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Theriogenology

Theriogenology 63 (2005) 1811–1823

www.journals.elsevierhealth.com/periodicals/the

Treatment of chronic endometritis in dairy cows with an intrauterine application of enzymes A field trial

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Received 14 December 2003; received in revised form 1 March 2004; accepted 21 May 2004

Abstract

The use of proteolytic enzymes has been established in the non-antibiotic treatment of mastitis in dairy cattle. The objective of this study was to evaluate, if enzymes are efficacious in the treatment of chronic endometritis. In a controlled field trial, cows with vaginal discharge 21–27 days in milk (DIM) were randomly assigned to two treatment groups. Endometritis was classified into three categories, depending on the type of vaginal discharge: clear mucus with flakes of pus (E1), mucopurulent discharge or fluctuating contents in the uterus (E2), and purulent discharge (E3). In group ENZYMES ($n = 191$), cows received an intrauterine treatment with a salve containing the enzymes trypsin (16 mg), chymotrypsin (16 mg), and papain (8 mg). Cows in group PGF ($n = 225$) were treated with 0.5 mg of cloprostenol. Cows that did not show any clinical signs of chronic endometritis were regarded as healthy control group (HC, $n = 699$). In groups ENZYMES and PGF, all cows were re-examined 35–41 DIM. In group ENZYMES, cows were re-treated with enzymes if signs of endometritis were found, while in group PGF all cows received a second dose of cloprostenol, regardless of their clinical findings. Cure rate after the first treatment, defined as the absence of vaginal discharge at the re-examinations, was 59.7 and 68.0% in groups ENZYMES and PGF, respectively ($P > 0.05$). Reproductive performance measures showed no significant differences between the two treatment groups. Service rate was significantly lower for ENZYMES and PGF, respectively, compared to HC. Conception rates to all services and percentages of cows pregnant by 250 DIM were significantly lower in group ENZYMES compared to HC, while no further differences were found between PGF and HC. In both treatment groups, cure rate and

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reproductive performance measures were better for cows categorized E1 or E2, than for cows categorized E3, respectively. Conception rate to all services for cows with endometritis category E1 was higher in group PGF than in group ENZYMES ($P < 0.05$). The results of this field trial suggest that prostaglandin $F_{2\alpha}$ is still the treatment of choice for chronic endometritis in dairy cattle.

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Keywords: Chronic endometritis; Prostaglandin $F_{2\alpha}$; Proteolytic enzymes; Reproductive performance; Dairy cattle

1. Introduction

Chronic endometritis in cows is one of the most common postpartum disorders on dairy farms. Depending on management and environmental conditions, i.e. deficiencies in hygiene at calving, dystocia, inadequate feeding of dry cows, and infectious diseases, the average incidence of chronic endometritis is up to 37.5% [1–4]. The main clinical symptom of chronic puerperal endometritis in dairy cattle is purulent vaginal discharge, an enlarged uterus [3,5] in combination with an enlarged cervical diameter [7]. The general attitude of the cows is usually unaffected. However, in literature, there is no standardized definition of chronic endometritis [6,7]. The incidence of chronic endometritis also depends on the sensitivity of the diagnostic method and the time postpartum when the examination was performed. Later in lactation fewer cows with endometritis are found [8–10]. In veterinary practice, palpation per rectum and vaginoscopic examinations are routinely used for examination. Although vaginoscopic examination is more sensitive to detect abnormal vaginal discharge [6,7,11], in large dairy herds many practitioners abandon vaginoscopy because of logistical and/or hygienic aspects. Recent research has shown that modern diagnostic tools, such as uterine cytology and ultrasound are more sensitive to detect chronic and subclinical endometritis [12–14]. These studies revealed that vaginoscopy has also a limited diagnostic value. However, cytology and ultrasound are not established in veterinary practice for the routine diagnosis of chronic endometritis in cattle.

Most authors agree that chronic endometritis is associated with depressed reproductive performance in the current lactation [3,9,15]. This is causing significant economic losses to the dairy farm up to US\$ 106 per cow per year [8,16].

The treatment of chronic endometritis in dairy cattle has been under discussion for several years. While some authors stated that chronic endometritis has a tendency to recover without intervention with no negative impact on reproductive performance [9,10], others described a depressed fertility even for cases of subclinical endometritis [7,12,13]. For the treatment of chronic endometritis in dairy cows, the use of prostaglandin $F_{2\alpha}$ is established in veterinary routine therapy [17–19]. Also, an intrauterine treatment with antibiotics has been found effective in recent publications [10,20]. The intrauterine treatment with disinfectants, e.g. Lugols iodine is still widespread in Europe, although several studies have shown no benefits from this treatment on reproductive performance [3,9,19,21]. An ideal treatment of endometritis should eliminate bacteria in the uterine cavity and in the subendometrial layers, should not inhibit the normal uterine defense mechanisms, and have no withdrawal period for milk [22]. However, in public and political

opinion the use of antibiotics and hormones in food-producing animals is increasingly under critical discussion.

The use of proteolytic enzymes for the intramammary treatment of mastitis has been described by Zander [23] and Krüger et al. [24]. Chymotrypsin, trypsin and papain have fibrinolytic and proteolytic activity in inflamed tissue. This effect is supposed to support the cellular defence mechanism. In vitro, enzymes inhibit the growth behaviour and survival of micro organisms, e.g. *Staphylococcus* spp, *Streptococcus* spp, *Escherichia coli* [24]. Most frequently isolated bacteria from the uterus of cows with chronic endometritis are *Arcanobacterium pyogenes*, *E. coli*, streptococci and other aerobic bacteria as well as anaerobic bacteria *Fusobacterium necrophorum* and *Bacteroides* spp [25].

The objective of this study was to evaluate the use of proteolytic enzymes for the treatment of bovine chronic endometritis in a field trial in comparison with a repeated treatment with prostaglandin F_{2α}-analogue cloprostenol in a 14-day interval. The treatment of chronic endometritis with enzymes has never been evaluated in a controlled field study before. The criteria for the efficacy of the treatment protocols were clinical cure rate, defined as no vaginal discharge at the re-examination and reproductive performance measures in the current lactation.

2. Material and methods

The study was conducted on a commercial dairy herd in Brandenburg, Germany. A total of 750 cows were housed in groups in freestall facilities with slotted floor and cubicles. Two weeks before calving, cows were moved into tiestalls with rubber mats and slotted floor. Seven days after calving, cows were grouped in freestall facilities. Cows were fed with mixed rations and additional concentrates according to their current milk yield. Average milk yield was 8200 kg per cow per year, with 4.16% fat and 3.53% protein.

All cows were examined by external inspection and palpation of the genital tract per rectum between 21 and 27 days in milk (DIM; Exam 1). Size of the uterine horns, fluctuating contents, and vulval discharge were recorded as well as size of the ovaries and presence of follicles or corpora lutea. Vaginal discharge and/or fluctuating contents in the uterus were regarded as clinical signs for chronic endometritis. Endometritis was classified in three categories: clear mucus with flakes of pus (E1), mucopurulent discharge or fluctuating contents in the uterus (E2), and purulent discharge with or without palpable contents in the uterus (E3).

Enrolment of cows with signs of endometritis into one of the two treatment groups was according to the last digit of the ear-tag number of the cows. Cows with even ear-tag numbers were enrolled in group ENZYMES and with uneven ear-tag numbers in group PGF, respectively. Cows destined for culling were excluded from the trial.

Cows in group ENZYMES received an intrauterine treatment with 20 g of Masti Veyxym[®] (Veyx Pharma, Schwarzenborn, Germany), containing the enzymes chymotrypsin (16 mg), trypsin (16 mg), and papain (8 mg), respectively, and additionally 200,000 IU of retinolpalmitat (vitamin A) and 240 mg of α-tocopherolacetat (vitamin E). The product was applied by a disposable catheter as used for artificial insemination. All cows with endometritis were re-examined 14 days later (35–41 DIM) by palpation of the genital

tract per rectum (Exam 2). Cows with signs of endometritis were re-treated locally with enzymes at Exam 2. These cows were re-examined 14 days later (49–55 DIM; Exam 3) and received a third treatment with enzymes if signs of endometritis were found.

Cows in group PGF received an injection with 0.5 mg of the prostaglandin $F_{2\alpha}$ -analogue cloprostenol (PGF Veyx forte[®], Veyx Pharma, Schwarzenborn, Germany) intramuscularly. At Exam 2 (35–41 DIM), all cows in group PGF were re-examined and re-treated, regardless of their clinical findings at Exam 2. Cows with signs of endometritis at Exam 2 received a third examination 49–55 DIM (Exam 3) and were treated with cloprostenol if signs of endometritis were found.

Cows without signs of endometritis at Exam 1 were regarded as healthy control group (HC). In this group, no intrauterine treatment or treatment with cloprostenol was administered until 72 DIM.

The voluntary waiting period was set at 72 DIM. All cows were examined 68–74 DIM by palpation of the genital tract per rectum. Cows with signs of endometritis were treated according to their study group. In all groups, cows with a corpus luteum received cloprostenol to induce estrus. Cows were artificially inseminated on observed estrus by an AI-technician. Pregnancy diagnosis (PD) was performed 38–44 days after AI. Cows not inseminated by 100 DIM and cows inseminated but not pregnant at PD were examined by palpation of the genital tract per rectum (fertility exam). The fertility exam was repeated in 14-day intervals until the cows were inseminated. Cows with signs of endometritis were treated with 0.5 mg of cloprostenol, regardless of their study group. Cows with a corpus luteum received 0.5 mg of cloprostenol to induce estrus. Cystic ovarian disease (COD) was defined as fluid-filled structure about 2.5 cm in diameter on the ovary by repeated palpation per rectum in 14-day intervals. If the suspected structure for COD was confirmed at the second examination, cows received GnRH (0.02 mg of buserelin, Receptal[®], Intervet Deutschland GmbH, Oberschleißheim, Germany). Cows without any functional structures on the ovaries at two consecutive fertility exams were regarded as acyclic cows. These cows received GnRH to induce cyclic activity and the growth of a follicle.

The outcome of the treatment was assessed by clinical cure rate, defined as no vaginal discharge at the re-examination after first treatment and by reproductive performance measures in the current lactation. Reproductive performance measures and their definitions are described in Table 1.

Statistical analysis was performed by using SPSS[®] for Windows (version 10.0.7, SPSS Inc., Munich, Germany). Clinical cure rate, service rate, first service conception rate, conception rate to further services, conception rate to all services, and cows pregnant were compared using Chi-square analysis [26]. Days to first service and days open were compared using Mann–Whitney *U*-test. The level of significance was set at $\alpha = 0.05$.

3. Results

A total of 1115 cows and heifers, which calved between August 2001 and March 2003, were enrolled in the study. Prevalence of chronic endometritis 21–27 DIM was 37.3% ($n = 416$). The classification of endometritis at Exam 1 in groups ENZYMES and PGF is shown in Table 2.

Table 1
Reproductive performance measures

Parameter	Definition
Service rate	$\frac{\text{Cows inseminated } 72 - 92 \text{ DIM} \times 100}{\text{No. of cows } > 72 \text{ DIM}}$
Days to first service	Date of first service–Date of calving
Days open	Date of successful AI–Date of calving
First service conception rate	$\frac{\text{Cows pregnant to 1st AI} \times 100}{\text{No. of cows inseminated}}$
Conception rate to further services	$\frac{\text{Cows pregnant to 2nd or greater AI} \times 100}{\text{Total no. of AI} - \text{No. of 1st AI}}$
Conception rate to all services	$\frac{\text{No. of cows pregnant} \times 100}{\text{Total no. of AI}}$
Cows pregnant within 250 DIM	$\frac{\text{No. of cows pregnant within 250 DIM}}{\text{Total no. of cows enrolled}}$

Before Exam 2 was performed 35–41 DIM, five cows in group ENZYMES and eight cows in group PGF left the herd. Another two cows in group ENZYMES and four cows in group PGF were not examined at Exam 2 due to logistical reasons. Clinical cure rate after the first treatment, i.e. no endometritis at Exam 2 was 59.7 and 68.0% in groups ENZYMES and PGF, respectively. The differences in cure rates for all cows and for categories E1 and E3 at Exam 1 were statistically not significant between the groups. For cows with endometritis categorized as E2, cure rates were significantly higher in group PGF (67.1%) than in group ENZYMES (47.3%). Diagnoses at Exam 2 are shown in Table 3.

After Exam 2, four cows in group ENZYMES were withdrawn from the study because of treatments not according to the study protocol. Another six cows in group ENZYMES and three cows in group PGF were not examined at Exam 3 because of culling or logistical reasons. Cure rates at Exam 3 were lower for cows with endometritis at Exam 2 in group ENZYMES (56.7%) than for cows in group PGF (77.2%, $P < 0.05$).

At 68–74 DIM, cows not already culled, bred or excluded from breeding were examined by palpation of the uterus and the ovaries per rectum. The percentage of cows with signs of endometritis at this examination was 13.6 (18/132), 8.1 (13/160), and 4.6% (24/521) in groups ENZYMES, PGF, and HC, respectively. The difference between groups ENZYMES and HC was significant ($P < 0.05$). The examination of the ovaries revealed that the percentage of cows with cystic ovarian disease was significantly higher (11.3%) in group PGF than in groups ENZYMES and HC, respectively (3.0, and 5.5%, respectively).

Table 2
Classification of endometritis at 21–27 DIM (Exam 1) in groups ENZYMES and PGF

Classification	ENZYMES ($n = 191$)	PGF ($n = 225$)
Endometritis 1 (E1, %)	102 (53.4)	131 (58.2)
Endometritis 2 (E2, %)	74 (38.7)	76 (33.8)
Endometritis 3 (E3, %)	15 (7.9)	18 (8.0)

Table 3

Diagnoses at 35–41 DIM (Exam 2) for three categories of endometritis at 21–27 DIM (Exam 1) in groups ENZYMES and PGF, respectively

Group	Diagnosis Exam 1		Diagnosis Exam 2					Missing ^a
			No endometritis	Endometritis E1	Endometritis E2	Endometritis E3		
ENZYMES	E1	<i>n</i> = 102	<i>n</i> (%)	72 (70.6)	17 (16.7)	9 (8.8)	0 (0.0)	4 (3.9)
	E2	<i>n</i> = 74	<i>n</i> (%)	35 (47.3) a	23 (31.1)	12 (16.2)	2 (2.7)	2 (2.7)
	E3	<i>n</i> = 15	<i>n</i> (%)	7 (46.7)	1 (6.7)	4 (26.7)	2 (13.3)	1 (6.7)
	Total	<i>n</i> = 191	<i>n</i> (%)	114 (59.7)	41 (21.5)	25 (13.1)	4 (2.1)	7 (3.7)
PGF	E1	<i>n</i> = 131	<i>n</i> (%)	97 (74.0)	26 (19.8)	2 (1.5)	0 (0.0)	6 (4.6)
	E2	<i>n</i> = 76	<i>n</i> (%)	51 (67.1) b	16 (21.1)	5 (6.6)	0 (0.0)	4 (5.3)
	E3	<i>n</i> = 18	<i>n</i> (%)	5 (27.8)	5 (27.8)	4 (22.2)	2 (11.1)	2 (11.1)
	Total	<i>n</i> = 225	<i>n</i> (%)	153 (68.0)	47 (20.9)	11 (4.9)	2 (0.9)	12 (5.3)

Values in columns with different letters (a, b) differ significantly ($P < 0.05$). E1, E2, E3 = endometritis category 1–3, respectively.

^a Missing = cows culled or not examined.

The percentage of cows receiving cloprostenol to induce estrus, i.e. a palpable corpus luteum was diagnosed, was significantly lower in group ENZYMES than in groups PGF and HC, respectively (43.9, 57.5, and 59.3%, respectively). The percentage of cows with no cyclic activity, i.e. no palpable corpus luteum or follicle, was 12.1, 6.3, and 8.1% in groups ENZYMES, PGF, and HC, respectively ($P > 0.05$).

For the analysis of reproductive performance, 187, 225, and 699 cows were eligible in groups ENZYMES, PGF, and HC, respectively. Table 4 summarizes the reproductive performance measures for the three groups. Between the two treatment groups no significant differences were found regarding service rate, days to first service, first service conception rate, conception rate to all services, days open and cows pregnant within 250 DIM. Conception rate to further services was significantly lower in group ENZYMES than

Table 4

Reproductive performance measures for groups ENZYMES, PGF, and HC, respectively

Measure	ENZYMES (<i>n</i> = 187)	PGF (<i>n</i> = 225)	HC (<i>n</i> = 699)
Cows inseminated (%)	167/187 (89.3)	198/225 (88.0)	627/699 (89.7)
Service rate ^a (%)	67/150 (44.7) a	90/180 (50.0) a	329/555 (59.3) b
Days to first service (mean and S.D. ^b)	90.5 ± 23.3	91.2 ± 24.0	88.0 ± 22.8
No. of inseminations	412	453	1348
First service conception rate (%)	64/167 (38.3)	81/198 (40.9)	256/627 (40.8)
Conception rate to further services (%)	71/245 (29.0) a	97/255 (38.0) b	305/721 (42.3) b
Conception rate to all services (%)	135/412 (32.8) a	178/453 (39.3)	561/1348 (41.6) b
Days open (mean and S.D. ^b)	122.5 ± 47.9	121.9 ± 44.2	115.1 ± 42.0
Cows pregnant within 250 DIM (%)	135/187 (72.2) a	178/225 (79.1)	561/699 (80.3) b

Values in rows with different letters (a, b) differ significantly ($P < 0.05$).

^a The difference in the dominator to the number of cows in the group is due to cows bred or culled before 72 days postpartum.

^b S.D. = standard deviation.

in group PGF. While service rate was significantly higher in group HC than in groups ENZYMES and PGF, respectively, conception rate to further services and conception rate to all services were higher in group HC than in group ENZYMES, but did not differ significantly between group HC and group PGF.

Fig. 1 shows the percentage of cows pregnant in the course of lactation. Within the first 150 DIM the percentage of cows pregnant was similar for the two treatment groups but lower than in group HC. Afterwards the percentage of cows pregnant increased faster in groups PGF and HC than in group ENZYMES.

The percentage of cows culled was 27.8 (52 cows), 20.9 (47), and 19.7% (138) in groups ENZYMES, PGF, and HC, respectively ($P > 0.05$). The percentage of cows culled for reproductive reasons was 7.0 (13 cows), 2.7 (6), and 3.8 % (27) in groups ENZYMES, PGF, and HC, respectively ($P > 0.05$).

The influence of the category of chronic endometritis on reproductive performance is summarized in Table 5. All reproductive performance measures were numerically worse for cows with endometritis category E3 compared to category E1 and E2. However, the number of cows in category E3 was low in both treatment groups. Between the groups significant differences in reproductive performance measures were found for conception rate to all services in category E1 (34.5 and 44.8% in ENZYMES and PGF, respectively). In group PGF, the percentage of cows pregnant within 250 DIM was significantly higher for cows with E1 than for cows with E3.

4. Discussion

The prevalence of chronic endometritis during the study period was within the range observed in other studies that based diagnosis of endometritis on palpation of the genital tract per rectum [3,4]. LeBlanc et al. [6] have emphasized that diagnoses by a combination of external inspection, vaginoscopic examination and palpation of the cervical diameter have a higher predictive value regarding time to next pregnancy than palpation of the uterus

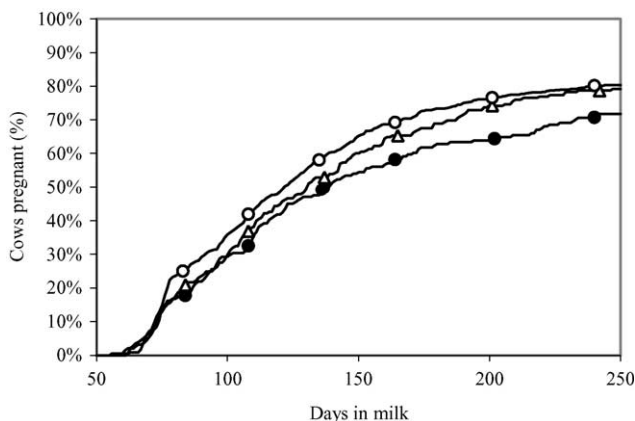


Fig. 1. Cows pregnant in the course of lactation for groups ENZYMES (●), PGF (△), and HC (○), respectively.

Table 5
Reproductive performance measures for endometritis category E1–E3 at Exam 1 (21–27 DIM)

Group	Exam 1	Days to first service (means and S.D. ^a)	Service rate (%)	First service conception rate (%)	Conception rate to all services (%)	Days open (means and S.D. ^a)	Cows pregnant within 250 DIM (%)
ENZYMES	E1	90.5 ± 23.3	40.8	39.3	34.5 a	121.0 ± 49.6	71.0
	E2	90.7 ± 24.2	50.0	41.2	32.4	119.6 ± 45.0	76.7
	E3	88.4 ± 17.7	45.5	10.0	24.2	156.0 ± 44.6	57.1
PGF	E1	91.4 ± 24.6	52.9	43.2	44.8 b	120.1 ± 43.8	84.7 a
	E2	89.6 ± 21.5	47.5	41.5	40.0	125.5 ± 44.3	76.3
	E3	96.9 ± 29.8	42.9	20.0	15.0	121.9 ± 52.1	50.0 b

Values in columns with different letters (a, b) differ significantly ($P < 0.05$). E1, E2, E3 = endometritis category 1–3, respectively.

^a S.D. = standard deviation.

alone. With a more precise diagnostic method i.e. vaginoscopic examination, cytology or ultrasound, probably more cows with signs of mild endometritis would have been found [6,13,14].

Clinical cure rates at the re-examination did not differ statistically between the two treatment groups. However, numerically cure rate was higher in PGF. The number of cows with endometritis at Exam 3 and at 68–74 DIM, respectively, was higher in ENZYMES than in PGF. For both groups cows with signs of mild endometritis, i.e. category E1, showed higher cure rates than cows with E2 or E3, regardless of the treatment applied. For cows with endometritis category E2, cure rate was significantly higher in group PGF compared to group ENZYMES. This indicates that for cows with moderate endometritis (E2), the use of prostaglandin $F_{2\alpha}$ is more effective than the use of enzymes. On the other hand, for cows with E3, group ENZYMES had numerically higher cure rates than group PGF. For a valid interpretation the number of cows with E3 was too small in both groups. To evaluate the absolute clinical efficacy of both treatments, a group of cows with endometritis but untreated would have been necessary. It has been often discussed that chronic endometritis in dairy cattle has a tendency to cure without intervention [6]. The study was conducted on a commercial dairy herd and the farmer was concerned about leaving a group of cows with endometritis untreated while using a treatment of unknown efficacy in another group.

Cure rate was based only on clinical symptoms with the limitations of the diagnostic methods used in veterinary practice. Therefore, the efficacy of a treatment for endometritis in dairy cows should be evaluated also by reproductive performance. To assess the outcome of the treatment protocols, reproductive performance measures for the two treatment groups and the healthy control group were compared. Service rate and days to first service were not different between the two treatment groups. These parameters are influenced by recovery after uterine infections and also by reproductive management. In this study, all cows were examined at the end of the voluntary waiting, except those cows that had already been inseminated. If a corpus luteum was diagnosed, estrus was induced by prostaglandin $F_{2\alpha}$. This is an effective and common method to improve estrus detection rates [27,28]. In comparison to healthy control cows, service rate was significantly lower in both treatment groups. Stevenson et al. [29] described that chronic endometritis has a negative effect on cyclic activity and estrus behaviour. In the present study, the number of cows with endometritis (ENZYMES) and cystic ovarian disease (PGF) at the end of the voluntary waiting period showed significant differences compared to group HC and might have depressed service rate and days to first service in the two treatment groups. Endometritis has been described as a risk factor for cystic ovarian disease [30,31]. Bosu and Peter [32] have suggested that increased secretion of prostaglandin $F_{2\alpha}$ and cortisol after intrauterine infections might be associated with the formation of cystic ovaries. Therefore, it can be hypothesized that the repeated treatment with prostaglandin $F_{2\alpha}$ could be responsible for a higher rate of cystic ovaries in group PGF. In contrast, López-Gatius and López-Béjar [33] showed that the repeated application of prostaglandin $F_{2\alpha}$ in the preservice period reduced the risk for cystic ovarian disease significantly. The percentage of cows without cyclic activity at the end of the VWP was not influenced by endometritis. However, the limited diagnostic accuracy of palpation of the ovaries has been shown [34,35]. Thus, these results should not be overestimated.

It can be assumed that chronic endometritis left alterations in the uterine lining and decreased the ability to conceive. Lee et al. [15] collected data from 1059 lactations and demonstrated that endometritis was associated with decreased conception rate and increased days open. Recent studies [12,13] have shown that subclinical endometritis also has a negative impact on first service conception rate and the percentage of cows pregnant within a study period. Assuming that chronic endometritis decreases first service conception rate, the results in this study indicate an equal efficacy of both treatment strategies for first service conception rate.

The conception rate for further services, conception rate to all services, and the proportion of cows pregnant was lower in group ENZYMES compared to group HC, while no differences were found between groups PGF and HC. It can be assumed that cows that did not conceive to first AI were more likely to have chronic lesions in the endometrial lining and that differences in therapeutic efficiency were even more pronounced in this group of cows. Based on this assumption, the treatment with cloprostenol was more effective than the treatment with enzymes. It has to be mentioned that in group PGF all cows received two treatments with cloprostenol while only 36.7% of the cows were re-treated in group ENZYMES. A second treatment for all cows in group ENZYMES might have increased the subclinical cure rate and following increased conception rates and percentage of cows pregnant. The observation that first service conception rate showed no differences between the three groups while conception rate for all services was lowest in group ENZYMES can be verified in the percentage of cows pregnant in the course of lactation. Within the first 150 DIM there are no differences between the three groups. After that, the percentage of cows pregnant increases more rapidly in groups PGF and HC than in group ENZYMES. Percentage of cows pregnant by 250 DIM showed no significant differences between groups ENZYMES and PGF, but between groups ENZYMES and HC. The percentage of cows culled for unsatisfying reproductive performance did not differ between the three groups. In literature, the percentage of cows culled for reproductive reasons varies widely. Esslemont and Kossaibati [2] found 36.5% of culling were for reproductive reasons, while Etherington et al. [36] reported of only 4.4% of all cows being culled caused by low reproductive performance. Bartlett et al. [8] found a positive association between endometritis and culling. However, many factors influence the culling decision in a herd [37,38]. The influence of the postpartum treatment on events after more than 150 DIM remains speculative.

It has been discussed controversially in the literature if cows with signs of mild endometritis should be treated or not [9,10,22]. Due to reasons mentioned above, we had no untreated control group in this study. However, reproductive performance in the healthy control group can be regarded as a “gold standard” for this particular herd. Besides numerically differences between HC and cows with E1 in groups ENZYMES and PGF, respectively, statistically significant differences were found for service rate (ENZYMES, PGF, respectively versus HC) and cows pregnant (ENZYMES versus HC). This observation indicates that for days to first service, first service conception rate, conception rate to all services and days open both treatments resulted in reproductive performance for cows with E1 as good as the “gold standard”. However, it cannot be concluded that spontaneous recovery from endometritis without any therapy would have achieved the same results. With the assumption that enzymes had no therapeutic effect in this study, this

data confirm that even untreated chronic endometritis E1 had a negative impact on the percentage of cows pregnant (ENZYMES vs HC) and can be treated efficaciously with prostaglandin $F_{2\alpha}$. On the opposite, with the hypothesis that $PGF_{2\alpha}$ had no therapeutic effect on E1, but endometritis recovers without intervention, the results suggest that enzymes had some negative effects on subsequent fertility as described for other intrauterine treatments [12,22]. So far, there is no evidence that enzymes damage the endometrial tissue.

The analyses of cure rates after the first treatment and reproductive performance measures revealed that for some parameters the treatment of chronic endometritis with prostaglandin $F_{2\alpha}$ was superior to the intrauterine treatment with enzymes, while other parameters showed no significant differences.

Due to growing consumers concerns towards hormones and antibiotics further research is required to develop new strategies for the treatment of chronic endometritis. The first evaluation of proteolytic enzymes showed no superiority for the described treatment regime compared to the treatment with $PGF_{2\alpha}$.

5. Conclusion

In veterinary practice, the treatment of chronic endometritis is usually based on prostaglandin $F_{2\alpha}$ and its analogues or an intrauterine infusion of antibiotics. However, the use of hormones and antibiotic drugs in food producing animals is under critical public discussion. This requires research for new therapeutic strategies. The present study is the first that evaluated the treatment of chronic endometritis with proteolytic enzymes. From the results of this study it can be concluded that prostaglandin $F_{2\alpha}$ is still the treatment of choice for chronic endometritis in dairy cows.

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