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Evaluation of vaginoscopy and transrectal examination of cervical diameter for the diagnosis of clinical endometritis in dairy cows

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Für meine Eltern

Carla und Hartwig Leutert

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1. INTRODUCTION

Uterine diseases postpartum negatively influence subsequent reproductive performance in dairy cows. This has been shown in numerous clinical studies (e.g. McDougall et al., 2007; Runciman et al., 2008) and summarized in several reviews (Sheldon et al., 2006; LeBlanc 2008). One important disease is the clinical endometritis. It is defined as an inflammation of the endometrium occurring later than 21 days in milk. A clinically useful case definition of clinical endometritis based on factors that were prognostic for impaired reproductive performance was established in two recent studies (LeBlanc et al., 2002a; Sheldon et al., 2006). The only clinical findings with predictive value for a decreased fertility were mucopurulent or purulent vaginal discharge after the 26 days in milk and a cervix diameter >7.5 cm after 20 days in milk (LeBlanc et al., 2002a).

Vaginal discharge can be clinically examined by several diagnostic methods such as the gloved hand, the metricheck device and vaginoscopy (Sheldon et al., 2002; McDougall et al., 2007; Pleticha et al., 2009). The vaginal examination with a vaginoscope is a common method to diagnose vaginal discharge (LeBlanc et al., 2002a; Sheldon et al., 2006). Williams et al. (2005) established a 4-point scoring system (0 = clear mucus, 1 = mucus containing flecks of pus, 2 = discharge containing less than 50 percent pus, 3 = discharge containing more than 50 percent pus) to classify vaginal mucus. This scoring was used in recent studies (Sheldon et al., 2006; Kaufmann et al., 2010; Dubuc et al., 2010). Also, the presence of mucopurulent or worse vaginal discharge may not be reflective of endometrial inflammation. Clinical evaluation of the reproductive tract can reveal the presence of endometritis, vaginitis, and cervicitis, and these conditions may not be differentiable by looking only at the vaginal discharge (Dubuc et. al., 2010).

A delayed involution of the cervix determined by transrectal palpation was considered as a simple and objective clinical finding for the diagnosis of clinical endometritis (LeBlanc et al., 2002a). The cervix is an important barrier against the invasion of bacteria into the uterus (Bekana et al., 1996; Bekana et al., 1997). Thus, cervical closure and regaining the firm structure of the cervix after parturition is important for reproductive performance (van Engelen et al., 2007). However, transrectal palpation of the uterus to assess its size lacks diagnostic accuracy, as large uteri may reflect physical damage or variations associated with breed, age or nutrition (Dohmen et al., 2000; LeBlanc et al., 2002a; Sheldon et al., 2006).

Every diagnostic method should be validated before use. An important obstacle in validating different diagnostic methods and describing test characteristics is the lack of a gold standard to verify inflammation of the uterus (Drillich et al., 2007). Cytological examination of the endometrium has been used as a definitive diagnosis for clinical endometritis and enables to differentiate clinical endometritis from vaginitis or cervicitis (Barlund et al., 2008). This diagnostic method is time consuming and expensive for routine use (Sheldon et al., 2006). Recent studies worked with the cytological results as well as the pregnancy status at 150 days in milk as reference to report the sensitivity and specificity (LeBlanc et al., 2002a; Barlund et al., 2008). Sensitivity and specificity of vaginoscopy compared to cytological findings calculated by Barlund et al. (2008) were 53.9% and 95.4%, respectively. Comparing results of the vaginoscopic examination with the pregnancy status at 150 days in milk sensitivity and specificity were 20% and 88% (LeBlanc et al., 2002a) and 7.1% and 87.4% (Barlund et al., 2008), respectively. Dubuc et al. (2010) calculated a sensitivity of 4.9% and 50.8% at 35 ± 3 days in milk with thresholds for cervical diameter of >7.5 cm and >5.0 cm, respectively. The reference applied in that study was the pregnancy status at 120 days in milk. Using the pregnancy status as reference to calculate diagnostic accuracy should be considered carefully due to numerous variables influencing the reproductive performance of a dairy cow (Kasimanickam et al., 2004).

There is science-based evidence both from accepted clinical (e.g. rectal palpation) and advanced diagnostic methods (e.g. radiography, ultrasound), that the investigator is a relevant source of measurement errors (Kelton et al., 1991; Schneider et al., 2002; Andermann et al., 2007). Currently, almost no information is available on the repeatability of the vaginoscopic examination and the assessment of the cervical diameter estimated by transrectal palpation.

Therefore, the objective of the first study performed for my thesis was to evaluate the repeatability of vaginoscopy. Specifically, I set out 1) to determine inter- and intraobserver repeatability of scoring vaginal discharge utilizing a vaginoscope and a 4-point classification system, 2) to test the influence of a previous transrectal manipulation of the uterus and the level of experience of the investigator on vaginal examination outcome and 3) to study sensitivity and specificity of the human capability to visually assess color shades by means of two in vitro experiments. The results of that study were published in:

Journal of Dairy Science (Impact factor: 2.564)

Volume 95, Issue 1, January 1012, Pages 206-212

Evaluation of vaginoscopy for the diagnosis of clinical endometritis in dairy cows.

Leutert C., von Krueger X., Plöntzke J., Heuwieser W.

The objective of the second study performed for my thesis was to determine the reliability of a manual assessment of the cervical diameter through transrectal palpation. Specifically, I set out to 1) evaluate the sensitivity and specificity of the manual assessment of the cervical diameter through transrectal palpation using a reference standard, 2) determine the interobserver repeatability, and 3) establish an in vitro experiment to study the human ability to assess diameters of known diameters through palpation. The results of that study were published in:

Journal of Dairy Science (Impact factor: 2.564)
Volume 96, Issue 2, February 2013, Pages 1063-1070
Evaluation of transrectal examination of cervical diameter by palpation in dairy cows.
Leutert C., Suthar V., Heuwieser W.

The two papers are presented in the form and style detailed in the guide for authors of the journal of dairy science.

2. PUBLICATION 1

EVALUATION OF VAGINOSCOPY FOR THE DIAGNOSIS OF CLINICAL ENDOMETRITIS IN DAIRY COWS

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3. PUBLICATION 2

EVALUATION OF TRANSRECTAL EXAMINATION OF THE CERVICAL DIAMETER BY PALPATION IN DAIRY COWS

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4. DISCUSSION

The objective of the research in my thesis was to evaluate two diagnostic methods being used for the diagnosis of clinical endometritis, the vaginoscopic examination and the transrectal palpation of cervical diameters. They are common diagnostic techniques for endometritis in clinical practice (LeBlanc et al., 2002a).

4.1. VAGINAL EXAMINATION BY VAGINOSCOPE

The objective of the first study was to evaluate the validity of vaginoscopic examination for the diagnosis of clinical endometritis in dairy cattle.

The overall prevalence of clinical endometritis in this study was 42.6% and within this similar to a previous trial conducted in two herds under comparable conditions by Westermann et al., (2010), who reported a prevalence of 39.7% and 42.2%. Animals with a vaginal discharge score 1, 2 and 3 were classified as affected with clinical endometritis and treated with $PGF_{2\alpha}$ twice within two weeks. Three investigators examined cows between 21 and 27 days in milk twice by vaginoscopic examination. The time lag between the investigators was less than one minute and between the two vaginoscopic examinations within one investigator less than 10 minutes. I tried to minimize the time lag between the examinations to assure virtually identical conditions for each investigator and for both vaginoscopic examinations. This way, however, it was not possible to fully exclude memorization of vaginoscopic examination results for a given cow.

The palpation of the uterus is the most common means of diagnosis of endometritis in clinical practice and transrectal examination is often performed at the same time as a vaginoscopy in order to increase the sensitivity of the diagnostic process (LeBlanc et al., 2002a; Runciman et al., 2008). Thus the effect of previous transrectal palpation was analyzed. A uterine stimulation caused by previous vaginal diagnostic tests improved detection of vaginal discharge, which was previously reported (McDougall et al., 2007; Pleticha et al., 2009). It may be assumed that the first vaginoscopic examination stimulated uterine contractions and led to the slight rise of prevalence of clinical endometritis at the second vaginoscopic examination, regardless of the investigator. Furthermore, a uterine stimulation might also be caused by previous transrectal palpation. My data clearly demonstrated that the detection of vaginal discharge by vaginoscopy could not be enhanced by a preceding palpation of the

uterus before vaginoscopic examination. No significant effect of a transrectal palpation of the uterus on the prevalence of clinical endometritis diagnosed by vaginoscopic examination ($P > 0.05$) was revealed by the relative risk analysis.

Frequency distribution of vaginal findings of the inexperienced investigators was similar to that of the experienced investigators. To make sure that experience was limited to less than 20 animals for each investigator, new investigators were utilized weekly. They were designated and summarized as investigator 3.

The in vivo trial was limited to evaluate vaginoscopic examination due to the nature of a field trial. Memorization of the cows as well as corresponding vaginal findings might have influenced the intraobserver repeatability. The cows were not randomly regrouped between the two vaginoscopic examinations to avoid additional stress and movement of the cows between herds, which might have influenced vaginal findings. However, each investigator spent only a short time per cow, i.e. 10 ± 5 seconds, which made me speculate that the likelihood of memorization was limited. Furthermore no communication between investigators was allowed and the documentation was made on separated data capture forms. In the in vitro trial it was possible to eliminate those limitations. Factors, affecting the results of the visual assessment, were minimized and a higher number of investigators were enrolled in vitro compared to the field study.

Interobserver repeatability was as high in vitro as in vivo, demonstrated by the median Kappa coefficients (experiment 1: K = 0.55, experiment 2: K = 0.47). Intraobserver repeatability was slightly higher in the in vitro trial compared to the in vivo trial. Intraobserver repeatability in the first in vitro experiment showed a substantial to almost perfect agreement and for the majority of the investigators in the second experiment a substantial agreement (experiment 1: K = 0.82, experiment 2: 0.61). The data confirm the assumption that diagnostic outcomes by examinations in field might be influenced by various factors (Vyskocil et al., 2008), such as time constraints or movement of the animal. The repeated examination in the in vitro trial was conducted after 4 weeks. This way, I was able to exclude potential memorization of findings as discussed for the field study. For most investigators the intraobserver repeatability calculated in both in vitro trials was higher compared to the in vivo trial. I assume that the short time interval between first and second vaginoscopic examination in vivo did have only a limited effect on the relatively high repeatability (K = 0.55 - 60). In the second experiment the images were assed through a vaginoscope and illuminated with a flashlight. The inter- and intraobserver repeatability of the second experiment was lower compared to the first experiment. These results demonstrate, that the use of a vaginoscope and a flashlight complicates the visual assessment.

The objective of the in vitro trial was to generate a reference standard, as no reference standard exists for the diagnosis of clinical endometritis (Sheldon et al., 2006). The in vitro approach enabled an exact calculation of sensitivity and specificity of the visual assessment and to quantify limitations (i.e. false positive and false negative cases) of a diagnostic method based on visual assessment. The importance of sensitivity and specificity of vaginoscopic examination has been emphasized recently (Westermann et al., 2010)

In both in vitro experiments, sensitivity and specificity were high. In the second experiment the sensitivity and specificity were slightly lower compared with the first experiment (99.6% and 96.7% vs. 96.3% and 90.1%). As already mentioned above, the use of flashlight and vaginoscope increases error rates due to the limited brightness of the flashlight and the reduced field of vision. Comparing sensitivity and specificity of visual assessment in my in vitro experiments to results generated in vivo when the pregnancy status at 150 days in milk or cytological results were used as reference method (LeBlanc et al., 2002a; Barlund et al., 2008), the sensitivity and specificity in vitro were considerably higher, except the specificity in the experiment 1 (87.4% vs. 85.5%). Le Blanc and Barlund (2002a; 2008) defined animals with mild purulent uterine discharge (vaginal discharge score 1) as not associated with reduced pregnancy rate and thus as healthy. My calculation of sensitivity and specificity was adjusted for comparison with the mentioned studies, defining vaginal discharge score 0 and 1 as healthy. In general these studies and my data demonstrate that the sensitivity and specificity of a vaginoscopic examination of vaginal discharge are high. Sensitivity and specificity calculated with the help of pregnancy status at 150 days in milk as reference should be interpreted carefully due to the considerable time lag between diagnosis and pregnancy confirmation and the multitude of factors that may influence pregnancy status (Kasimanickam et al., 2004; Barlund et al., 2008).

4.2. TRANSRECTAL PALPATION OF CERVICAL DIAMETER

The objective of the second study was to evaluate accuracy and repeatability of the transrectal palpation of cervical diameters in dairy cows. Next to the vaginal findings, a diameter of >7.5 cm has been demonstrated to be a reliable predictor for reproductive performance (LeBlanc et al., 2002a). Different thresholds between 5 cm and 7.5 cm for cervical diameter have been discussed (Oltenacu et al., 1983; LeBlanc et al., 2002a; Dubuc et al., 2010). The cervical diameter between healthy cows and cows with abnormal discharge had a maximal difference of only 10 mm at 3 weeks postpartum (Oltenacu et al., 1983). A relationship between fertility and cervical diameter determined by ultrasound was demonstrated recently (López-Helguera

et al., 2012).

The overall prevalence of cows with a cervical diameter of >7.5 cm was 13.1 % (n = 8). No cow between 21 and 27 days in milk showed a cervical diameter of >7.5 cm. Oltenacu et al. (1983) reported a prevalence of 26% between 12 and 26 days in milk with a cut point of >5 cm for cows in first lactation and ≥6 cm for cows in second or greater lactation. More recent studies described a prevalence of cervical diameter with >7.5 cm of 6.6% (LeBlanc et al., 2002a) between 20 and 33 days in milk and 4.4% (Dubuc et al., 2010) at 35 ± 3 days in milk, respectively. The objective of this experiment was to study the accuracy and repeatability of transrectal assessments of the cervical diameter. As the cervical diameter shrinks to 5.3 ± 1.0 cm at 48 hours postpartum (van Engelen et al., 2007), I decided to include the early postpartum period (2 to 5 days in milk) to increase the variation of cervical diameters. Cows between 21 and 27 days in milk differed only slightly (4.3 ± 0.6 cm), with a maximum of 6.7 cm. These data are in line with a study examining 53 cows 3 times postpartum and reporting a cervical diameter of 3.1 ± 0.3 at 22 to 28 days postpartum (López-Helguera et al., 2012). In addition, the authors of that study did not report any cows at 15 to 35 days postpartum with a cervical diameter of >7.5 cm. The examination of cows between 2 and 5 days in milk will lead to false-positive cases, considering the definition of endometritis specified by LeBlanc et al. (2002a) for the fourth week after calving, as physiological involution of a healthy uterus requires at least 3 weeks (Morrow et al., 1969; LeBlanc et al., 2002b; Gilbert et al., 2005).

I was able to calculate sensitivity and specificity, by creating a reference standard with ultrasound-based measurements in vivo and wooden cylinders in vitro. With the help of two in vivo experiments it could be demonstrated that ultrasound-based measurements could be used as a reliable reference standard. The correlation within one investigator was 0.97. Between 2 investigators, both correlation (0.99, $P = 0.01$, n = 61) and repeatability (mean: 0.01 cm, 95% CI: −0.48 to 0.51 cm) were excellent. The coefficient of variation between independent measurements was 3.4%. As the cervix of a cow is not perfectly round images were measured vertically and horizontally and the mean was calculated for further analysis. The overall sensitivity and specificity for all 3 investigators, considering ultrasound in vivo as the reference standard, were 37.5% and 96.2%. Cervical diameters of ≥ 6 cm confused the investigators and led to false-positive findings. This observation was confirmed in the in vitro trial. The variability of the manual assessment increased with increasing diameter (diameter of 10.5 cm: mean ± SD = 13.2 ± 4.0 cm; Figure 2a). As the manual assessment of diameters by palpation remains a challenge, especially cervical diameters ≥ 6 cm, and several publications did report few or no cows with a cervical diameter of > 7.5 cm ((Dubuc et al., 2010, López-Helguera et al., 2012), categorizing cows into healthy or metritic groups based

on the threshold for cervical diameters by LeBlanc (2002a) should be considered.

Calculated sensitivity (28.6%, 42.9% and 42.9%) and specificity (100%, 96.2% and 92.6%) in vivo for investigator 1, 2 and 3, respectively, were limited due to the small number of cows (n = 8) with a large cervix and possible confounding by a different texture of uterine tissue early postpartum. For the comparison with previous studies I calculated sensitivity and specificity in vitro. The ability of investigators to manually assess different diameters was reasonable and could be improved through training, as demonstrated in group A (before training: 73.2 %, after training: 85.9 %). Dubuc et al. (2010) calculated a sensitivity of 4.9 % and 50.8 % at 35 ± 3 days in milk, considering thresholds of a cervical diameter >7.5 cm and >5.0 cm, respectively, using predicted pregnancy status 120 days after parturition, as reference. As mentioned above, calculating sensitivity based on confirmed pregnancy status as a reference has to be interpreted critically.

The estimates of cervical diameters obtained through transrectal palpation conducted independently by 3 investigators did not show a significant difference ($P = 0.12$) but did show low correlation (r = 0.79, 0.52 and 0.49; all $P < 0.001$) and high variation (CV = 19.4%, 33.3% and 33.8%). Repeatability was estimated by calculating the mean and 95% CI of the arithmetic differences between repeated measurements on the same subject, according to Bland and Altman (1986). Plotting the differences between ultrasound measurements and estimates obtained manually against the mean of the 2 values showed that disagreements between the methods were evenly distributed across the range. Ultrasound based measurements were, on average, 0.6 cm greater than estimates obtained by transrectal palpation. The 95% CI (lower and upper limits of agreement) for the point estimates of the mean difference were −2.4 and 3.6 cm. By using the Bland and Altman (1986) method, approximately 95 % of data points were within the lower and upper limits of agreement.

The time lag between investigators was minimized to less than 1 minute to ensure identical conditions. The number of investigators in the in vivo experiment was limited to 3 to minimize strain on the animals and avoid the risk of rectal lesions. As discussed previously, transrectal stimulation causes uterine tone to increase contractions (McDougall et al., 2007; Pleticha et al., 2009), which might affect the cervical diameter. Like above I designed an in vitro trial to exclude the limitations. Besides the availability of an absolute reference standard, a greater number of investigators were used and a greater variation of different diameters was examined (3.5 to 10.5 cm in 0.3 cm increments). I did choose wood as the material for the cylinders to ensure that the diameter did not change during several palpations.

This is the first report related to dairy cattle to demonstrate that the human ability to manually assess different diameters by palpation can be trained (Figures 1 and 2). Correlation and variation clearly improved after training. Further research is warranted to determine how this effect can be implemented in in vivo situations.

5. SUMMARY

EVALUATION OF VAGINOSCOPY AND TRANSRECTAL EXAMINATION OF CERVICAL DIAMETER FOR THE DIAGNOSIS OF CLINICAL ENDOMETRITIS IN DAIRY COWS

The two present studies of my thesis were performed to evaluate 1) the visual assessment of vaginal discharge by vaginoscopy and 2) the manual assessment of cervical diameter through palpation in dairy cows. Both studies are based on an in vivo and in vitro trial.

In the first study Holstein-Friesian cows (n = 380) were examined by vaginoscope between 21 and 27 days in milk by three investigators twice. Vaginal discharge was categorized on a 4-point classification system (0 = clear mucus, 1 = mucus containing flecks of pus, 2 = discharge containing less than 50 percent pus, 3 = discharge containing more than 50 percent pus). Cows with a vaginal discharge score of 0 were classified as healthy whereas cows with a vaginal discharge score of 1 to 3 were classified as having a clinical endometritis. My results illustrate that vaginal discharge score on a scale from 0 to 3 has moderate intra- (Cohen´s kappa coefficient = K; K = 0.55 – 0.60) and interobserver (K = 0.44) repeatability. The prevalence of clinical endometritis was comparable between the three investigators (1st VE: 42.6%, 34.8%, 38.7%; 2nd VE: 46.8%, 36.9%, 43.7%). Transrectal palpation (RR = 0.96 - 1.03) or experience of the investigator (RR = 0.9 - 1.1) did not affect results of vaginoscopic examination (VE). In an in vitro trial sensitivity and specificity of a visual assessment were determined utilizing 33 images showing yellow and pink areas in certain percentages as a reference standard. Pus was represented as yellow and the mucosa including clear mucus as pink areas. These images were visually assessed by 30 investigators via power point (experiment 1) and by 23 investigators via a simulated vaginoscopic examination (experiment 2) utilizing the same 4-point classification system. Sensitivity was 99.6% and 96.3% and specificity was 96.7% and 90.1% in experiment 1 and 2, respectively.

In the second study, three investigators examined 64 Holstein- Friesian cows between 2 and 5 days in milk and between 21 and 27 days in milk by transrectal palpation. For calculation of sensitivity and specificity, ultrasound-generated measurements were used as reference standard. A cervix >7.5 cm was categorized as large. The Pearson coefficient of correlation

between the results of the 3 investigators and ultrasound-generated measurements was moderate (r = 0.71, 0.74 and 0.51). The estimates generated by palpation by the 3 different investigators did not differ and were similar to measurements obtained by ultrasound. The coefficient of variation between the investigators and ultrasound was high (20.9, 18.7 and 32.0%). The mean difference between the investigators and the ultrasound was 0.60 cm (95% confidence interval: −2.4 to 3.6 cm). Sensitivity was 28.6%, 42.9% and 42.9% and specificity was 100%, 96.2% and 92.6% for the ability of the 3 investigators, respectively, to detect a cervix with a diameter of 7.5 cm by palpation. Overall, sensitivity and specificity for all 3 investigators, considering ultrasound measurements as reference, were 37.5% and 96.2%, respectively.

In vitro, 24 wooden cylinders were used to represent cervical diameter and to examine the reliability, as well as sensitivity and specificity, of manual assessment of different diameters. The Pearson coefficient of correlation between the results of the investigators (n = 11) and the actual diameters of the cylinders was 0.78. The coefficient of variation between the investigators and the cylinders was 27.8%. The variation in the results was greater for cylinders with a larger diameter (3.5 cm diameter: mean ± standard deviation = 2.6 ± 0.9 cm; 10.5 cm diameter: mean ± standard deviation = 13.2 ± 4.0 cm). The estimate obtained by palpation for the 7.5 cm cylinder was 7.4 ± 2.1 cm. Sensitivity was 79.4 % and specificity 92.5%. After training one group of investigators, sensitivity and specificity improved to 85.9% and 94.4%, respectively.

The results show that a visual assessment conducted by vaginoscopic examination is not perfect but can be considered a reasonable measurement of vaginal discharge and as a practical tool to distinguish healthy from diseased cows. My data provide clear evidence that manual assessment of diameters by palpation remains a challenge.

6. ZUSAMMENFASSUNG

EVALUIERUNG DER VAGINOSKOPIE UND DER TRANSREKTALEN UNTERSUCHUNG DES ZERVIX-DURCHMESSERS ZUR DIAGNOSTIK KLINISCHER ENDOMETRITIDEN BEI MILCHKÜHEN

Die hier vorliegenden Studien wurden durchgeführt, um 1) die visuelle Beurteilung des vaginalen Ausflusses mit Hilfe eines Vaginoskops und 2) die manuelle Untersuchung des Zervix Durchmessers zu evaluieren. Beide Studien basieren auf einem in vivo und einem in vitro Versuch.

In der ersten Studie wurden 380 Holstein-Friesian Kühe von drei Untersuchern zweimalig zwischen dem 21 und 27 Tag in Milch mittels Vaginoskopie (VE) untersucht. Der Scheidenausfluss wurde mit Hilfe einer 4-Punkt-Klassifikation beurteilt (0 = klarer Schleim, 1 = Schleim mit Flecken von Eiter, 2 = Ausfluss mit weniger als 50 Prozent Eiter, 3 = Ausfluss mit mehr als 50 Prozent Eiter). Kühe mit einem Ausfluss von 0 wurden als gesund und Kühe mit einem Ausfluss von 1 bis 3 als an klinischer Endometritis erkrankt eingestuft. Die Wiederholbarkeit der Ergebnisse bei einer Skala von 0 bis 3 sind moderat für den einzelnen Untersucher und zwischen den verschiedenen Untersuchern. Die Prävalenz der klinischen Endometritis war zwischen den drei Untersuchern vergleichbar (VE 1: 42,6 %, 34,8%, 38,7 %; VE 2: 46,8 %, 36,9 %, 43,7 %). Die transrektale Palpation (RR = 0,96 - 1,03) sowie die Erfahrung des Untersuchers (RR = 0,9 - 1,1) hatten keinen Einfluss auf die Ergebnisse der vaginoskopischen Untersuchung.
In einer in vitro Studie wurden 33 Bilder als Referenzstandard verwendet, um die Sensitivität und die Spezifität zu ermitteln. Die Bilder zeigen gelbe und rosa Bereiche in bestimmten Prozentsätzen als Referenzstandard. Eiter wurde als gelb und die Schleimhaut, einschließlich des klaren Schleims, als rosa Bereiche dargestellt. Diese Bilder wurden visuell durch 30 Untersucher via Power Point (Experiment 1) und 23 Untersucher via einer simulierten vaginoskopischen Untersuchung (Experiment 2) unter Verwendung der gleichen 4-Punkt-Klassifikation beurteilt. Die Sensitivität lag bei 99,6 % und 96,3%, die Spezifität bei 96,7% und 90,1 % im Versuch 1 und 2.

In der zweiten Studie haben drei Untersucher 64 Holstein-Friesian Kühe zwischen dem 2 und 5 Tag in Milch und zwischen dem 21 und 27 Tag in Milch durch transrektale Palpation untersucht. Für die Berechnung der Sensitivität und Spezifität wurden Ultraschall generierte Messungen als Referenzstandard verwendet. Eine Zervix >7,5 cm wurde als zu groß eingestuft. Der Korrelationskoeffizient nach Pearson zwischen den Ergebnissen der drei Untersucher und der Ultraschalluntersuchung lagen bei r = 0,71, 0,74 und 0,51. Die Ergebnisse der drei Untersucher wichen nur geringgradig voneinander ab und waren vergleichbar mit den Ultraschallergebnissen. Der Variationskoeffizient zwischen den Untersuchern und der Ultraschalluntersuchung war hoch (20,9%, 18,7% und 32,0%). Die mittlere Differenz zwischen den Untersuchern und der Ultraschalluntersuchung lag bei 0,6 cm (95 % Konfidenzintervall: -2,4 bis 3,6). Die Sensitivität betrug 28,6%, 42,9% und 42,9%, die Spezifität 100%, 96,2% und 92,6% für die drei Untersucher und der Toleranzgrenze von 7,5 cm. Die Sensitivität und Spezifität für alle 3 Untersucher mit dem Ultraschall als Referenzstandard lag bei 37,5% und 96,2%.
In vitro wurden 24 hölzerne Zylinder als Modelle verwendet. Es wurden Sensitivität und Spezifität sowie die Wiederholbarkeit der manuellen Untersuchung unterschiedlicher Durchmesser ermittelt. Elf Untersucher haben die Durchmesser der 24 Modelle in 10 Durchgängen manuell geschätzt. Der Korrelationskoeffizient nach Pearson zwischen den Ergebnissen der Untersucher (n = 11) und den tatsächlichen Durchmessern der Zylinder betrug 0,78. Der Variationskoeffizient zwischen den Untersuchern und den Zylindern betrug 27,8%. Die Ergebnisse variierten stärker bei Modellen mit einem größeren Durchmesser (3,5 cm Durchmesser: Mittelwert ± Standardabweichung = 2,6 ± 0,9 cm; 10,5 cm Durchmesser: Mittelwert ± Standardabweichung = 13,2 ± 4,0 cm). Die Ergebnisse der Untersucher für die 7,5 cm-Zylinder lagen bei 7,4 ± 2,1 cm. Die Sensitivität betrug 79,4% und die Spezifität 92,5%. Mit Training konnte die Sensitivität und Spezifität auf 85,9% und 94,4% verbessert werden.

Die Ergebnisse belegen, dass die Befunde der vaginoskopischen Untersuchung zwar nicht perfekt ausfallen, die Vaginoskopie aber dennoch als angemessene Untersuchungsmethode von vaginalem Ausfluss angesehen werden sollte. Bezüglich der zweiten Studie zeigen meine Daten eindeutig, dass die Beurteilung von Durchmessern durch Palpation eine Herausforderung darstellt.

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8. PUBLICATIONS

Publications in journals (first author):

Evaluation of vaginoscopy for the diagnosis of clinical endometritis in dairy cows.
Leutert C., von Krueger X., Plöntzke J., Heuwieser W.
J Dairy Sci. 2012 Jan; 95(1): 206-12. doi: 10.3168/jds.2011-4603.

Evaluation of transrectal examination of cervical diameter by palpation in dairy cows.
Leutert C., Suthar V., Heuwieser W.
J Dairy Sci. 2013 Feb; 96(2): 1063-70. doi: 10.3168/jds.2012-6061. Epub 2012 Nov

Posters at conferences (first author):

Evaluation of vaginoscopy für diagnosis of endometritis in dairy cows
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10. DECLARATION OF INDEPENDENCE

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

This is to declare that I conducted all of the studies described herein myself and the manuscripts were produced independently. I confirm that I have used only the specified resources and tools to complete this thesis. My personal contributions to the research projects presented under this cumulative doctoral thesis are summarized in the following table.

Contribution	Research project 1	Research project 2
Study design	++[1]	+++
Data collection	+++	+++
Data analyses	+++	++
Manuscript writing	+++	+++
Manuscript editing	++	+++

[1] Score: + = < 50%; ++ = 50 to 70%; +++ = > 70%

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