen Untersuchung die Herzfrequenz gemessen. Jede Kuh wurde dreimal untersucht und blieb dabei unbehandelt (Gruppe CON), erhielt eine Epiduralanästhesie (Gruppe EPID) oder ein Schmerzmittel (Gruppe NSAID). Die Dauer der Rückenkrümmung wurde während und nach der Untersuchung gemessen. Die Rückenkrümmung war in Gruppe EPID am kürzesten und in Gruppe CON am längsten. Die Abwehrreaktionen unterschieden sich zwischen den Phasen Säubern der Scham und während der Untersuchung in Gruppe EPID nicht. Kühe der Gruppe CON zeigten die stärksten Abwehrreaktionen, Kühe der Gruppe EPID die schwächsten. Die mittlere Herzfrequenz stieg während des Säuberns der Scham und der vaginalen Untersuchung an, nach der Untersuchung fiel sie ab. Sie unterschied sich zwischen den drei Behandlungsgruppen nicht.

Die Ergebnisse dieser Dissertation belegen, dass die Verhaltensreaktionen während der vaginalen Untersuchung mittels ARS bewertet werden können. Eine grundlegende Übereinstimmung zwischen verschiedenen Beobachtern und eine fast perfekte Übereinstimmung zwischen den Beobachtungen eines Beobachters beweisen, dass der ARS in der Praxis einfach und zuverlässig angewendet werden kann.

Darüber hinaus zeigen die Ergebnisse, dass vaginale Untersuchungen zu stärkerer Abwehr führen als das bloße Berühren oder Fixieren der Tiere in der Liegebox. Deshalb ist anzunehmen, dass diese Untersuchungen mit Stress für die Kühe verbunden sind. Kühe begegnen diesem Stress mit verschiedenen Verhaltensreaktionen und einem Anstieg der Herzfrequenz. Dennoch werden in der tierärztlichen Praxis vaginale Untersuchungen bisher kaum als invasive Untersuchungen angesehen. Zwar bleibt die vaginale Untersuchung von Kühen nach wie vor ein wichtiges Instrument des Reproduktionsmanagements (Diagnose von puerperalen Störungen). Es ist außerdem nicht angezeigt, die Kühe zwecks Linderung des Unwohlseins vor der Untersuchung medikamentös zu behandeln. Dennoch sollte dem Untersucher stets bewusst sein, dass die Untersuchungen für die Kühe mit Stress verbunden sind und sie entsprechend vorsichtig durchführen. Die Untersuchung mit Metricheck® ist dabei weniger invasiv als die Untersuchung mit der Hand.

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7. VERÖFFENTLICHUNGEN

7.1. Wissenschaftliche Originalien

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7.2. Vorträge

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7.3. Posterpräsentationen

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C. Fischer-Tenhagen, M. Pilz, W. Heuwieser (2012*). Assessment of stress responses and heart rate during vaginal examination in dairy cows.* 1st Dairy Cattle Welfare Symposium, Guelph, Canada.

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M. Pilz, C. Fischer-Tenhagen, G. Thiele, S. Schröder, W. Heuwieser (2012). *Maintenance of pregnancy in a Holstein Friesian dairy cow with an abscess of the uterine wall.* 45. Jahrestagung Physiologie und Pathologie der Fortpflanzung, Berlin. Reprod. Domest. Anim. 47 (s2) S. 41-42.

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9. SELBSTÄNDIGKEITSERKLÄRUNG

Hiermit bestätige ich, dass ich alle Studien selbständig durchgeführt und die vorliegende Arbeit selbständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe.

Tab. 1. Eigener Anteil[1] an den Forschungsprojekten der vorliegenden Dissertation

	Studie 1[*]	Studie 2[#]
Studienplanung	+++	+++
Datenerhebung	+++	+++
Datenanalyse	+++	+++
Verfassen des Manuskripts	+++	+++
Editieren des Manuskripts	++	++

Legende: +++: > 70 %

++: 50-70 %

+: < 50 %

[*]Pilz, M., Fischer-Tenhagen, C., Thiele, G., Tinge, H., Lotz, F., Heuwieser, W. (2012). Behavioural reactions before and during vaginal examination in dairy cows. Appl. Anim. Behav. Sci. 138, 18-27.

[#]Pilz,M., Fischer-Tenhagen, C., Grau, M., Heuwieser, W. (2014). Behavioural and physiological assessment of stress reactions during vaginal examination in dairy cows. Tierärztl. Prax. (G) 42: 88-94.

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