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# Clinical and subclinical endometritis in dairy cattle: Prevalence, Indicators, and Therapy

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# 1. INTRODUCTION

The key for an optimal fertility in dairy herds is a healthy uterine environment. A healthy uterus is the basis for high submission and conception rates. Endometritis is one of the most common uterine disorders in dairy cows, causing decreased fertility and high economic losses.

Studies focussing on clinical endometritis (CE) report on prevalences of the disease ranging from 18 to 37 % (Bartlett et al., 1986, Drillich et al., 2002, Drillich et al., 2005a, Etherington et al., 1984, Markusfeld, 1987, Peeler et al., 1994). Studies on subclinical endometritis (SCE) found prevalences in the range between 12 and 94 % (Barlund et al., 2008, Gilbert et al., 2005, Hammon et al., 2006, Kasimanickam et al., 2004, 2005, Raab, 2004). Different study designs and inconsistent definitions of CE and SCE hinder a valid comparison of the results of these studies. The prevalence of clinical und subclinical endometritis depends on the occurrence of early post partum uterine diseases (Benzaquen et al., 2007, Drillich et al., 2001), the time of examination, and the diagnostic technique (Drillich et al., 2002, Falkenberg and Heuwieser, 2005, LeBlanc et al., 2002a).

Recently, a consortium of authors defined CE as the presence of purulent (> 50 % pus) or mucopurulent (approximately 50 % pus, 50 % mucus) uterine exudate in the vagina, 21 days or more post partum, and not accompanied by systemic signs. Current studies have focused on a more sophisticated diagnosis of endometrial alterations beyond clinical signs of endometritis. New techniques have been described for the diagnosis of SCE. The inflammation of the endometrium is characterized by the proportion of polymorphonuclear (PMN) cells in a cytological sample taken from clinically healthy cows. Cytological samples can be obtained by flushing the uterine lumen (Gilbert et al., 2005, Mateus et al., 2002, Sheldon et al., 2006), taking an endometrial biopsy (Bonnett et al., 1993, Chapwanya et al., 2010, Kinsel, 1996) or by using the cytobrush technique (Kasimanickam et al., 2004, Lincke, 2006, Raab, 2004). This latter technique uses a small brush that is inserted into the uterus to collect endometrial cells and to determine the proportions of PMN in the sample.

Both CE and SCE result in increased days to first service as well as decreased conception and pregnancy rates followed by an increased risk of culling (Gilbert et al., 2005, Kasimanickam et al., 2004, LeBlanc et al., 2002a, Raab, 2004).

Knowledge of prevalence and effects of SCE in the post partum period raises the question on the prevalence and effects of SCE around breeding. I conducted a study that focused on the uterine environment around the time of breeding. The objective of the study was to investigate the prevalence of bovine SCE four hours after artificial insemination and its effect on first service conception rate (FSCR). To evaluate an effect of the cytobrush technique I integrated a matched pair study. Conception rates of cows included in the study were compared with cows not examined for SCE with the cytobrush technique but inseminated at the same time by the same AI technician. Results have been published in:

Theriogenology (Impact Factor: 2,041)
Volume 71, Issue 2, 15 January 2009, Pages 385-391

**Prevalence of bovine subclinical endometritis 4 h after insemination and its effects on first service conception rate**

T.B. Kaufmann, M. Drillich, B.-A. Tenhagen, D. Forderung, W. Heuwieser

Bacteriological contaminations of the uterus after parturition and metabolic changes in the transition period are important etiological factors for the occurrence of metritis and endometritis. Negative energy balance is known to influence the number as well as the functional properties of PMN. There is evidence that periparturient depression of functional properties and number of PMN is of great importance for the pathogenesis of endometritis, as recently reviewed (Földi et al., 2006, Sheldon et al., 2009).

Previous studies demonstrated negative impacts of elevated concentrations of non-esterified fatty acids (NEFA), beta-hydroxybutyric acid (BHBA), bilirubin, and urea on the function of PMN (Hammon et al., 2006, Hoeben et al., 1997, Hoeben et al., 2000, Klucinski et al., 1988). Negative effects have also been described on uterine environment (Butler, 1998, Elrod and Butler, 1993, Elrod et al., 1993, Rhoads et al., 2004) and the prevalence of metritis, the typical pre-stage of endometritis (Duffield et al., 2009, Hammon et al., 2006, Huzzey et al., 2007, Kaneene et al., 1997, Walsh et al., 2007). Most of these studies, however, were in vitro studies. Evidence from studies under field conditions is weak. Thus, further research needs to be conducted to confirm these findings considering different management and housing situations as well as different concentrations of infectious agents on dairy farms.

An early identification of cows at risk for CE and SCE could provide new targets for intervention and the basis for changes in management practices to prevent these diseases. Furthermore, the identification of predictors for CE and SCE will contribute to a better understanding of the underlying mechanisms in the pathogenesis of these diseases. To my knowledge, there is only a single recent publication on the identification of metabolic predictors for endometritis (Hammon et al., 2006). However, predictor cut points for use in dairy practice were not established.

Therefore, I conducted a study to investigate the relationship between elevated serum concentrations of NEFA, BHBA, bilirubin and urea in the periparturient period and the prevalence of CE and SCE. Specifically, I set out to determine sensitivity and specificity of these metabolic parameters for the detection of CE and SCE, establishing optimal predictor cut points for practical use. Results have been published in:

BMC Veterinary Research (inofficial Impact Factor: 2,01)
Volume 6, 27 October 2010, Page 47
**The correlation between periparturient serum concentrations of non-esterified fatty acids, beta-hydroxybutyric acid, bilirubin, and urea and the occurrence of clinical and subclinical bovine endometritis**
T.B. Kaufmann, M. Drillich, B.-A. Tenhagen, D. Forderung, W. Heuwieser

Most prevalent pathogens involved in the etiology of endometritis are *Arcanobacterium (A.) pyogenes*, *Escherichia (E.) coli, Fusobacterium necrophorum*, and Prevotella species (Földi et al., 2006, Sheldon et al., 2006, Sheldon et al., 2002).
Suggested treatment protocols for CE include the intrauterine application of antibiotics (Drillich et al., 2005b, LeBlanc et al., 2002b) or the administration of $PGF_{2\alpha}$ and its analogues (Drillich et al., 2005a, Drillich et al., 2005b, Heuwieser et al., 2000, LeBlanc et al., 2002b). The local application of disinfectants (e.g. Lugol's solution) has been described. Effects on future fertility of the cows, however, were negative (Knutti et al., 2000). A systemic antibiotic treatment of cows with CE as described for acute metritis (Chenault et al., 2004, Drillich et al., 2001, Heuwieser et al., 2000, Zhou et al., 2001), has not been reported in recent literature. Pharmacokinetic data for a systemic application of ceftiofur in cows early post partum suggested an efficacious concentration of the antibiotic in the endometrium in cows with CE (Drillich et al., 2006, Okker et al., 2002). Pharmacokinetic data on intrauterine applied antibiotics, i.e. amoxicillin, have rarely been published in recent literature and only utilizing in vitro models (Braun et al., 2009). In contrast to systemically applied antibiotics, it has been assumed that the presence of pus and organic debris in the uterine fluids decreases the efficacy of local antibiotics (Paisley et al., 1986).
Thus, I conducted a study to test the hypothesis that in cows with clinical endometritis 21 to 27 days post partum a systemic treatment with 1.0 mg of ceftiofur on three consecutive days results in improved clinical cure and conception rates compared with a control protocol consisting of two treatments with the $PGF_{2\alpha}$ anaolgue cloprostenol in a 14 d interval. Results have been published in:

Animal reproduction science (Impact Factor: 1,89)
Volume 121, Issue 1-2, August 2010, Pages: 5-62
**Systemic Antibiotic Treatment of Clinical Endometritis in Dairy Cows with Ceftiofur or two Treatments of Cloprostenol in a 14 day interval**
T. B. Kaufmann, S. Westermann, M. Drillich, J. Plöntzke, W. Heuwieser

The three papers are presented in the format outlined in the guide for authors of the respective journal.

## 2. PUBLICATION I

# Prevalence of subclinical endometritis 4 h after insemination and its effect on first service conception rate

Subclinical endometritis four hours after insemination

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## 2.1 Abstract

The objective of this study was to investigate the prevalence of subclinical endometritis 4 h after AI and its effect on first service conception rate (FSCR) in dairy cows.

A total of 201 Holstein-Friesian cows with no signs of clinical endometritis were examined 4 h after first AI for signs of subclinical endometritis. Endometrial samples were collected from the uterus using the cytobrush technique. The proportion of polymorphonuclear cells (PMN) in the cytological sample was used to characterize an inflammation of the endometrium. Cows were categorized into 3 groups according to the proportion of PMN in the sample. Cows with 0 % PMN (n = 115) were assigned to group Zero, cows with >0 to15 % PMN (n = 59) to group Medium, and cows with >15 % PMN (n = 27) to group High. Pregnancy diagnosis was performed between days 38 to 44 after AI by palpation of the uterus and its contents per rectum.

The FSCR was significantly higher in group Medium than in groups Zero and High (57.6 % vs. 39.1 % and 29.6 %). Statistical analysis revealed an interaction between parity and PMN group. Primiparous cows were at higher risk of being classified into group Medium than multiparous cows (OR = 2.27, *P* = 0.01). Primiparous cows in group Zero had lower odds of pregnancy after first AI than primiparous cows in group Medium (OR = 0.3, *P* = 0.02). A comparison with cows that were not examined for subclinical endometritis showed that the collection of endometrial samples itself had no effect on FSCR.

## 2.2 Keywords

subclinical endometritis, insemination, first service conception rate, cytobrush, dairy cattle

## 2.3 Introduction

The key for excellent fertility in dairy herds is a healthy uterine environment, optimal estrus detection efficiency and ideal timing for breeding. This study focuses on the uterine environment at the time of breeding. The incidence and impact of clinical and subclinical endometritis (SE) in the postpartum period has been the objective of several studies [5,7,10,12,27].

A case definition for clinical endometritis based on the risk of impaired fertility was introduced by LeBlanc et al. [12]. Clinical endometritis was characterized by a cervical diameter >7.5 cm and the presence of purulent or mucopurulent vaginal discharge after 20 days postpartum (dpp). Affected cows were 30 % less likely to become pregnant at first service and 70 % more likely to be culled for reproductive failure [12].

Recent studies have focused on a more sophisticated diagnosis of endometrial alterations beyond clinical signs of endometritis. New techniques have been described for the diagnosis of subclinical endometritis (SE). The inflammation of the endometrium is characterized by the proportion of polymorphonuclear cells (PMN) in a cytological sample taken from clinically healthy cows. Cytological samples can be obtained by flushing the uterine lumen [5,14,19] or by using the cytobrush technique [10,13,16]. This latter technique uses a small brush that is inserted into the uterus to collect endometrial cells and to determine the proportions of PMN in the sample.

Studies on SE found a prevalence of SE in the range between 12 % and 94 % [2,5,7,9,10,16]. Different study designs and inconsistent definitions for SE hinder a valid comparison of the results of these studies. The time of examination in these studies varied from 21 to 60 dpp. Furthermore, different threshold values of PMN were used for the definition of SE. For example, Hammon et al. [7] used a threshold value of >25 % in the cytological sample at 28 (± 3) dpp whereas Kasimanickam et al. [9] found >18 % PMN at 20 to 33 dpp as indicative of SE. Other authors [2,5,13,16] set the threshold at 5 % or 8 % PMN. All cited studies, except [8], found negative effects of SE on reproductive performance, i.e. decreased first service conception rate and/or prolonged days open. Subclinical endometritis in the postpartum period, however, can resolve before insemination. Gilbert et al. [5] found decreasing proportions of cows with SE from two weeks postpartum (100 %) to eight weeks postpartum (41 %). In contrast, cows without SE at the end of the postpartum period can be positive for SE later in lactation. Of 152 cows identified as healthy 21 to 27 dpp (≥5 % PMN), 12.5 % were identified with SE and 14.5 % with clinical endometritis 14 days later [16]. It remained unclear in that study, if subclinical and clinical endometritis were caused by the cytological examination itself. In the cited studies, however, cytological examinations were conducted at the end of the postpartum period, not in the breeding period. To our knowledge there are few studies that investigated the impact of cytological evidence of inflammation immediately prior to insemination on fertility in dairy cows [26]. Although not statistically significant, the cited researchers did notice a negative relationship between uterine inflammation and conception.

The objective of the current study was to investigate the prevalence of bovine subclinical endometritis 4 h after AI and its effect on first service conception rate (FSCR). To evaluate an effect of the diagnostic technique itself, conception rates of cows included in the study were compared with conception rates of cows not examined for SE.

## 2.4 Materials and Methods

### *2.4.1 Study farm*

The study was conducted between December 2005 and July 2006 on a commercial dairy farm in Germany. Cows were housed in a free-stall barn with slotted floors and cubicles, lined with rubber mats. Herd average milk yield was 9,259 kg (4.08 % fat, 3.48 % protein) per cow per year. Cows were fed a total mixed ration.

### *2.4.2 Study design*

The voluntary waiting period was set at 65 dpp. Estrus detection was performed by the herd manager and the staff of the farm by visual observation of signs of estrus. The cows were visually observed twice a day for 20 min. Cows detected in estrus in the morning were presented the same day to the AI technician, who performed the breeding soundness evaluation. Cows detected in estrus in the afternoon were presented to the AI technician the next morning. An AI technician from the local breeding station visited the farm daily between 10 to 12 a.m. During the weekly visit of the veterinarian, cows not observed in estrus were examined by palpation of the uterus and the ovaries per rectum. Cows with a corpus luteum received 0.5 mg of cloprostenol (PGF Veyx forte, Veyx Pharma GmbH, Schwarzenborn, Germany, im) to induce estrus. These cows were presented to the AI technician three days later and were bred if found suitable for AI by the AI technician. No timed breeding protocols, e.g. Ovsynch, and no timed AI were performed.

During scheduled weekly visits of the researchers all inseminated cows were examined 4 h after AI both externally and by use of a vaginoscope for clinical signs of endometritis. Subsequently, an endometrial sample was collected using the cytobrush technique as described by Kasimanickam et al. [10]. The brush (Gynobrush, Heinz Herenz, Hamburg, Germany) used for collecting the endometrial sample was 20 mm in length and 6 mm in diameter. It was protected by a one-way catheter and inserted via the cervix into the uterine body. Inside the uterus, the catheter was retracted and the brush was rolled along the uterine wall. Thereafter, the brush was retracted into the catheter to protect it during the passage through the genital tract. After collecting the sample, the brush was rolled onto a clean glass microscopical slide. The slide was immediately fixed on farm and stained (LT-SYS®, Labor und Technik, Berlin, Germany) in the laboratory. A total of 300 cells were counted under a microscope (x 400 magnification) to determine the proportion of PMN. According to the proportion of PMN in the endometrial sample, cows were categorized into three groups. Group Zero included cows with 0 % PMN, group Medium with >0 to 15 % PMN, and group

High with >15 % PMN. The upper breakpoint (>15 %) was chosen between the two threshold values (>10 % PMN and >18 % PMN) described by Kasimanickam et al. [10].
Cows that were inseminated at days when no examinations with the cytobrush were performed served as a control group for the evaluation of a possible effect of the cytobrush technique itself. First service conception rate of cows examined by cytobrush was compared with FSCR of non-examined cows. Pregnancy diagnosis was performed between 38 and 44 days after AI by a veterinarian by palpation of the uterus and its contents per rectum.

#### *2.4.3 Statistical analyses*

Statistical analyses were performed using SPSS (Version 12.0.1, SPSS Inc.). First service conception rate was compared between cows examined or not examined with the cytobrush technique by Chi-Square analysis. The FSCR was compared between PMN groups (Zero, Medium, High), parity classes (primiparous vs. multiparous cows), and classes for interval from calving to first insemination (median days to first service, MDFS) (interval < MDFS vs. interval > MDFS) by Chi-square analyses in a first step. Interaction between parity class and PMN group was analyzed by logistic regression with parity class as outcome variables (0 = primiparous, 1 = multiparous) and PMN group as independent variable with three categories (0 = reference group Medium, 1 = group Zero, 2 = group High). Group Medium was chosen as reference, because FSCR was highest in this group. Because interactions between parity class and PMN group were found, in a next step odds ratio for pregnancy after first service (0 = not pregnant, 1 = pregnant) was analyzed by binary logistic regression models for primiparous and multiparous cows. PMN group (three categories) and days to first service (0 = ≤ median, 1 = > median) were the independent variables. Level of significance for all analyses was $\alpha = 0.05$. The confidence interval was set at 95 %.

## 2.5 Results

A total of 201 Holstein-Friesian cows were enrolled in the study. No effect on FSCR was found for the collection of endometrial sample with the cytobrush technique. First service conception rate was 43.3 % for 201 cows examined with the cytobrush technique and 41.7 % for 103 cows with no sample taken after AI ($P$ >0.05).
Cows in group Medium had higher FSCR than cows in groups Zero and High, respectively (57.6 % vs. 39.1 and 29.6 %, $P$ <0.05). The difference between Zero and High was found not significant. First service conception rate was higher in primiparous cows than in multiparous cows (54.3 vs. 35.8%, $P$ <0.05). Median days to first service were 78 days ($1^{st}$ quartile 74.0

days, 3rd quartile 109.5 days). No significant differences in FSCR were found for cows with fewer or more than 78 days to first service (Table 1).

An interaction was found between parity class and PMN group, with primiparous cows at higher risk for being classified into group Medium than multiparous cows (OR= 2.27, CI 1.20-4.32, $P$ = 0.01). First service conception rates for the PMN groups stratified by parity are shown in Table 1. Logistic regression revealed that primiparous cows in group Zero had significantly lower odds of conception at first service than primiparous cows in group Medium. No effect of PMN group on FSCR was found for multiparous cows (Table 2).

In 5 cytological samples spermatozoa were found. Two of those cows got pregnant after first AI. We were not able to identify phagocytized sperm in the cytological samples.

Table 1: First service conception rate for cows classified by PMN group, days to first service and parity class.

| Parameter | | Total | | Primiparous | | Multiparous | |
|---|---|---|---|---|---|---|---|
| | | Number of cows n (%) | FSCR % | Number of cows n (%) | FSCR % | Number of cows n (%) | FSCR % |
| PMN group[1] | | | | | | | |
| | Zero | 115 (57.2) | 39.1 [a] | 34 (53.1) | 44.2 | 25 (60.0) | 36.1 |
| | Medium | 59 (29.4) | 57.6 [b] | 43 (42.0) | 70.6 | 72 (20.8) | 40.0 |
| | High | 27 (13.4) | 29.6 [a] | 4 (4.9) | 25.0 | 23 (19.2) | 30.4 |
| Days to first service | | | | | | | |
| | ≤ 78 dpp | 101 (50.2) | 36.6 | 41 (50.6) | 45.5 | 60 (50.0) | 39.5 |
| | > 78 dpp | 100 (49.8) | 50.0 | 40 (49.4) | 54.5 | 60 (50.0) | 60.5 |
| Total | | | 43.3 | 81 (100) | 54.3 [c] | 120 (100) | 35.8 [d] |

[a,b,c,d;] Categories within parameters with different superscripts are different ($P < 0.05$)

[1]PMN group: Zero = 0 % PMN, Medium = >0 to 15 % PMN, High = >15 % PMN

Table 2: Logistic regression for the odds of pregnancy after first service in primiparous and multiparous cows with PMN group and days to first service as covariates.

| | | Primiparous | | Multiparous | |
|---|---|---|---|---|---|
| Covariate | df | Odds Ratio (95 % CI) | P-value | Odds Ratio (95 % CI) | P-value |
| PMN group | 2 | - | 0.33 | - | 0.91 |
| Medium | 1 | reference | - | reference | - |
| Zero | 1 | 0.30 (0.11 - 0.80) | 0.02 | 0.92 (0.36 - 2.37) | 0.86 |
| High | 1 | 0.15 (0.01 - 1.65) | 0.12 | 0.77 (0.23 – 2.63) | 0.68 |
| DTFS (bin) [1] | 1 | 1.78 (0.69 - 4.57) | 0.23 | 1.88 (0.86 – 4.10) | 0.11 |

[1] DTFS (bin) = Days to first service (binominal): ≤ 78 dpp = 0, > 78 dpp = 1

## 2.6 Discussion

The finding that collection of samples with the cytobrush technique had no effect on FSCR indicates that this technique is suitable for endometrial examination 4 h after AI.

Studies in various species reported that natural breeding or AI is followed by an immigration of leukocytes into the lumen and tissue of the uterus [6,11,17,18,23]. Semen induces a neutrophil response in the uterus of the mare after insemination [23]. Post-breeding inflammatory reaction in the horse peaked between 6 and 12 h after AI and decreased within 24 h [11]. Güvenc et al. [6] performed insemination into the uterine body or into the tip of the horn. Neither the site of insemination, nor the sperm dose affected the magnitude of the uterine inflammatory reaction. Rozeboom et al. [18] showed that post breeding uterine PMN migration in gilts reaches its magnitude between 6 and 12 h after AI and persists for 24 h. Ribeiro et al. [17] performed ovariohysterectomy 10 to12 h after insemination to obtain cytological samples in the bitch. Significantly higher numbers of PMN were found on the surface of the endometrium of the artificially inseminated uteri compared to uteri that had been not inseminated. The bovine uterus, however, shows only a weak post-mating inflammatory response [28]. A recent study showed that sperm cells do not trigger a detectable immunological response in the bovine uterus, irrespectively of the sperm preparation, i.e. freshly ejaculated and frozen-thawed spermatozoa. The cows were flushed 6 h after insemination and a biopsy was taken [25]. The current study, however, does not provide information on the status of the endometrium before AI. It would have been preferable to sample cows immediately before AI, to gain information if the PMN found in the samples originate from a pre-AI subclinical endometritis or a post mating PMN influx. Because of concerns of the AI technician and the owner of the farm that sampling before AI could negatively affect conception rate, samples were only taken after AI. Sampling cows immediately before AI could expose semen to small amounts of blood caused by taking the samples. The spermatotoxic effects of red blood cells [15] could have influenced the FSCR. To obviate an interference of a possible post-mating PMN influx and SE at AI we scheduled the time of sampling 2 h before the peak of the PMN influx described in the species mentioned above.

The significance of the results of our study, however, is limited to observations post breeding. Further research is required to investigate the correlation between pre- and post AI influx of PMN indicative for SE. For veterinarians in the field it is of highest interest to detect cows with SE before breeding. Further research is required to avoid misclassification of uterine health, i.e. false positive findings and false negative findings. The current study is a first step in the understanding of SE around breeding.

The subject of the study was to determine the prevalence of SE 4 h after AI and to evaluate its effect on FSCR in dairy cows. With a PMN level >15 % set as a threshold value indicative of SE, the prevalence of SE in the current study was 13.4 %. This prevalence was lower than in recent postpartum studies on SE with a prevalence from 16 to 94 % [5,9,10,16]. The threshold value for SE varied in the cited studies from 5 % to 18 %. Using a threshold value of 5 %, 10 % or 18 %, the prevalence of SE in the present study would have been 22 %, 17 % and 12 %, respectively. An explanation for the lower prevalence of SE in this study compared to other studies could be a recovery competence of the uterus from the end of the postpartum period until the time of AI. A comparison of the results from the current study with results from literature, however, is difficult because of different timeframes for the examinations (21-60 dpp in literature, median 78 dpp in the current study). Furthermore, there is no consensus about the proportion of PMN that defines SE [2,5,7-10,12,13,16,19].

Williams et al. [26] investigated the effect of subclinical endometritis immediately prior to AI on conception. Although not significant ($r = -0.24$, $P > 0.10$) the authors found a negative correlation between uterine inflammation and conception. Inflammation was classified depending on the percentage of PMN but the precise relationship between class and conception remained unclear. The classification of PMN groups chosen was different from the current study. Furthermore, results were not analysed for primiparous and multiparous cows separately. It is possible that these methodological differences evoked the differences in outcome.

The present study showed that cows in group Medium had the highest FSCR. The role of PMN in inflammatory processes is the rapid inactivation and elimination of foreign and altered autologous structures. In addition, they exert various regulatory functions such as release of cytokines, priming, activation or inhibition of cells, or influencing the expression of surface molecules [1]. The antibacterial action of PMN can be measured by their chemotactic, phagocytic and killing ability. Subandrio et al. [21] examined the three aspects of PMN function. They failed to demonstrate that there was any consistent relationship between the PMN function and the reproductive performance of the cows. In the present study more than half of the examined cows (57.2 %) were assigned to group Zero. This might support recent findings that only marginal amounts of PMN can be detected in the bovine uterus as a post-mating inflammatory response [25]. This result, however, is in contrast to observations by Subandrio et al. [21] that the number of PMN in the uterus is elevated during estrus or after treatment with estradiol.

Büchi et al. [3] examined postcoital bacterial contamination and the resulting inflammatory response of the uterus in mares. They found 16.3 % of the inseminated mares having neither bacteriological nor cytological positive samples. Two explanations for the occurrence of the bacteriological and cytological negative samples were given. Elimination of bacteria and healing of the endometrium could have taken place before the sampling of the mares. In that study, however, samples were taken 24 to 69 h after AI. The authors also discussed that with the collection of material from the uterus it is possible to miss bacterial contamination or inflammatory regions. The authors assumed a focal distribution of leukocytes and bacteria in the uterus. Ellenberger et al. [4], however, revealed that in 82 % of examined uteri with endometritis the whole endometrium is effected. Therefore, we assume that the Cytobrush technique is suitable for identifying most cases of endometrial inflammation.

Another explanation for a non-detected influx of PMN in group Zero could have been a low concentration of estrogen during estrus. It is not fully understood how the changes in estrogen concentration relate to changes in PMN function [20]. It can be hypothesized that low estrogen levels result in insufficient immunological capacity of the uterus to maintain pregnancy. This could also explain that cows in group Zero had a reduced FSCR compared with group Medium. This hypothesis needs to be evaluated by analyses of estrogen concentrations and PMN responses in cows in estrus. On the other hand, it is questionable that more than 50 % of the cows had an insufficient estrogen production.

Further analyses revealed that parity had a significant effect on the assignment of cows to the three groups. Primiparous cows had a higher risk of being classified into group Medium. In addition, primiparous cows had higher FSCR than multiparous cows. This is in accordance to the findings, that in general primiparous cows have a higher FSCR than multiparous cows [8,22]. The logistic regression model revealed no significant difference between group High and group Medium with regard to FSCR in primiparous cows. This might be explained by the small number of primiparous cows in group High. A larger data set involving more primiparous cows in all groups might have resulted in a significant difference between group Medium and group High in primiparous cows. This hypothesis, however, will have to be proven in another study.

The logistic regression model stratified by parity showed for multiparous cows no effect of PMN group on FSCR. In multiparous cows the potential positive effect of a medium amount of PMN, as demonstrated for primiparous cows, could have been offset by the metabolic situation of the cows. Wathes et al. [24] found that metabolic profiles and fertility differ between primiparous and multiparous cows.

It has been demonstrated that days to first service has a significant impact on FSCR in dairy cows [24]. In the current study, however, this parameter did not affect conception rates. The reason could be that the timeframe between the end of the voluntary waiting period and median days to first service was tight. The management decision to induce estrus in cows with a palpable corpus luteum influenced days to first service. If AI had occurred within a larger timeframe postpartum another outcome could not be ruled out.

Subclinical endometritis at AI might impair the uterine environment and hamper the implantation and development of the embryo. Based on the results of this study, we suggest defining the presence of >15 % PMN 4 h after AI as indicative of SE. However, this classification is based on a limited set of data and is not in accordance with threshold values found in studies on SE in the postpartum period. Further research is required to confirm our findings.

## 2.7 Conclusion

This study demonstrated that cows with high proportions of endometrial PMN (>15 % PMN) after AI have a decreased FSCR, which might be indicative of SE. Further analyses showed that results were affected by parity. In multiparous cows the proportion of PMN had no effect on conception rates. In primiparous cows, FSCR was highest for cows with PMN >0 to 15%.
Furthermore, the results of the current study show that the cytobrush technique is an appropriate method for the collection of endometrial samples around the time of AI.
The significance of the results of the current study, however, is limited to observations post breeding. Further research is required to investigate the correlation between pre- and post AI influx of PMN indicative for SE. The current study is a first step in the understanding of SE around breeding.

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# 3. PUBLICATION II

## Correlations between periparturient serum concentrations of non-esterified fatty acids, beta-hydroxybutyric acid, bilirubin, and urea and the occurrence of clinical and subclinical bovine endometritis

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## 3.1 Abstract


### *3.1.1 Background*

Postpartum endometritis in cattle is a multifactorial disease with high economic impact. Both clinical endometritis (CE) and subclinical endometritis (SCE) result in decreased reproductive performance. Results from in vitro studies led to the implication that non-esterified fatty acids (NEFA), beta-hydroxybutyric acid (BHBA), bilirubin, and urea could be used as predictors for endometritis in veterinary practice. In this field study, we set out to establish optimal predictor cut points of these metabolic parameters for the detection of CE and SCE. Serum samples were collected one week prior to parturition (wk -1), in the first week postpartum (wk +1) and between 28 and 35 days postpartum (wk +5) from 209 Holstein-Friesian cows. At wk +5, all cows were examined for signs of CE and SCE.

### *3.1.2 Results*

Higher concentrations of urea at wk +1 were associated with increased odds of CE (OR = 1.7, P = 0.04) in primiparous (PP) cows. A predictor cut point of 3.9 mmol/L (sensitivity: 61%, specificity: 70%) was determined. In multiparous (MP) cows, the logistic regression model revealed that higher concentrations of NEFA at wk -1 were associated with increased odds of CE and SCE (healthy vs. CE: OR = 9.1, P = 0.05; healthy vs. SCE: OR = 12.1, P = 0.04). A predictor cut point of 0.3 mmol/L (sensitivity: 38%, specificity: 87% and sensitivity: 35%, specificity: 89%, respectively) was determined. Increasing concentrations of urea at wk +5 were associated with decreased odds of CE (healthy vs. CE: OR = 0.6, P = 0.01; SCE vs. CE: OR = 0.5, P = 0.03). A predictor cut point of 3.8 mmol/L (sensitivity: 52%, specificity: 81%) was determined. For BHBA and bilirubin relationships with CE or SCE were not detected.

### *3.1.3 Conclusions*

The corresponding combinations of sensitivity and specificity of the determined predictor cut points were not satisfactory for practical use. Thus, the analysed parameters, i.e. NEFA, BHBA, bilirubin, and urea, at the chosen time points, i.e. at wk -1, at wk +1, and at wk +5 relative to calving, are unsatisfactory for disease prediction. Further research is required to clarify the questions raised by the current study.

## 3.2 Background

Postpartum endometritis in cattle is a multifactorial disease with high economic impact. Inflammation of the bovine uterus has been demonstrated to decrease reproductive performance. Both clinical and subclinical endometritis were associated with increased days to first service as well as decreased conception and pregnancy rates resulting in an increased risk of culling [1-4].

Bacteriological contaminations of the uterus after parturition and metabolic changes in the transition period are important etiological factors. Negative energy balance, is known to influence the number and functional properties of polymorphonuclear (PMN) cells [5, 6]. There is evidence that periparturient depression of functional properties and number of PMN is of great importance for the pathogenesis of endometritis, as recently reviewed [7-9].

The identification of risk factors for endometritis will contribute to a better understanding of the underlying mechanisms in the pathogenesis of endometritis. An early identification of cows at risk for endometritis could provide new targets for intervention and the basis for changes in management practices to prevent this disease. Decreased dry matter intake (DMI) prior to parturition is associated with mobilization of lipids, which are released as non-esterified fatty acids (NEFA) from adipose tissue [10]. Decreased DMI and increased NEFA levels are associated with periparturient suppression of the immune function, resulting in a greater susceptibility of cows to infection [11]. Cows with clinical or subclinical ketosis have shown impaired phagocytic activity of PMN isolated from milk and blood [12-14]. Elevated serum BHBA concentrations in the first two weeks postpartum were indicative of an increased risk (OR = 3.35) of metritis [15], a common pre-stage for CE and SCE, and decreased pregnancy risk (OR = 0.48)[16]. A reduction of probability of pregnancy of 20 to 50% was found, depending on the magnitude and duration of elevated serum BHBA (≥1,000 µmol in wk +1 postpartum, P = 0.04 or ≥1,400 µmol in wk +2 postpartum, P = 0.01)[16]. A relationship between the plasma concentration of bilirubin and the respiratory burst activity of bovine PMN in vitro was found [17]. It was hypothesized by the authors that bilirubin has potential as a diagnostic marker of impaired neutrophil function and consequently for identification of cows at risk around parturition. Furthermore, elevated urea concentrations had an effect of decreasing uterine pH during the luteal phase [18-20]. Bovine endometrial cells in culture responded directly to increasing urea concentrations with alteration in pH gradient [21]. This effect might facilitate subsequent bacterial growth and lower local immune defense.

Few publications are available describing relationships between metabolic disorders and the prevalence of CE and SCE under field conditions. Hammon et al. [5] reported significantly elevated concentrations of NEFA and BHBA around parturition for cows with CE and SCE compared with healthy cows. Decreased PMN function and elevated plasma levels of NEFA prior to parturition and elevated plasma levels of BHBA postpartum were associated with uterine disorders later in lactation. However, predictor cut points have not been determined yet.

The objective of this study was to investigate the relationship between elevated serum concentrations of NEFA, BHBA, bilirubin and urea in the periparturient period and the prevalence of CE and SCE. Specifically, we set out to determine sensitivity and specificity of these metabolic parameters for the detection of CE and SCE, establishing optimal predictor cut points for daily practical use.

## 3.3 Results

### *3.3.1. Prevalence of endometritis*

Overall prevalence of CE and SCE was 18.7 and 12.4%, respectively. Prevalence of CE and SCE at wk +5 in PP cows was 23.4% and 7.8%, respectively and in MP cows 15.9% and 15.2%, respectively.

### *3.3.2 Primiparous cows*

Serum concentrations of urea at wk +1 differed between health categories. All other metabolic parameters did not differ between health categories in PP cows (See additional file 1: Table 1 - Descriptive statistics of serum concentrations of NEFA, BHBA, bilirubin, and urea in relation to health categories for primiparous cows). Binary logistic regression revealed that in PP cows (n = 77), higher concentrations of urea at wk +1 were associated with increased odds of CE (P = 0.04) at wk +1 (Table 3). An optimal predictor cut point of 3.9 mmol/L (sensitivity 61%, specificity 70%, AUC 0.68) was determined.

### *3.3.3 Multiparous cows*

Serum concentrations of NEFA at wk -1 and wk +5 and of urea at wk +5 differed between health categories. All other metabolic parameters did not differ between health categories in MP cows (See additional file 2: Table 2 - Descriptive statistics of serum concentrations of NEFA, BHBA, bilirubin, and urea in relation to health categories for multiparous cows). Higher concentrations of NEFA at wk -1 were associated with increased odds of CE (P = 0.05) and SCE (P = 0.04) at wk +5 compared with healthy cows (Table 3). An optimal predictor cut point of 0.3 mmol/L for the discrimination between healthy and CE cows (sensitivity 38%, specificity 87%, AUC 0.66) and healthy and SCE cows (sensitivity 35%, specificity 89%, AUC 0.65) was determined. Higher concentrations of urea at wk +5 were associated with decreased odds of CE compared with healthy (P = 0.01) and SCE (P = 0.03) cows (Table 3). An optimal predictor cut point of 3.8 mmol/L for the discrimination between healthy and CE cows (sensitivity 52%, specificity 81%, AUC 0.70) was determined. For the discrimination between SCE and CE cows an optimal predictor cut point of 3.5 mmol/L (sensitivity 48%, specificity 90%, AUC 0.72) was determined.

## 3.4 Tables

Table 1
Descriptive statistics of serum concentrations of NEFA, BHBA, bilirubin, and urea in relation to health categories (healthy, clinical endometritis, subclinical endometritis) for primiparous cows.

| | Weeks relative to calving | Metabolite concentration | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Healthy (n = 53) | | | Clinical endometritis (n = 18) | | | Subclinical endometritis (n = 6) | | |
| | | Median | 1e quartile | 3e quartile | Median | 1e quartile | 3e quartile | Median | 1e quartile | 3e quartile |
| NEFA (mmol/L) | -1 | 0.15 | 0.10 | 0.35 | 0.18 | 0.12 | 0.41 | 0.28 | 0.10 | 0.64 |
| | +1 | 0.52 | 0.36 | 0.71 | 0.53 | 0.31 | 0.73 | 0.42 | 0.33 | 0.64 |
| | +5 | 0.17 | 0.12 | 0.31 | 0.18 | 0.13 | 0.30 | 0.22 | 0.15 | 0.47 |
| BHBA (µmol/L) | -1 | 596 | 458 | 709 | 590 | 530 | 772 | 652 | 502 | 652 |
| | +1 | 648 | 431 | 868 | 699 | 453 | 699 | 698 | 611 | 841 |
| | +5 | 561 | 467 | 736 | 550 | 380 | 814 | 603 | 443 | 774 |
| Bilirubin (µmol/L) | -1 | 3.7 | 3.0 | 5.4 | 3.6 | 2.6 | 5.1 | 4.0 | 2.0 | 7.2 |
| | +1 | 7.6 | 5.5 | 9.3 | 8.8 | 4.6 | 16.8 | 7.1 | 4.2 | 8.9 |
| | +5 | 3.0 | 2.4 | 3.8 | 2.9 | 2.5 | 3.4 | 2.7 | 1.9 | 3.7 |
| Urea (mmol/L) | -1 | 3.3 | 2.5 | 4.0 | 3.5 | 3.1 | 3.9 | 3.4 | 2.6 | 4.6 |
| | +1 | 3.2[a] | 2.7 | 4.2 | 4.0[b] | 3.2 | 5.0 | 3.2[a] | 2.5 | 3.7 |
| | +5 | 2.9 | 4.0 | 5.9 | 4.7 | 3.6 | 5.7 | 4.8 | 3.9 | 6.3 |

Within rows median values with different superscript are different ($P < 0.05$) revealed by Kruskal-Wallis-H-test.

Table 2
Descriptive statistics of serum concentrations of NEFA, BHBA, bilirubin, and urea in relation to health categories (healthy, clinical endometritis, subclinical endometritis) for multiparous cows.

| | | Metabolite concentration | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Weeks relative to calving | Healthy (n = 91) | | | Clinical endometritis (n = 21) | | | Subclinical endometritis (n = 20) | | |
| | | Median | 1e quartile | 3e quartile | Median | 1e quartile | 3e quartile | Median | 1e quartile | 3e quartile |
| NEFA (mmol/L) | -1 | 0.12[a] | 0.09 | 0.21 | 0.18[b] | 0.12 | 0.41 | 0.17[b] | 0.11 | 0.47 |
| | +1 | 0.55 | 0.40 | 0.76 | 0.43 | 0.39 | 0.71 | 0.67 | 0.40 | 0.75 |
| | +5 | 0.20[a] | 0.13 | 0.28 | 0.22[a/b] | 0.14 | 0.35 | 0.30[b] | 0.19 | 0.47 |
| BHBA (µmol/L) | -1 | 650 | 538 | 806 | 695 | 565 | 817 | 707 | 540 | 833 |
| | +1 | 861 | 693 | 1286 | 864 | 679 | 1115 | 974 | 748 | 1562 |
| | +5 | 660 | 561 | 958 | 658 | 529 | 1248 | 691 | 529 | 863 |
| Bilirubin (µmol/L) | -1 | 3.2 | 2.4 | 5.0 | 4.5 | 3.1 | 6.1 | 4.7 | 2.9 | 5.7 |
| | +1 | 6.6 | 4.8 | 10.4 | 6.9 | 5.1 | 8.4 | 8.1 | 6.1 | 8.1 |
| | +5 | 3.2 | 2.6 | 4.4 | 3.0 | 2.4 | 6.1 | 3.7 | 2.9 | 4.6 |
| Urea (mmol/L) | -1 | 4.2 | 3.4 | 5.3 | 4.8 | 3.6 | 5.2 | 3.8 | 3.2 | 5.0 |
| | +1 | 4.1 | 3.3 | 5.2 | 4.5 | 4. 0 | 4.8 | 4.2 | 3.1 | 5.3 |
| | +5 | 4.8[a] | 4.0 | 5.7 | 3.8[b] | 3.2 | 4.8 | 4.6[a] | 3.9 | 5.5 |

Within rows median values with different superscript are different ($P < 0.05$) revealed by Kruskal-Wallis-H-test.

Table 3
Logistic regression model showing the effect of metabolic parameters on the risk of health category for cows at week 5 postpartum[1]

| Parity group | Metabolic parameter | Time of Sampling[a] | Health category[b] (n) | P value | Odds ratio | 95% confidence interval |
|---|---|---|---|---|---|---|
| Primiparous cows | Urea | +1 | H (53) vs. CE (18) | 0.04 | 1.65 | 1.03-2.65 |
| | Urea | +1 | SCE (6) vs. CE (18) | 0.09 | 3.07 | 0.84-11.29 |
| Multiparous cows | NEFA | -1 | H (91) vs. SCE (20) | 0.04 | 12.07 | 1.07-136.27 |
| | NEFA | -1 | H (91) vs. CE (21) | 0.05 | 9.08 | 1.00-82.28 |
| | Urea | +5 | SCE (20) vs. CE (21) | 0.03 | 0.49 | 0.26-0.93 |
| | Urea | +5 | H (91) vs. CE (21) | 0.01 | 0.57 | 0.37-0.86 |
| | NEFA | +5 | H (91) vs. SCE (20) | 0.06 | 4.09 | 0.93-17.95 |

[1]Only combinations were a difference (P < 0.05) between median values for NEFA, BHBA, bilirubin and urea in the health categories was found by Kruskal-Wallis-H-test are shown.
[a] relative to calving in weeks
[b] Health category: healthy (H), clinical endometritis (CE) and subclinical endometritis (SCE)

## 3.5 Discussion

Previous studies demonstrated negative impacts of elevated concentrations of NEFA, BHBA, bilirubin, and urea on PMN function [35,39,40,51], uterine environment [9,23,24,74] and the prevalence of metritis [21,35,41,44,96]. These findings led to our hypothesis that elevated concentrations of these metabolic parameters might serve as indicators for the presence of endometritis. Differences between PP and MP cows in the regulation of fat tissue mobilization [97] and the DMI around parturition [41] have been described. Therefore, in our study metabolic profiles were analyzed separately for MP and PP cows. In MP cows, NEFA concentrations measured at wk -1 were a significant predictor for cows at higher risk of CE or SCE (CE: $P = 0.05$, SCE: $P = 0.04$) with lower concentrations in healthy cows. Sensitivities and specificities, however, were low (CE: sensitivity 38%, specificity 87%; SCE: sensitivity 35%, specificity 89%). Yet, our data generated in the field support evidence from earlier in vitro studies [54,81,101] showing that high concentrations of NEFA can affect functions of bovine blood PMN. Also this study confirms previous findings [35] on an association between energy status prior to calving and uterine health in the postpartum dairy cow utilizing a different diagnostic technique (i.e. cytobrush) for SCE that has been described as more reliable than the uterine lavage [3]. To our knowledge this is the first study to describe an exact predictor cut point for NEFA to discriminate between healthy and diseased cows. Elevated concentrations of BHBA and other ketone bodies have been shown to impair the proliferation of bone marrow cells (>1.0 mmol/L) [38], the proliferation of lymphocytes (6.25 mmol/L) in vitro [30], the in vitro chemotactic differentials of leukocytes (>1.6mmol/L) [92] and the respiratory burst activity of PMN (2.5 mmol/L) [39] in cattle. Field studies found elevated concentrations of BHBA during early lactation in cows with CE and SCE compared to healthy cows [35] and elevated BHBA concentrations (>1.2 mmol/L) in the first week postpartum indicative of an increased risk of subsequent metritis [21]. Surprisingly, our data do not confirm the diagnostic value of BHBA concentrations to predict endometritis. Differences in experimental designs, methodologies and disease definitions might have contributed to this discrepancy. In the field studies cited metritis or endometritis was diagnosed earlier in lactation (21 to 28 days postpartum [35] and before 15 days postpartum [21]) than in the current study (28 to 35 days postpartum). Furthermore, SCE was diagnosed by uterine lavage [35] and a higher threshold of PMN (>25% vs. >18% in our study) was used. Alterations of uterine pH caused by elevated concentrations of urea [23,24] have been demonstrated to affect the viability of embryos [14,55,75]. This study investigated the relationship between serum concentrations of urea and uterine health. Multiparous cows with CE had lower (healthy vs. CE: $P = 0.01$, SCE vs. CE: $P = 0.03$) plasma concentrations of urea at wk +5. This could be explained by a possible lower feed intake of cows with CE. A recent study [41] showed that cows with uterine disease occurring in the 3 weeks postpartum consumed less dry matter during the transition period compared to healthy cows. A direct comparison, however, is not valid because cows in our study were examined later in lactation (28 to 35 d postpartum) but the similar criteria were used in both studies to define uterine disease. Also, it is not clear whether the reduction in DMI is the reason for uterine disease or a consequence. Information concerning the effect of a lower urea concentration, as found in cows with CE in this study, on the uterine environment or on PMN function is to our knowledge not available. On the other side, PP cows with CE had higher serum concentrations of urea at wk +1. We can only speculate about the reason for the opposing results concerning urea concentrations in PP and MP cows with CE in this study. In cows with a ruminal flora not adapted to lactational rations, the dietary protein supply exceeds the energy availability in the bovine rumen, which results in higher urea concentrations [43]. The hypothesis of socially subordinate cows not being able to cope with frequent restructuring of the social hierarchy resulting in reduced DMI [41] may have worsened the situation. Primiparous cows in our study may have been longer socially subordinate than MP cows during the postpartum period through regrouping of the cows and the first encounter with MP cows. Socially subordinate cows are less motivated to compete for access to the feed [41]. Thus, it is possible that in our study PP cows faced a more abrupt diet transition than MP cows not only due to a change in ration but also in terms of DMI.

For the determination of the optimal predictor cut points the statistical software MedCalc utilizes the Youden’s index [100]. The index balances sensitivity and specificity equally. It is important to note that depending on the goals of diagnosing cows with CE or SCE one could use a different approach such as choosing a threshold based on high sensitivity or high specificity.

## 3.6 Conclusions

The combinations of sensitivity and specificity for the predictors cut points determined in this study (in PP cows: urea at wk +1, in MP cows: NEFA at wk -1 and urea at wk +5) were low (35 to 61% and 70 to 90%, respectively) and unsatisfactory for practical use. Therefore, we cannot recommend the use of NEFA, BHBA, bilirubin or urea at wk -1, wk +1 or wk+5 relative to calving as predictors for CE and SCE in dairy cows, yet. This study was designed as a first approach to establish predictor cut points for daily practical use. Further research, with tighter sampling intervals around parturition, measurement of body weight, and monitoring of actual DMI, is required to determine the effect of a low urea concentration on the uterine environment and the relation between dry matter intake and CE and SCE.

## 3.7 Methods

### *3.7.1 Study farm*

The study was conducted on a commercial dairy farm in Brandenburg, Germany, housing 900 Holstein-Friesian cows. A total of 209 cows (77 primiparous cows, 132 multiparous cows) that calved between December 2005 and June 2006 were enrolled in the study. The cows were housed in a free-stall barn with slotted floors and cubicles lined with rubber mats. Herd average milk yield was 9,259 kg (4.1% fat, 3.5% protein) per cow per year. A total mixed ration containing (in % dry matter) 35.4.% corn silage, 23.1% grass silage, 8.8% rye silage, 7.8% sugar beet pulp, 6.4% rumen protected rape seed, 5.8% brewer's grain, 5.4% minerals, 3.7% rape forage cake and 3.6% soya meal, was mixed and offered twice daily after milking at about 08:00 and 16:00. Water was available ad libitum.

### *3.7.2 Study design*

Blood samples were collected from all cows in the last week prior to parturition (wk -1), the first week postpartum (wk +1) and between 28 and 35 d postpartum. Blood sampling was performed at the same time every day, i.e. within 1h after the morning feed, by puncture of the vena coccygea mediana. A vacutainer system (Venoject II, Terumo Europe N.V., Leuven, Belgium) was used. Samples were kept at 4°C until centrifugation (10 min, 1.000 x g) within 8h and serum was stored in two aliquots at –25°C until analysis.

Serum concentrations of NEFA, BHBA and bilirubin were measured using colometric enzymatic reactions (NEFA C Test, Wako Chemicals GmbH, Neuss, Germany; Autokit 3-HB, Wako Chemicals GmbH, Neuss, Germany and Bilirubin total (NBD) Konelab/T Series, Thermo Fisher Scientific GmbH, Dreieich, Germany, respectively) with an automated wet chemistry analyzer (Olympus AU 400, Olympus, Hamburg, Germany). Urea concentrations were measured quantitatively using the Konelab T Series instrument for chemical analysis (Urea, Thermo Scientific, Dreieich, Germany). The laboratory was previously validated using the University of Guelph (Pearson correlation r = 0.91, n = 200). All analyses were performed according to the manufacturers' instructions.

At wk +5, all cows were examined for signs of CE by external and internal inspection. Internal inspection was performed by vaginoscopy. Based on the definition of Sheldon et al. [85], CE was characterized by the presence of purulent (>50% pus) or mucopurulent (approximately 50% pus, 50% mucus) discharge in the vagina. Cows with CE were treated intramuscularly with 0.5 mg of cloprostenol (PGF Veyx forte, Veyx Pharma GmbH, Schwarzenborn, Germany). Three weeks later the treated cows were re-examined and re-treated if not cured. If purulent or mucopurulent discharge was not found at wk +5 by vaginoscopy an endometrial cytological sample was taken using the Cytobrush technique [49]. The prevalence of SCE was determined using a threshold value of 18% PMN [47].

#### *3.7.3 Statistical analysis*

Analyses were performed for PP and MP cows separately. For statistical analyses, cows were assigned to one of three categories (CE, SCE, or healthy). Differences in each blood parameter at wk -1, wk +1 and wk +5 between the health categories were analyzed using Kruskal-Wallis-H-test. Blood parameters with differences ($P < 0.05$) were analyzed by binary logistic regression models to assess the association between a metabolic parameter and health categories. Separate models were calculated for CE vs. healthy, SCE vs. healthy and CE vs. SCE, respectively. The metabolic parameters were included as predictors. If an association ($P < 0.05$) was found, receiver operating characteristic (ROC) analysis was used to determine the optimal predictor cut point (i.e. Youden’s index) and corresponding sensitivity and specificity to discriminate between the two health categories. The area under the ROC curve (AUC) was used to assess the distinguishing ability of the metabolic parameter (the higher the value the better the distinguishing ability with values between 0.5 and 1.0) [28]. ROC analyses were performed by MedCalc software [1]. All other analyses were performed by using SPSS software [2].

## 3.8 Authors' contributions

TBK participated in the design of the study, carried out the clinical assessments and data acquisition, performed data analyses and drafted the manuscript. MD conceived the study, participated in its design and coordination, and helped to draft the manuscript. BAT investigated the data analyses performed by TBK. WH participated in the design of the study and coordination, and helped to draft the manuscript.
All authors read and approved the final manuscript.

## 3.9 Acknowledgements

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# 4. PUBLICATION III

## Systemic Antibiotic Treatment of Clinical Endometritis in Dairy Cows with Ceftiofur or two Doses of Cloprostenol in a 14 day interval

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## 4.1 Abstract

The objective of the study was to compare the systemic antibiotic treatment of clinical endometritis in dairy cows with ceftiofur with a treatment protocol consisting of 2 doses of prostaglandin $F_{2\alpha}$ analogue cloprostenol in a 14 d interval. On 2 commercial dairy farms, housing a total of 1,900 Holstein cows, all cows that calved between June 2008 and January 2009 were examined 21 to 27 dpp by vaginoscopy. Cows with clinical signs of endometritis, i.e. vaginal discharge containing flecks of pus, mucopurulent material or purulent mucus, were randomly allocated to one of two treatment groups. Cows in group CEF (n=141) received 1 mg/kg BW of ceftiofur (i.m.) on 3 consecutive days. Cows in group CLP (n=140) received 0.5 mg of cloprostenol (i.m.) at the day of enrolment and 14 d later. All cows were re-examined by vaginoscopy 42 to 48 dpp. Proportion of cows cured, i.e. cows with clear, translucent or no mucus, 42 to 48 dpp (74.2 and 80.2% in groups CEF and CLP, respectively) was not affected by treatment group (*P* = 0.09). The voluntary waiting period was set at 40 dpp. Artificial insemination (AI) submission rate, days to first service, first service conception rate, days open and proportion of cows pregnant did not differ between the groups. In conclusion, the systemic treatment with 1.0 mg/kg BW of ceftiofur on 3 consecutive days in cows with signs of clinical endometritis 21 to 27 dpp was equivalent to an intervention protocol consisting of 2 doses of cloprostenol in a 14 d interval.

## 4.2 Keywords

endometritis, antibiotic treatment, prostaglandin, reproductive performance

## 4.3 Introduction

Endometritis is one of the most common disorders in dairy cows, causing decreased fertility and high economic losses. Recently, a consortium of authors defined clinical endometritis as the presence of purulent (> 50% pus) or mucopurulent (approximately 50% pus, 50% mucus) uterine exudate in the vagina, 21 d or more postpartum, and not accompanied by systemic signs [85]. A scoring scheme for vaginal discharge ranging from clear or translucent mucus (clinically healthy) to purulent discharge has been suggested by Williams et al. (2005). The relevance of mild endometritis, defined by mucus containing flecks of pus, and its effect on reproductive performance is contradictory [15,57,71]. Although it has been demonstrated that mild clinical endometritis does not affect fertility [57], studies on subclinical endometritis found that in cows with translucent, clear or no mucus but elevated number of polymorphonuclear neutrophils (PMN) in endometrial samples, reproductive performance is negatively affected [47,49,88].

The most prevalent pathogens involved in the etiology of endometritis are *Arcanobacterium pyogenes*, *Escherichia coli, Fusobacterium necrophorum*, and Prevotella sp. [29,85,87]. Suggested treatment protocols for clinical endometritis include the intrauterine administration of antibiotics [19,58,80] or the administration of $PGF_{2\alpha}$ and its analogues [19,20,37,58]. The intrauterine infusion of antiseptics, e.g. Lugol's solution, has been tested, but some authors found negative effects on future fertility of the cows [37,53]. Furthermore, treatment approaches with proteolytic enzymes [19], the administration of estradiol [68,86] or GnRH [42] have been described.

A systemic antibiotic treatment of cows with clinical endometritis, however, as described for acute metritis [12,17,102], has not been reported in the recent literature. In an older study, Masera et al. (1980) found in cows with chronic endometritis higher concentrations of oxytetracycline in plasma after intramuscular administration than after intrauterine administration. For both routes, oxytetracycline was detectable in endometrial tissue. Pharmacokinetic data for a systemic administration of 1 mg/kg BW ceftiofur suggest an efficacious concentration of the antibiotic in the endometrium in early postpartum cows, i.e. within 3 dpp, up to 24 h after treatment [16,65]. In Europe, ceftiofur is approved for treatment of acute metritis during the first 10 dpp at a dosage of 1 mg/kg BW. Pharmacokinetic data on intrauterine applied antibiotics have rarely been published in the recent literature [8]. Bretzlaff et al. (1983) and Masera et al. (1980) described that the intrauterine administration of oxytetracycline achieves sufficient concentration in the endometrium, but little penetration to deeper layers of the uterus. In contrast to systemically applied antibiotics, it has been assumed that the presence of pus and organic debris in the uterine fluids decreases the efficacy of intrauterine antibiotics [66]. Clinical studies, however, demonstrated the efficacy of an intrauterine infusion of antibiotics in cows with clinical endometritis [58,80].

The hypothesis tested in the study was that in cows with clinical endometritis 21 to 27 dpp a systemic treatment with 1 mg/kg BW of ceftiofur on 3 consecutive days results in improved clinical cure and conception rates compared with a protocol consisting of two treatments with the $PGF_{2\alpha}$ analogue cloprostenol in a 14 d interval.

## 4.4 Materials and methods

### *4.4.1 Study animals*

The study was conducted between June 2008 and January 2009 on two commercial dairy farms in Brandenburg, Germany. Herd sizes were 851 (herd A) and 1068 (herd B) cows, respectively. Animals were housed in free-stall facilities with cubicles, rubber mats and slatted floors all year round. Calving pens were straw-bedded. On both farms, cows were fed a total mixed ration based on maize silage, grass silage and concentrates. Herd average milk yields were 9,200 kg (fat 4.2%, protein 3.3%) and 9,300 kg per lactation (fat 4.2%, protein 3.3%) in herd A and B, respectively.

### *4.4.2 Study design*

The participating farmers and the local veterinarian were informed about all relevant characteristics of the study and agreed with the study design (informed consent). The veterinarians and AI technicians involved in the study, however, were not informed about the allocation of cows to the treatment groups. All cows were examined by vaginal inspection 21 to 27 dpp. The vulva was cleaned with a dry paper towel before a speculum (Hauptner and Herberholz, Solingen, Germany) was inserted into the vagina. Each cow was examined with a speculum that had been autoclaved and single packed again at the Clinic for Animal Reproduction, Berlin. Specula were used only once on the farm and autoclaved again before the next use. Each speculum was unpacked and moistened with 0.9% saline immediately before examination. The speculum was inserted into the vagina up to the exterior cervical os; illumination was provided by an electric torch. Classification of uterine health (healthy; mild, moderate, severe endometritis) was made according to a vaginal discharge score (**VDS**) suggested by Williams et al. (2005). Clear or translucent mucus is equivalent to score 0 for healthy cows. Score 1 describes mucus containing flecks of white or off-white pus. Score 2 describes discharge containing less than 50% white or off-white mucopurulent material. In score 3 the discharge is composed of more than 50% white or yellow pus or is sanguineous. To avoid an effect of an antibiotic treatment not related to the objective of the study, cows that received a systemic antibiotic treatment prior to 21 DIM, e.g. for acute metritis were excluded from the study. Additionally, cows with vaginal lesions or a caesarean section and cows that were not supposed to be bred after calving were excluded from the study. Cows that received a systemic antibiotic treatment between 21 DIM and the re-examination 42 to 48 DIM for purposes not related to the study, e.g. acute mastitis, were retrospectively withdrawn.

Cows diagnosed with VDS > 0 at 21 to 27 DIM and not meeting the exclusion criteria were enrolled in the study. Animals were systematically allocated to one of two groups according to their ear tag numbers. In group **CEF** (even ear tag numbers), cows received a treatment with 1 mg/kg of estimated BW of ceftiofur (Excenel RTU, Pfizer Tiergesundheit, Berlin, Germany, i.m.) on 3 consecutive days, beginning at the day of enrollment. In the U.S. and in Europe, ceftiofur is approved for acute postpartum metritis. Administration of ceftiofur to cows with clinical endometritis would be considered an extra label use. Thus, for the study an experimental test permit was obtained from the responsible authorities.

In group **CLP** (odd ear tag numbers), cows received 0.5 mg of cloprostenol (PGF Veyx forte, Veyx Pharma, Schwarzenborn, Germany, i.m.) at the day of enrollment and were re-treated 14 d later. All cows enrolled in groups CEF and CLP were re-examined 42 to 48 DIM by vaginal examination as described above, except cows that had already been bred by AI.

On both farms the voluntary waiting period (**VWP**) was set at 40 DIM. Estrus detection was performed by the farm staff twice a day. Cows found in estrus were examined by an AI technician by vaginoscopy and rectal palpation and bred by AI, if confirmed in estrus and VDS 0. Animals not inseminated by 70 DIM were examined by the local veterinarians by vaginoscopy and rectal palpation for abnormalities of the genital tract and treated according to their findings. Cows with a corpus luteum received 0.5 mg of cloprostenol to induce estrus whereas cows with cystic ovaries (fluid-filled structure >2.5 cm in diameter, cow not seen in estrus) received 0.05 mg of GnRH analogue lecirelin (Dalmarelin, Selectavet, Weyarn-Holzolling, Germany, i.m.). Cows without a corpus luteum detectable and without pathological findings of the ovaries and uterus received an Ovsynch protocol [72]. These examinations were repeated in a 14 d interval. Pregnancy diagnosis was performed between 42 and 49 d after AI by transrectal palpation of the uterus and its contents by the same veterinarian on both farms.

#### *4.4.3 Reproductive parameters*

Clinical cure rate was defined as the proportion of cows with no signs of clinical endometritis, i.e. VDS 0, at the re-examination 42 to 48 DIM. Cows that had already been inseminated before the re-examination were regarded as cured. Reproductive performance was characterized by days from calving to first AI, 21 d submission rate (number of cows inseminated for the first time within 21 d after VWP divided by number of all cows after VWP), first service conception rate (FSCR) (number of cows pregnant after first AI divided by number of cows inseminated $\times$ 100), days to pregnancy, proportion of cows pregnant by 200 DIM (number of cows pregnant by 200 DIM divided by number of cows enrolled $\times$ 100).

Cows not pregnant by 200 DIM were regarded as open, even if they remained in the herd and conceived later in lactation. Cows inseminated but culled before pregnancy diagnoses were regarded as not pregnant. All diagnoses and treatments were documented on case report forms. At the end of the study, case report forms were checked for compliance.

#### *4.4.4 Statistical analyses*

Data were analyzed using SPSS (Version 16.0, SPSS Inc. Munich, Germany). The prevalence of endometritis was compared between herd A and herd B and between primiparous and multiparous cows by $\chi^2$-analysis. Binary logistic regression models were calculated for the risk of clinical cure at the re-examination and for the risk of conception at first AI as outcome variables. Survival analyses for the hazards of insemination and pregnancy within 200 DIM, respectively, were performed using Cox-Regression, censoring cows that were not inseminated and not pregnant, respectively. For logistic regression and Cox-Regression, cows culled before pregnancy diagnoses were regarded as inseminated but not pregnant. For logistic regression models as well as for Cox-Regression, VDS 21 to 27 DIM (1 = mucus with flecks, 2 = < 50% pus, 3 = > 50% pus), group (0 = CLP, 1 = CEF), herd (0 = herd A, 1 = herd B), and parity class (0 = primiparous, 1 = multiparous) were included as factors. In addition, for logistic regression model induction of estrus at first AI with $PGF_{2\alpha}$ or Ovsynch (0 = no induction of estrus, 1 = induced estrus) was included as factor. Interactions of treatment group x herd have been included in the analyses.
Adjusted odds ratios, hazard ratios, confidence intervals (**CI**), and *P*-values are reported. For logistic regression as well as for survival analyses, CI was set at 95%. For all statistical analyses level of significance was set at $\alpha = 0.05$.

## 4.5 Results

A total of 1,164 Holstein-Friesian cows that had calved between June and November 2008 were examined by vaginoscopy at 21 to 27 DIM (657 cows in herd A, 507 cows in herd B). The prevalence of endometritis 21 to 27 DIM was 39.7 and 42.2% in herd A and B, respectively, and did not differ between the herds ($P = 0.39$).

After checking the exclusion criteria, 193 cows were withdrawn from further analyses because of receiving a systemic antibiotic treatment for e.g. acute metritis or meeting other exclusion criteria. One cow with incomplete treatments (group CLP) was retrospectively withdrawn from the study. Finally, a total of 281 cows with endometritis score 1 to 3 were eligible for the final analyses (herd A: 70 in CEF, 75 in CLP; herd B: 71in CEF, 65 in CLP).

Proportion of primiparous cows was 41.8 and 38.6% in CEF and CLP, respectively ($P$ = 0.58).

The overall proportion of cows cured 42 to 48 DIM was 74.2 and 80.2% in CEF and CLP, respectively (herd A: 68.7% in CEF, 77.6% in CLP; herd B; 80.0% in CEF, 83.3% in CLP). The proportion of cows diagnosed with VDS 1 to 3 at the re-examination did not differ between the 2 groups (Table 1). A total of 28 cows were not re-examined because of culling (2 in CEF, 5 in CLP) or logistic reasons (7 in CEF, 14 in CLP). The clinical cure at the re-examination was affected by vaginal discharge score, with a lower likelihood for clinical cure for cows with VDS 3 compared with VDS 1 at first examination (Table 2).

First service conception rate was greater (32.1 vs 20.9%) in CLP than in CEF. No differences between treatments were found in 21 d submission rate, days to first service, proportion of cows bred by 200 DIM, days to pregnancy and the cumulative proportions of cows pregnant or culled within the observation period (Table 3). Estrus was induced at first AI (by the use of $PGF_{2\alpha}$ or Ovsynch) in 42 and 37 cows in CEF and CLP, respectively.

Further analysis by logistic regression revealed no effect of treatment group on FSCR, but effects of herd ($P$ = 0.05) and interactions of treatment group by herd ($P$ = 0.02). No effects were found for VDS assigned 21 to 27 DIM, induction of estrus at first AI or parity class (Table 4).

Cox regression analyses found no effect of treatment group on the likelihood of insemination within 200 DIM, however, an effect of VDS and parity class was found. The chance for insemination was lower for cows with VDS 2 or 3 compared with score 1 (HR = 0.74, $P$ = 0.04, and HR = 0.67, $P$ = 0.01, respectively) and lower for multiparous cows compared with primiparous cows (HR = 0.58, $P$ < 0.01). The chance for pregnancy within 200 DIM was lower for multiparous cows compared with primiparous cows (HR = 0.63, $P$ < 0.01). No effect was found for treatment group, VDS, and herd (Table 5).

## 4.6 Tables

Table 1.

Classification of vaginal discharge score 21 to 27 DIM and 42 to 48 DIM in cows treated with 1.0 mg/kg BW of ceftiofur on 3 consecutive days or 0.5 mg of cloprostenol at 21 to 27 and 35 to 41 DIM.

| Treatment | Examination | | Vaginal discharge score (%)[b] | | | |
|---|---|---|---|---|---|---|
| group[a] | DIM | n | Score 0 | Score 1 | Score 2 | Score 3 |
| CEF | 21-27 | 141 | - | 44.7 | 31.2 | 24.1 |
| | 42-48 | 133 | 74.2 | 14.4 | 3.0 | 8.3 |
| CLP | 21-27 | 140 | - | 36.4 | 32.1 | 31.4 |
| | 42-48 | 120 | 80.2 | 14.0 | 3.3 | 2.5 |

[a]CEF = cows with endometritis 21 to 27 DIM received 1.0 mg/kg BW of ceftiofur on 3 consecutive days; CLP= cows with endometritis 21 to 27 DIM received 0.5 mg of cloprostenol 21 to 27 and 35 to 41 DIM.

[b]Vaginal discharge score: 0 = clear or translucent mucus at vaginal examination, no signs of endometritis; 1 = mucus with flecks of white or off-white pus; 2 = discharge with less than 50% white or off-white mucopurulent material; 3 = discharge with more than 50% white or yellow pus.

Table 2.
Results of binary logistic regression analysis for the risk of clinical cure at a re-examination in cows with clinical endometritis 21 to 27 DIM treated with 1.0 mg/kg BW of ceftiofur on 3 consecutive days or 0.5 mg of cloprostenol at 21 to 27 and 35 to 41 DIM.

| | Clinical cure at re-examination | | |
|---|---|---|---|
| Factor | OR[a] | CI 95%[b] | *P* |
| Treatment group[c] | 0.49 | 0.22-1.13 | 0.09 |
| Vaginal discharge score[d] | | | < 0.01 |
| Score 1 | | Reference score | |
| Score 2 | 1.15 | 0.50-2.65 | 0.74 |
| Score 3 | 0.24 | 0.11-0.49 | < 0.01 |
| Parity class[e] | 0.68 | 0.35-1.32 | 0.25 |
| Herd[f] | 1.17 | 0.14-9.57 | 0.88 |
| Treatment group x herd | 1.45 | 0.40-5.24 | 0.57 |
| Constant | 14.87 | | < 0.01 |

[a]OR = adjusted odds ratio.
[b]CI = confidence interval.
[c]Treatment group: 0 = group CLP, cows with endometritis 21 to 27 DIM received 0.5 mg of cloprostenol 21 to 27 and 35 to 41 DIM; 1 = group CEF, cows with endometritis 21 to 27 DIM received 1.0 mg/kg BW of ceftiofur on 3 consecutive days.
[d]Vaginal discharge score at 21 to 27 DIM: 1 = mucus containing flecks of white or off-white pus, 2 = discharge containing less than 50% white or off-white mucopurulent material, 3 = discharge is composed of more than 50% white or yellow pus.
[e]Parity class: 0 = primiparous, 1 = multiparous.
[f]Herd: 0 = herd A, 1 = herd B.

Table 3.
Descriptive reproductive performance traits in cows with clinical endometritis 21 to 27 DIM treated with 1.0 mg/kg BW of ceftiofur on 3 consecutive days or 0.5 mg of cloprostenol at 21 to 27 and 35 to 41 DIM.

| | Treatment group[a] | |
|---|---|---|
| Outcome | CEF | CLP |
| 21 d submission rate, % (n/n) | 36.8 (50/136) | 29.3 (39/133) |
| 95% CI[b] | 28.3-44.5 | 21.2-36.7 |
| Median days to first AI | 69.0 | 69.0 |
| IR[c] | 51.8-79.8 | 54.0-85.0 |
| Cows inseminated at 200 DIM, % (n/n) | 95.0 (134/141) | 93.6 (131/140) |
| 95% CI | 91.1-98.3 | 89.2-97.3 |
| First AI conception rate, %, (n/n) | 20.9 (28/134) | 32.1 (42/131) |
| 95% CI | 13.6-27.4 | 23.7-39.7 |
| No. of inseminations | 378 | 350 |
| Proportion pregnant at 200 DIM, %, (n/n) | 65.2 (92/141) | 64.3 (90/140) |
| 95% CI | 57.0-75.8 | 56.0-71.9 |
| Inseminations per pregnancy | 4.4 | 3.9 |
| Median days open | 101 | 94 |
| IR | 79.0-128.8 | 65.0-121.8 |

[a]CEF = cows with endometritis 21 to 27 DIM received 1.0 mg/kg BW of ceftiofur on 3 consecutive days; CLP= cows with endometritis 21 to 27 DIM received 0.5 mg of cloprostenol 21 to 27 and 35 to 41 DIM.
[b]CI = confidence interval
[c]IR = interquartile range

Table 4.
Results of binary logistic regression analysis for the risk of conception after first AI in cows with clinical endometritis 21 to 27 DIM treated with 1.0 mg/kg BW of ceftiofur on 3 consecutive days or 0.5 mg of cloprostenol at 21 to 27 and 35 to 41 DIM.

| | Conceiving after first AI | | |
|---|---|---|---|
| Factor | OR[a] | CI 95%[b] | *P* |
| Treatment group[c] | 0.93 | 0.44-1.97 | 0.86 |
| Vaginal discharge score[d] | | | 0.86 |
| Score 1 | | —— Reference score —— | |
| Score 2 | 1.20 | 0.61-2.37 | 0.59 |
| Score 3 | 1.04 | 0.50-2.17 | 0.91 |
| Induced estrus[e] | 0.54 | 0.27-1.07 | 0.08 |
| Parity class[f] | 0.75 | 0.41-1.40 | 0.34 |
| Herd[g] | 6.24 | 1.03-37.90 | 0.05 |
| Treatment group x herd | 0.23 | 0.07-0.77 | 0.02 |
| Constant | 0.57 | | 0.38 |

[a]OR = adjusted odds ratio.
[b]CI = confidence interval.
[c]Treatment group: 0 = group CLP, cows with endometritis 21 to 27 DIM received 0.5 mg of cloprostenol 21 to 27 and 35 to 41 DIM; 1 = group CEF, cows with endometritis 21 to 27 DIM received 1.0 mg/kg BW of ceftiofur on 3 consecutive days.
[d]Vaginal discharge score: 1 = mucus containing flecks of white or off-white pus, 2 = discharge containing less than 50% white or off-white mucopurulent material, 3 = discharge is composed of more than 50% white or yellow pus.
[e]Induced estrus: 0 = no induction of estrus, 1 = estrus induced by administration of $PGF_{2\alpha}$ or Ovsynch.
[f]Parity class: 0 = primiparous, 1 = multiparous.
[g]Herd: 0 = herd A, 1 = herd B.

Table 5.
Results of the survival analyses (Cox-Regression) for the hazard of insemination and pregnancy in cows with clinical endometritis 21 to 27 DIM treated with 1.0 mg/kg BW of ceftiofur on 3 consecutive days or 0.5 mg of cloprostenol at 21 to 27 and 35 to 41 DIM.

| | Insemination | | | Pregnancy | | |
|---|---|---|---|---|---|---|
| Factor | HR[a] | CI 95%[b] | *P* | HR | CI 95% | *P* |
| Treatment group[c] | 1.04 | 0.75-1.46 | 0.80 | 1.08 | 0.72-1.62 | 0.72 |
| Vaginal discharge score[d] | | | 0.02 | | | 0.95 |
| Score 1 | —— Reference score —— | | | —— Reference score —— | | |
| Score 2 | 0.74 | 0.55-0.99 | 0.04 | 0.98 | 0.69-1.39 | 0.92 |
| Score 3 | 0.67 | 0.49-0.90 | 0.01 | 0.95 | 0.66-1.36 | 0.76 |
| Parity class[e] | 0.58 | 0.45-0.75 | <0.01 | 0.63 | 0.46-0.85 | <0.01 |
| Herd[f] | 0.77 | 0.36-1.66 | 0.50 | 1.56 | 0.61-3.93 | 0.35 |
| Treatment group x herd | 1.01 | 0.62-1.64 | 0.98 | 0.76 | 0.43-1.37 | 0.36 |

[a]HR = adjusted hazard ratio.
[b]CI = confidence interval.
[c]Treatment group: 0 = group CLP, cows with endometritis 21 to 27 DIM received 0.5 mg of cloprostenol 21 to 27 and 35 to 41 DIM; 1 = group CEF, cows with endometritis 21 to 27 DIM received 1.0 mg/kg BW of ceftiofur on 3 consecutive days.
[d]Vaginal discharge score: 1 = mucus containing flecks of white or off-white pus, 2 = discharge containing less than 50% white or off-white mucopurulent material, 3 = discharge is composed of more than 50% white or yellow pus.
[e]Parity class: 0 = primiparous, 1 = multiparous.
[f]Herd: 0 = herd A, 1 = herd B.

## 4.7 Discussion

The treatment of clinical endometritis has been the objective of numerous studies in recent years (e.g.[19,20,37,53,58,80]. The administration of $PGF_{2\alpha}$ and its analogues has been described as an adequate intervention [19,20,37,58]. Even though $PGF_{2\alpha}$ is widely used as an intervention strategy for endometritis, science-based evidence of its effect on cows with endometritis but without corpus luteum has to be demonstrated, yet. For this reason we administered two doses of cloprostenol in a 14 d interval to ensure that in all cyclic cows at least once a corpus luteum was present [37]. Studies on the intrauterine administration of antibiotics showed that this strategy was effective [79] and superior than or equal to the use of prostaglandins [20,58]. The treatment of clinical endometritis with a systemic administration of antibiotics has not been published in recent literature.

In up to 88% of postpartum cows, i.e. up to 28 dpp, bacteria were isolated from the genital tract and in 64% of cows uterine pathogens, e.g. *E. coli*, have been diagnosed [99]. In uterine samples of cows with endometritis 60.3% were positive for bacteria, whereas only 41.8% of the healthy samples showed bacterial growth [69]. Furthermore, the density of pathogen growth was higher in cows with endometritis than in healthy cows. Therefore, an antibiotic treatment of endometritis seems to be rational. The hypothesis tested in the present study was that a systemic antibiotic treatment with ceftiofur would result in improved clinical cure and conception rates followed by a greater proportion of cows pregnant at 200 DIM compared with a group treated with cloprostenol. This hypothesis, however, has to be rejected for cows included in this study. It should be emphasized that cows with a history of acute metritis and treated with antibiotics prior to the diagnosis of clinical endometritis were withdrawn from the study. Proportion of cows cured did not differ between the 2 groups; reproductive performance traits were not affected by treatment. Considering the numerical differences and the statistical power of the study it cannot be completely excluded that this may have been caused by a type II error.

Pathogens predominantly associated with endometritis, i.e. *E. coli* and *A. pyogenes* [15,99] should be susceptible to ceftiofur [82]. The systemic administration of ceftiofur in early postpartum cows results in concentrations that exceed the minimum inhibitory concentrations for pathogens associated with uterine disorders [16,65,82] and has been described as effective in the treatment of metritis [12,17,82]. Information on the pharmacokinetics of ceftiofur in cows with clinical endometritis ≥21 DIM, however, is not available. It can be hypothesized that with the present study design concentrations of ceftiofur did not exceed the minimum inhibitory concentrations required to eliminate uterine pathogens. Further research is required to evaluate whether using a larger dose of 2.2 mg of ceftiofur per kg of BW, which is approved in the U.S., or a longer treatment period would result in improved cure rates or reproductive performance traits.

The knowledge of immunological response of endometrial cells to uterine pathogens has dramatically increased in the last years, as reviewed by Sheldon et al. (2009). Recent research suggests that the use of $PGF_{2\alpha}$ for the treatment of endometritis is efficient not only by induction of myometrial contractions and estrus [98], but also by regulation of inflammatory responses in the endometrium. It has been demonstrated that bacterial endotoxin lipopolysaccharide, expressed by uterine pathogens such as *E. coli* switch the endometrial epithelial secretion of prostaglandins from the F to the E series. This results in prolonged luteal phases and modulation of endocrine functions that are essential for reproduction [36,88]. Therefore, the exogenous administration of $PGF_{2\alpha}$ might compensate the reduced endogenous epithelial secretion of $PGF_{2\alpha}$. In contrast, other authors reported that lipopolysaccharide stimulates the secretion of $PGF_{2\alpha}$ [59]. The link between the described findings in the early postpartum period and the pathogenesis of clinical endometritis is still not fully understood. Results by Gabler et al. (2009) suggested an association between a dys-regulated cytokine and/or prostaglandin profile in the endometrium and the occurrence of clinical and subclinical endometritis. Furthermore, there is an ongoing discussion in human and veterinary medicine about the protective effects of microbial biofilms produced e.g. by *E. coli* and *Staphylococcus spp.* that decrease the efficacy of antimicrobials [56,90].

It is widely accepted that clinical endometritis has a negative impact on reproductive performance [57,80,89]. Furthermore, it has been demonstrated that the proportion of cows pregnant by 200 DIM decreased with an increasing VDS [26,71]. The present study partly confirmed findings that a VDS 2 and 3 (muco-purulent and purulent vaginal discharge) has a negative impact on fertility [64,71]. We found that the chance for insemination was lower in cows with VDS 2 and 3 compared with VDS 1. No effect, however, was found for VDS 2 and 3 compared to VDS 1 on FSCR and likelihood of pregnancy. It remains unclear, if this should be interpreted as a positive effect of both treatment protocols or an effect of delayed insemination in groups VDS 2 and 3. It is questionable if cows with VDS 1 need to be treated. LeBlanc et al. (2002b) found that mild clinical endometritis (VDS 1) has no negative impact on fertility. This, however, is contradictory to findings that even cows categorized as VDS 0 but with increased proportion of PMN, i.e. subclinical endometritis, showed a reduced fertility [47].

Most authors agree that vaginal examination with a speculum is accurate for the diagnosis of endometritis [57,80,85], although the absence of discharge in the vagina does not always reflect the absence of inflammation in the uterus [3,47,85]. Result of vaginal examination has a higher predictive value for time to pregnancy than rectal palpation [57]. In this study, clinical endometritis was diagnosed by vaginal examination with a speculum. Vaginal examination can also be performed by using a gloved hand or the Metricheck device

[64,71,87]. Finally, it remains unclear, if clinical cure rates and reproductive performance traits resulted from the administration of ceftiofur, cloprostenol or from self recovery, i.e. both treatment strategies were ineffective. Because of the owners concerns on economical losses in a group with no intervention strategy, it was not possible to include an untreated control group. Only few studies have been published that included an untreated control group [58,79]. Recently, Runciman et al. (2009) found that cows diagnosed with endometritis 7 to 28 DIM benefit from an intrauterine infusion of 500 mg of cephapirin. Treated cows showed an improved reproductive performance compared with untreated controls.

It can be assumed that management practices on the farms had an effect on fertility traits. Cows were bred after a relatively short VWP of 40 DIM. Therefore, it could be hypothesized that a longer VWP with an extended interval for reconstitution of the endometrium would have resulted in higher conception rates in one of the groups. This hypothesis is partly supported by the fact that time to insemination was affected by VDS, but FSCR and proportion of cows pregnant were not. Submission rate and days to first service, however, were similar in both groups. Therefore, VWP might have influenced the overall conception rates but did not affect the results of the comparison between the groups. The management practice to use $PGF_{2\alpha}$ or an Ovsynch protocol to induce estrus and ovulation in cows not bred by 70 DIM did not affect FSCR. The design of this study, however, does not allow conclusions about the efficacy of these management tools.

Some studies found that parity is another factor that affects conception rates [10,49,93] or the chance of insemination and pregnancy [16,71] while others did not [61]. Therefore, this trait was included in the logistic regression model and in Cox regressions. In the present study, multiparous cows had a lower likelihood of insemination and pregnancy, respectively, than primiparous cows. No effect, however, was found for parity on FSCR.

For veterinarians in dairy practice, treatment decision includes also considerations about a prudent use of drugs and the cost per treatment. In addition, veterinarians should be aware of a growing public concern about a possible association between the use of antibiotics in food producing animals and multi-drug resistances of bacteria against antibiotics in human medicine.

## 4.8 Conclusion

The present study demonstrated that the systemic treatment with 1.0 mg/kg BW of ceftiofur on 3 consecutive days in cows with signs of clinical endometritis 21 to 27 DIM was not superior to a treatment protocol consisting of 2 doses of cloprostenol in a 14 d interval. In two herds with a VWP of 40 dpp, reproductive performance did not differ between the two treatment protocols. Further research should focus on the mechanisms of action of prostaglandin $F_{2\alpha}$ in cows with endometritis on future fertility. More information on the pharmacokinetics of ceftiofur in cows with clinical endometritis is required to determine reasons for treatment failures in the present study and to provide more detailed information for future treatment opportunities, e.g. with different regimes of an antibiotic therapy.

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# 5. DISCUSSION

The objectives of the studies in my thesis were to investigate the prevalence and relevance of subclinical endometritis (SCE) at breeding in dairy cows (Kaufmann, Drillich et al., 2009), to determine predictors for the prevalence of clinical endometritis (CE) (Kaufmann, Drillich et al., submitted), and to evaluate a new strategy for the treatment of CE (Kaufmann, Westermann et al., in press).

In the first study, the prevalence of SCE was 13.4 %, with a PMN level of >15 % in the uterine sample set as threshold value indicative for SCE. This prevalence was lower than in recent studies on SCE with prevalences ranging from 16 to 94 % [32,46,47,73]. However, in the cited studies samples were taken in the post partum period, whereas samples in my study were taken four hours after breeding, and threshold values for SCE in the cited studies varied from 5 % to 18 %. Even if the lowest threshold value of 5 % is applied to my dataset, the outcome of 22 % cases of SCE would have been lower than the prevalences found in most post partum studies. An explanation for the lower prevalence of SCE in the first study compared to other studies could be the difference in the time of sampling. A self recovery competence of the uterus from the end of the post partum period until the time of breeding can be assumed [31,47].

I found the highest first service conception rate (FSCR) in cows assigned to group Medium (>0 to15 % PMN) compared to groups Zero (0 % PMN) and High (> 15 % PMN) (57.6 % vs. 39.1 and 29.6 %, $P$ <0.05). It could have been expected that an inflamed endometrium has a negative effect on fertility, but the finding of an absence of PMN affecting fertility is surprising. One explanation could be a low concentration of estrogen during estrus in cows in group Zero. It is not fully understood how the changes in estrogen concentration relate to changes in PMN function [91], but it can be hypothesized that low estrogen levels result in insufficient immunological capacity of the uterus to maintain pregnancy. On the other hand, it is questionable that more than 50 % of the cows had an insufficient estrogen production.

I set out to test the cytobrush technique in cows four hours after artificial insemination (AI) with regard to subsequent fertility. It can be discussed if the technique is accurate to identifying most cases of endometrial inflammation. If endometrial inflammation is restricted to circumscriptive regions of the uterine mucosa, it could be possible that collecting cells with the cytobrush from a small area results in false negative diagnoses. A recent histological study revealed that in 82 % of examined uteri with endometritis the entire endometrium was affected [22]. Furthermore, results from our studies showed that FSCR was similar after collection of samples with the cytobrush technique four hours after AI compared with cows not examined by cytobrush (43.3 vs. 41.7 %, $P$ >0.05). These findings indicate that the cytobrush technique is suitable for identifying most cases of SCE and has no negative effect on subsequent fertility if cows are examined immediately after AI.

The first study does not provide information on the status of the endometrium before AI. It would have been preferable to sample cows immediately before AI, to gain information if the PMN found in the samples originate from a pre-breeding SCE or a post-mating PMN influx. Due to plausible concerns of the dairyman this was not possible. To prevent an interference of a possible post-mating PMN influx and SCE at AI we scheduled the time of sampling two hours before the peak of the PMN influx described in other species [34,48,76,77,95]. The significance of the results of my study, however, is limited to observations post-breeding.

The hypothesis in the second study was that elevated concentrations of NEFA, BHBA, bilirubin and urea at week -1 (wk -1), wk +1 or wk +5 relative to calving might serve as indicators for the presence of CE and SCE diagnosed at wk +5. Specifically, I set out to determine optimal predictor cut points for practical use. Analyses of the data revealed that in primiparous cows, higher concentrations of urea at wk +1 were associated with increased odds of CE ($P$ = 0.04). An optimal predictor cut point of 3.9 mmol/L (sensitivity 61 %, specificity 70 %, AUC 0.68) was determined. In multiparous cows higher concentrations of NEFA at wk -1 were associated with increased odds of CE ($P$ = 0.05) and SCE ($P$ = 0.04) compared with healthy cows. An optimal predictor cut point of 0.3 mmol/L for the discrimination between healthy and CE cows (sensitivity 35 %, specificity 89 %, AUC 0.65) was determined. Higher concentrations of urea at wk +5 were associated with decreased odds of CE compared with healthy ($P$ = 0.01) and SCE ($P$ = 0.03) cows. An optimal predictor cut point of 3.8 mmol/L for the discrimination between healthy and CE cows (sensitivity 52 %, specificity 81 %, AUC 0.07) was determined. For the discrimination between SCE and CE cows an optimal predictor cut point of 3.5 mmol/L /(sensitivity 48 %, specificity 90 %, AUC 0.72) was determined. All other tested parameters did not differ between health categories (healthy vs. CE vs. SCE).

One can only speculate about the reason for the opposing results concerning the urea concentrations in primiparous and multiparous cows with CE in this study. It is known that in cows with a ruminal flora not adapted to lactational rations, the dietary protein supply exceeds the energy availability in the bovine rumen, which results in higher urea concentrations [43]. However, the hypothesis of socially subordinate cows not being able to cope with frequent restructuring of the social hierarchy resulting in reduced DMI [41] may have worsened the situation. Primiparous cows in the study may have been longer socially subordinate than multiparous cows during the post partum period through regrouping of the cows and the first encounter with multiparous cows. Socially subordinate cows are less motivated to compete for access to the feed [41]. Thus, it is possible that in the study primiparous cows faced a more abrupt diet transition than multiparous cows not only due to a change in ration but also in terms of DMI, which led to high concentrations of urea in wk +1 in cows that were diagnosed with CE in wk +5. Multiparous cows, on the other hand, with CE had lower plasma concentrations of urea at wk +5. This could be explained by a possible lower feed intake of diseased cows at the time of diagnosis. A recent study [41] showed that cows with uterine diseases in the three weeks post partum consumed less during the transition period compared to healthy cows. A direct comparison, however, is not valid because cows in the study were examined later in lactation (28 to 35 dpp) but similar criteria were used in both studies to define uterine disease. Also, it is not clear whether the reduction in DMI is the reason for uterine disease or a consequence. Compromised immune status as a result of inadequate nutrition may increase a cow's susceptibility to metritis [41] and probably CE.

The findings of the study also showed that it is possible to use urea at wk +1 in primiparous cows as predictor for CE. Furthermore, in multiparous cows, NEFA at wk -1 can be used as predictor for CE. Urea at wk +5 can be used as indicator for the presence of CE and SCE. However, I found NEFA as well as urea measured at the above mentioned times not suitable as predictors for CE and SCE in the field. The corresponding sensitivity and specificity were not satisfactory for practical use.

The hypothesis tested in the third study was that a systemic antibiotic treatment with ceftiofur results in improved reproductive performance traits compared with traits of a group treated with cloprostenol. In conclusion, that study revealed that fertility traits (21 day submission rate, days to first service, proportion of cows bred by 200 dpp, days to pregnancy and the cumulative proportions of cows pregnant or culled) did not differ between a protocol consisting of two doses of cloprostenol and a systemic antibiotic treatment with ceftiofur. The FSCR was even greater in the CLP than in CEF group (32.1 vs. 20.9 %, $P$ = 0.04). Thus, the hypothesis of a superior treatment with ceftiofur had to be rejected.

The systemic administration of ceftiofur in early post partum cows results in concentrations that exceed the minimum inhibitory concentration (MIC) for pathogens associated with uterine disorders [16,65,84] and has been described as effective in the treatment of acute metritis [13,17]. Information on the pharmacokinetics of ceftiofur in cows with CE ≥21 dpp, however, is not available. It can be hypothesized that in the present study design concentrations of ceftiofur did not exceed the MIC required to eliminate uterine pathogens.

Further analyses of the data, however, did show an effect of the vaginal discharge score (VDS) and parity class on the likelihood of insemination and pregnancy. The chance for insemination was lower in cows with VDS 2 or 3 (2 = muco-purulent, 3 = purulent vaginal discharge) compared with VDS 1 (1 = mucus with flecks) (Hazard Ratio (HR) = 0.74, $P$ = 0.04, and HR = 0.67, $P$ = 0.01, respectively). It has been demonstrated that the proportion of cows pregnant by 200 dpp decreased with an increasing VDS [27,70]. The third study partly confirmed findings that a VDS 2 and 3 has a negative impact on the likelihood of insemination [64,70]. No effect, however, was found for VDS 2 and 3 compared with VDS 1 on likelihood of pregnancy. It remains unclear if this should be interpreted as a positive effect of both treatment protocols or an effect of delayed insemination in groups VDS 2 and 3 regardless of treatment or no treatment. Furthermore, it is questionable if cows with VDS 1 need to be treated. LeBlanc et al. (2002b) found that mild clinical endometritis (VDS 1) has no negative impact on fertility. This, however, is contradictory to findings that even cows categorized as VDS 0 but with increased proportion of PMN, i.e. subclinical endometritis, showed a reduced fertility [47].

The effect of parity on reproductive performance traits has been described by several authors (e.g. [10,45,49,70,94] and was confirmed by my first two studies. In the first study, FSCR was higher in primiparous cows than in multiparous cows (54.3 vs. 35.8%, $P$ <0.05). The chance for insemination and pregnancy was lower in multiparous cows than in primiparous cows (HR = 0.58, P < 0.01 and HR = 0.63, $P$ < 0.01, respectively) in the second study. There is conclusive evidence that parity is a factor that should be included in the analyses of data in studies on bovine reproduction.

In conclusion, SCE at AI might impair the uterine environment and affect the implantation and development of the embryo. Based on the results of the first study, I suggest defining the presence of > 15 % PMN four h after AI as indicative of SCE. Furthermore, sensitivity and specificity for the predictor cut points determined in my second study (primiparous cows: urea at wk +1; multiparous cows: NEFA at wk -1, urea at wk +5) were low (sensitivities: 35 to 61 %; specificities: 70 to 90 %). Therefore, I cannot recommend the use of NEFA, BHBA, bilirubin or urea at wk -1, wk +1 or wk +5 relative to calving as predictor or indicator, respectively, for CE and SCE at wk +5 in dairy cows. Finally, the systemic treatment with 1.0 mg/kg BW of ceftiofur for three consecutive days in cows with CE 21 to 27 dpp was not superior to a treatment protocol consisting of two treatments with cloprostenol in a 14 d interval, as demonstrated by my third study.

# 6. SUMMARY – Clinical and subclinical endometritis in dairy cattle: prevalence, indicators, and therapy

The three studies were conducted to 1) investigate the prevalence of subclinical endometritis (SCE) around breeding and its effect on first service conception rate (FSCR) in dairy cows, to 2) determine the correlation between periparturient serum concentrations of non-esterified fatty acids (NEFA), beta-hydroxybutyric acid (BHBA), bilirubin, and urea and the prevalence CE and SCE and to 3) compare systemic antibiotic treatment of clinical endometritis (CE) in dairy cows with ceftiofur with a treatment protocol consisting of two doses of prostaglandin $F_{2\alpha}$.

For the first study, a total of 201 Holstein-Friesian cows with no signs of CE were examined four hours after first artificial insemination (AI) for signs of SCE. Endometrial samples were collected from the uterus using the cytobrush technique. The proportion of polymorphonuclear (PMN) cells in the cytological sample was used to characterize an inflammation of the endometrium. Cows were categorized into three groups according to the proportion of PMN in the sample. Cows with 0 % PMN (n = 115) were assigned to group Zero, cows with >0 to15 % PMN (n = 59) to group Medium, and cows with >15 % PMN (n = 27) to group High. Pregnancy diagnosis was performed between days 38 to 44 after AI by palpation of the uterus and its contents per rectum. The FSCR was significantly higher in group Medium than in groups Zero and High (57.6 % vs. 39.1 % and 29.6 %, $P$ < 0.05). Primiparous cows were at higher risk of being classified into group Medium than multiparous cows (OR = 2.27, $P$ = 0.01). A comparison with cows that were not examined for SCE showed that the collection of endometrial samples itself had no effect on FSCR.

In the second study serum samples were collected one week prior to parturition (wk -1), in the first week post partum (wk +1) and between 28 and 35 days post partum (wk +5) from 209 Holstein-Friesian cows to determine the concentrations of NEFA, BHBA, bilirubin, and urea. At wk +5, all cows were examined for signs of CE and SCE. Of 77 primiparous cows, 23.4 % were diagnosed with CE and 7.8 % with SCE. Of 132 multiparous cows, 15.9 % were diagnosed with CE and 15.2 % with SCE. For parameters that showed a correlation with SCE or CE, optimal predictor cut points were determined. Higher concentrations of urea at wk +1 were associated with increased odds of CE (OR = 1.7, $P$ = 0.04) in primiparous cows. A predictor cut point of 3.9 mmol/L (sensitivity: 61%, specificity: 70 %) was determined. In multiparous cows, higher concentrations of NEFA at wk -1 were associated with increased odds of CE and SCE (healthy vs. CE: OR = 9.1, $P$ = 0.05; healthy vs. SCE: OR = 12.1, $P$ = 0.04). A predictor cut point of 0.3 mmol/L (healthy vs. CE: sensitivity: 38 %, specificity: 87 %; healthy vs. SCE: sensitivity: 35 %, specificity: 89 %) was determined. Increasing concentrations of urea at wk +5 were associated with decreased odds of CE (healthy vs. CE:

OR = 0.6, *P* = 0.01; SCE vs. CE: OR = 0.5, *P* = 0.03). A predictor cut point of 3.8 mmol/L (sensitivity: 52 %, specificity: 81%) was determined. For BHBA and bilirubin relationships with CE or SCE were not detected. The corresponding combinations of sensitivity and specificity of the determined predictor cut points were not satisfactory for practical use. Thus, the analysed parameters at the chosen time points cannot be recommended as predictors for CE and SCE in dairy cows.

The third study was conducted on two commercial dairy farms, housing a total of 1900 Holstein cows. All cows were examined 21 to 27 days post partum (dpp) by vaginal inspection. Cows with clinical signs of endometritis, i.e. mucopurulent or purulent vaginal discharge were randomly allocated to one of two treatment groups. Cows in group CEF (n=141) received 1 mg/kg BW of ceftiofur (i.m.) on three consecutive days. Cows in group CLP (n=140) received 0.5 mg of cloprostenol (i.m.) at the day of enrolment and 14 days later. All cows were re-examined by vaginal inspection 42 to 48 dpp. Proportion of cows cured 42 to 48 dpp was 74.2 and 80.2 % in groups CEF and CLP, respectively (*P* > 0.05). AI submission rate, days to first service, days open and proportion of cows pregnant did not differ between the groups. Risk of conception after first insemination was higher (OR = 0.56, P = 0.045) in CLP than in CEF. In conclusion, the systemic treatment with 1.0 mg/kg BW of ceftiofur on three consecutive days in cows with signs of clinical endometritis 21 to 27 dpp was not superior to a treatment protocol consisting of two doses with cloprostenol.

# 7. ZUSAMMENFASSUNG – Die klinische und subklinische Endometritis beim Milchrind: Vorkommen, Indikatoren und Therapie

Die drei Studien wurden durchgeführt um 1) die Prävalenz der subklinischen Endometritis (SKE) um den Zeitpunkt der Besamung und ihren Effekt auf den Erstbesamungserfolg (EBE) zu untersuchen, um 2) die Korrelation zwischen den Konzentrationen von freien Fettsäuren (FFS), Beta-Hydroxybutyrat (BHB), Bilirubin und Harnstoff im Serum und der Prävalenz klinischer und subklinischer Endometritiden zu bestimmen und 3) die systemische Behandlung der klinischen Endometritis (KE) des Milchrindes mit Ceftiofur mit einem Behandlungsschema bestehend aus einer zweimaligen Gabe von Prostaglandin $F_{2\alpha}$ im Abstand von 14 Tagen zu vergleichen.

Für die erste Studie wurden 201 Milchrinder der Rasse Holstein-Friesian vier Stunden nach der künstlichen Besamung (KB) auf Anzeichen einer SKE untersucht. Die zytologische Untersuchung des Uterus wurde mittels des Cytobrush Verfahrens durchgeführt. Der Anteil der polymorphkernigen neutrophilen Granulozyten (PMG) aus dem gewonnenen Zellabstrich diente als Kriterium zur Charakterisierung der Entzündung des Endometriums. Abhängig vom Anteil der PMG im Zellabstrich wurden die Kühe drei Gruppen zugeteilt. Kühe mit 0 % PMG (n = 115) wurden in die Gruppe Null, Kühe mit >0 bis 15 % PMG (n = 59) in die Gruppe Medium und Kühe mit >15 % PMG (n = 27) in die Gruppe Hoch eingestuft. Trächtigkeitsuntersuchungen wurden zwischen Tag 38 und 44 nach der KB mittels rektaler Palpation des Uterus durchgeführt. Der EBE in der Gruppe Medium war signifikant höher als in den Gruppen Null und Hoch (57,6 % vs. 39,1 % und 29.6 %, $P < 0{,}05$). Primipara wurden mit einer höheren Wahrscheinlichkeit in die Gruppe Medium eingestuft als Multipara (OR = 2,27; $P = 0{,}01$). Der Vergleich von Kühen, die mittels des Cytobrush Verfahrens untersucht wurden und Kühen die nicht mit diesem Verfahren untersucht wurden, zeigte, dass die Probennahme mittels des Cytobrush Verfahrens keinen Einfluss auf den EBE hatte.

Für die zweite Studie wurden Serumproben in der Woche ante partum (wk -1), in der Woche post partum (wk +1) und zwischen Tag 28 und 35 post partum (wk +5) von 209 Holstein-Friesian Kühen entnommen. Aus den gewonnen Proben wurden die Konzentrationen von FFS, BHB, Bilirubin und Harnstoff bestimmt. In wk +5 wurden alle Kühe auf Anzeichen einer KE oder SKE hin untersucht. Von insgesamt 77 Primipara wurde bei 23,4 % eine KE sowie bei 7,8 % eine SKE diagnostiziert. Von insgesamt 132 Multipara wurde bei 15,9 % eine KE sowie bei 15,2 % eine SKE diagnostiziert. Für Stoffwechselparameter, die eine Korrelation mit der Diagnose KE oder SKE aufwiesen wurden Grenzwerte zur Vorhersage von KE oder SKE ermittelt. Höhere Harnstoff Konzentrationen in wk +1 wiesen einen Zusammenhang mit einer erhöhten Wahrscheinlichkeit auf die Diagnose KE bei Primipara auf (OR = 1,7;

*P* = 0,04). Ein Grenzwert von 3,9 mmol/L (Sensitivität: 61 %; Spezifität: 70 %) wurde ermittelt. Bei Multipara wiesen höhere NEFA Konzentrationen in wk -1 einen Zusammenhang mit einer erhöhten Wahrscheinlichkeit auf die Diagnose KE und SKE auf (gesund vs. KE: OR = 9,1; *P* = 0,05; gesund vs. SKE: OR = 12,1; *P* = 0,04). Ein Grenzwert von 0,3 mmol/L (gesund vs. KE: Sensitivität: 38 %; Spezifität: 87 %; gesund vs. SKE: Sensitivität: 35 %; Spezifität: 89 %) wurde ermittelt. Höhere Harnstoff Konzentrationen in wk +5 wiesen einen Zusammenhang mit einer verringerten Wahrscheinlichkeit auf die Diagnose KE auf (gesund vs. KE: OR = 0,6; *P* = 0,01 und SKE vs. KE: OR = 0,5; *P = 0,03*). Ein Grenzwert von 3,8 mmol/L (Sensitivität: 52 %; Spezifität: 81 %) wurde ermittelt. Die Serum Konzentrationen von BHBA und Bilirubin wiesen keine Zusammenhänge mit den Diagnosen KE oder SKE auf. Die Kombinationen der Sensivitäten und Speziftäten der ermittelten Grenzwerte waren für den praktischen Gebrauch unbefriedigend. Aus diesem Grunde können die analysierten Parameter zu den gewählten Zeitpunkten nicht als Prädiktoren bzw. Indikatoren für KE oder SKE empfohlen werden.

Die dritte Studie wurde auf zwei kommerziellen Milchviehbetrieben, mit insgesamt 1900 Milchrindern durchgeführt. Alle Kühe wurden zwischen Tag 21 und 27 post partum mittels Spekulum vaginal untersucht. Kühe mit klinischen Anzeichen einer Endometritis, d.h. mucopurulenter oder eitriger vaginaler Ausfluss, wurden randomisiert zwei Behandlungsgruppen zugeteilt. Kühe in der Gruppe CEF (n=141) erhielten 1,0 mg/kg KG Ceftiofur (i.m.) an drei aufeinanderfolgenden Tagen. Kühe in der Gruppe CLP (n=140) erhielten 0,5 mg Cloprostenol (i.m.) am Tag der Aufnahme und nochmals 14 Tage später. Die Abschlussuntersuchung fand mittels Spekulum bei allen Kühen zwischen Tag 42 und 48 post partum statt. Die Heilungsrate betrug 74,2 % bzw. 80,2 % in den Gruppen CEF und CLP (*P* > 0.05). Brunstnutzungsrate, Rastzeit, Güstzeit und Konzeptionsrate unterschieden sich nicht zwischen den Behandlungsgruppen. Die Wahrscheinlichkeit auf Konzeption nach Erstbesamung war in der CLP Gruppe höher als in der CEF Gruppe (OR = 0,56; *P* = 0,045). Zusammenfassend lässt sich sagen, dass die systemische Behandlung mit 1,0 mg/kg KG Ceftiofur an drei aufeinanderfolgenden Tagen bei Kühen mit KE 21 bis 27 Tage post partum einer zweimaligen Behandlung mit Cloprostenol im Abstand von 14 Tagen nicht überlegen ist.

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# 9. PUBLICATIONS

Publications in journals (first author):

Kaufmann, T.B.; Drillich M.; Tenhagen, B.-A.; Forderung, D.; Heuwieser, W. (2009): Prevalence of bovine subclinical endometritis 4 h after insemination and its effects on first service conception rate.
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J Dairy Sci 94 (1), 284-290

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Kaufmann, T.B.; Drillich, M.; Heuwieser, W. (2006):
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7th Middle-European Buiatrics Congress
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Kaufmann, T.B.; Westermann, S.; Drillich, M.; Heuwieser, W. (2009):
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4. Berliner Tagung "Fruchtbarkeit beim weiblichen Rind"
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Posters at conferences (first author):

Kaufmann, T.B.; Drillich, M.; Tenhagen, B.-A.; Heuwieser, W. (2008):
Relationship between Periparturient Serum Concentrations of Non-Esterified Fatty Acids (NEFA), Beta-Hydroxybutyrat (BHBA), Bilirubin and Urea and the Prevalence of Postpartum Bovine Endometritis.
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## DECLARATION OF INDEPENDENCE

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

Berlin, den 26.05.2010

Toschi Barbara Kaufmann