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

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Key words
Cattle, vaginal examination, behaviour, stress, animal welfare

Summary
Objectives and aim: The objective of this study was to determine the origin of an arched back in cows during vaginal examination. Moreover, we tested 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 of the perivaginal region and during 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 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.

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Zusammenfassung

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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, 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 activity 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 emission 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 analgesia 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

Animals and experimental design

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 10 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 2 m 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 disinfected with 90% ethanol. Epidural anaesthesia was conducted using a procaine solution (100 mg, Procasel 2%, Selectavet, Weyarn-Holzolling, Germany) and applied 25 minutes 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 1500 mg ketoprofen (15.0 ml Romefen 10%, Merial, Hallbergmoos, Germany; group NSAID) and was administered intravenously 20 minutes before vaginal examination started. 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 heart rate (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 minutes 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 minutes. The experiment was videotaped and behavioural reactions of the cows were analysed on the same day by the examiner. All vaginal examinations were conducted by one female examiner with a 2-year experience for these examinations. The frequency of examinations...
was adapted to her constitution. The circumference of her hand measured 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 stopwatch. 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.

Statistical analysis

Data from the heart rate receiver was downloaded via an infrared port into the software Polar Pro Trainer and converted into Excel spreadsheets (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 spreadsheets. 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 s in all treatments (CON, EPID, NSAID). Due to different degrees of dirtiness of the perivulvar region, the cleaning phase lasted $57.3 \pm 5.6$, $53.8 \pm 10.8$ and $56.4 \pm 10.1$ s 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 s 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 the three treatment groups were P = 1 – β = 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 s in groups CON, EPID and NSAID, respectively (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 s in groups CON, EPID 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 – β = 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 HR recordings – in nine cows of group CON.

Mean HR differed among cows in different treatment groups (p < 0.05). Mean HR post-examination decreased compared to mean HR during cleaning 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

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Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Cleaning</th>
<th>Vaginal examination</th>
<th>Post-examination</th>
<th>p-value5</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON1</td>
<td>91.1 ± 4.6b&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.2 ± 5.6&lt;sup&gt;∗&lt;/sup&gt;</td>
<td>96.4 ± 7.4&lt;sup&gt;b&lt;/sup&gt;c&lt;sup&gt;a&lt;/sup&gt;</td>
<td>92.7 ± 4.0&lt;sup&gt;∗&lt;/sup&gt;</td>
<td>0.026</td>
</tr>
<tr>
<td>EPID2</td>
<td>86.5 ± 6.7b&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.3 ± 8.6&lt;sup&gt;∗&lt;/sup&gt;&lt;sup&gt;a&lt;/sup&gt;c&lt;sup&gt;b&lt;/sup&gt;</td>
<td>91.4 ± 7.5&lt;sup&gt;d&lt;/sup&gt;d&lt;sup&gt;c&lt;/sup&gt;</td>
<td>87.4 ± 6.9&lt;sup&gt;d&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.002</td>
</tr>
<tr>
<td>NSAID3</td>
<td>90.4 ± 6.4b&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.0 ± 5.3&lt;sup&gt;∗&lt;/sup&gt;&lt;sup&gt;a&lt;/sup&gt;c&lt;sup&gt;b&lt;/sup&gt;</td>
<td>97.5 ± 5.3&lt;sup&gt;d&lt;/sup&gt;d&lt;sup&gt;c&lt;/sup&gt;</td>
<td>93.8 ± 6.2&lt;sup&gt;d&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0.173</td>
<td>0.037</td>
<td>0.058</td>
<td>0.030</td>
<td></td>
</tr>
</tbody>
</table>

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; 1500 mg ketoprofen)
4: within phases between treatments effects
5: within treatment between phases effects
<sup>a,b,c</sup>: values with same superscripts within lines differ (p < 0.05)
<sup>∗</sup>: values with same superscripts within rows differ (p < 0.05)
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 1–2 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 6–10 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 minutes before vaginal examination, which is even earlier than the 10–15 minutes time which is at least required (34). Ketoprofen was administered 20 minutes 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 EPID arched their back for nearly 1 minute 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 three 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 minute 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 a 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 non-linear 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).

Conflict of interest

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

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.

Acknowledgements

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References


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