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Evaluation of endometrial echotexture and cervical cytology in cows during and after treatment of endometritis

Küçükaslan, I ; Kaya, D ; Emre, B ; Bollwein, H ; Özyurtlu, N ; Mülazimoglu, B ; Aslan, S

Abstract: Ziel der Studie war, nach intrauteriner Verabreichung der antiseptischen Lösung Lotagen® (Metacresolsulfonsäure und Formaldehyd) bei Kühen, die nach Behandlung trächtig wurden, die Endometriumveränderungen mittels Echotexturanalyse zu beurteilen. Material und Methodik: 21 Kühe wurden drei Gruppen zugeteilt: geringgradige (E1, n = 7), moderate (E2, n = 8) und hochgradige Endometritis (E3, n = 6). Die Kontrollgruppe (C, n = 11) umfasste gesunde, unbehandelte Tiere ohne Endometritis. Mit einem computergestützten Bildanalyseprogramm wurden an sonographischen Endometriebildern (B-Modus) die Echostrukturparameter Kontrast (CON), Gradient (GR), Homogenität (HOM) und mittlere Graustufe (MGL) unmittelbar vor (0 h), 1 h, 6 h und 2, 3, 5 und 10 Tage nach der Behandlung analysiert. Ergebnisse: Zum Zeitpunkt (ZP) 0 h lag GR in Gruppe E2 niedriger ($p < 0,05$) als in Gruppe E1 und C. In Gruppe E2 und E3 stieg GR zwischen 0 h und 10 d, in Gruppe C nahm GR im selben Zeitraum ab ($p < 0,05$). Umgekehrt waren CON der Gruppe E2 zum ZP 0 h deutlich niedriger als an den folgenden ZP und niedriger als in Gruppe C ($p < 0,05$). Auch in Gruppe E3 war CON am Tag 10 höher als in Gruppe C ($p < 0,05$). HOM war an Tag 5 und 10 in den Gruppen E1, E2 und E3 niedriger ($p < 0,05$) als in Gruppe C. In Gruppe E2 sank HOM bis zum 5. Tag nach Behandlung ab ($p < 0,05$). Ferner war HOM in Gruppe E3 an den ZP 6 h, d 2 und d 10 höher als zum ZP 0 h ($p < 0,05$). Im Gegensatz zu GR wiesen Kontrolltiere an den ZP 6 h und d 10 höhere HOM-Werte auf als E3-Kühe. Die MGL-Werte der E2-Kühe überschritten die der Kontrolltiere bis zum Tag 10 ($p < 0,05$). Für Gruppe E3 galt dies für den ZP 6 h ($p < 0,05$). Bei E2-Tieren stieg MGL bis zum 2. Tag an und fiel dann ab ($p < 0,05$). Schlussfolgerung: Die Echostrukturparameter spiegeln die Veränderungen im Endometrium wider und können zur Beurteilung des Heilungsverlauf nach Endometritisbehandlung dienen.

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Evaluation of endometrial echotexture and cervical cytology in cows during and after treatment of endometritis

Bewertung der Echotexturparameter und Zervixzytologie des Endometriums bei Kühen während und nach der Behandlung von Endometritis

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and Gynaecology

Running Title: Echotexture proves endometrial changes after cure

Arbeitstitel: Die Echotextur beweist die endometriale Änderungen nach der Behandlung wider

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SUMMARY

Objective: The aim of this study was to evaluate changes in the endometrium by using echotexture parameters during and after treatment of endometritis with intrauterine administration of an intrauterine antiseptic solution Lotagen® (3% Metacresolsulphonic acid and formaldehyde) in cows which got pregnant after treatment. **Material and methods:** Cows were divided into 4 groups due to the severity of endometritis; **E1** (slight, n=7), **E2** (moderate, n=8), **E3** (severe, n=6) and control group (**CG**; without endometritis, no intrauterine administration, n=11). A software (Bs200 Pro®) was used to evaluate echotexture parameters

Contrast (**CON**), Gradient (**GR**), Homogeneity (**HOM**), Mean Gray Level (**MGL**) of images taken during the examinations at hours (h) 0, 1 and 6 and days (d) 2, 3, 5 and 10. Results: At 0h, GR was significantly lower in group E2 than in E1 and CG ($p < 0.05$). There was an increase in GR values between 0h and 10d in E3 group, but a decrease during the same time interval in CG ($p < 0.05$). In contrast, CON values of E2 were lower ($p < 0.05$) at 0h compared to other time points of examination and lower than in CG. HOM values were lower ($p < 0.05$) in E1, E2 and E3 than CG at days 5 and 10. HOM values were higher at 1h compared to 6h, 2d and 10d in group E3 ($p < 0.05$). By contrast to GR values, HOM values were higher in CG at 6h and 10d than they were in E3. MGL values of E2 were higher ($p < 0.05$) than CG until 10d and higher ($p < 0.05$) in E3 than CG at 6h after treatment. Conclusion and clinical relevance: Echotexture parameters determined by the evaluation of sonographic B-mode images reflect changes in the endometrium and could be used for the evaluation of the recovery period after treatment of endometritis

Keywords: cow, endometritis, echotexture, treatment

ZUSAMMENFASSUNG

Das Ziel dieser Studie war es, nach intrauteriner Verabreichung der antiseptischen Lösung Lotagen ® (Metacresolsulfon-Säure und Formaldehyd) bei Kühen, die nach der Behandlung trächtig geworden sind, die Veränderungen des Endometriums mittels Echotexturanalyse zu beurteilen. Material und Methodik: 32 Kühe wurden in Abhängigkeit vom Grad der Endometritis in 4 Gruppen eingeteilt: Tiere mit geringgradiger (E1, $n = 7$), moderater (E2, $n = 8$) und hochgradiger Endometritis (E3, $n = 6$) sowie gesunde Tiere ohne Endometritis (Kontrollgruppe CG; $n = 11$). Es wurde ein computergestütztes Bildanalyseprogramm verwendet, um an im sonographischen B-Modus aufgenommenen Bildern des Endometriums die Echostrukturparameter Kontrast (CON), Gradient (GR), Homogenität (HOM) und mittlere Graustufe (MGL) unmittelbar vor (0h) sowie 1h, 6h und 2, 3, 5 und 10 Tage nach der Behandlung zu analysieren. Ergebnisse: Unmittelbar vor der Behandlung (0h) war GR bei den Kühen der Gruppe E2 niedriger ($p < 0,05$) als bei denen der Gruppen E1 und CG. Es gab einen Anstieg der GR-Werte in der Stunde 0 und am 10.Tag bei der E3-Gruppe. Im Gegensatz dazu nahmen GR bei den Kühen der Gruppe CG im selben Zeitraum ab ($p < 0,05$). Auf der anderen Seite waren die CON-Werte der Gruppe E2 zum Zeitpunkt 0h deutlich niedriger als an den folgenden Untersuchungszeitpunkten und niedriger als CG ($p < 0,05$). Auch bei den Kühen der Gruppe E3 war CON am Tag 10 höher als bei den Kühen der Gruppe CG ($p < 0,05$). Die HOM-Werte waren an den Tagen 5 und 10 bei den Tieren der Gruppen E1, E2 und E3 niedriger ($p < 0,05$) als bei denen der Gruppe CG. Bei den Kühe der Gruppe E2

nahmen die HOM-Werte bis zum 5. Tag nach der Behandlung ab ($p < 0,05$). Ferner waren die HOM-Werte bei den E3-Tieren an den Zeitpunkten 6h, 2d und 10d im Vergleich zu den zum Zeitpunkt 0h gemessenen Werten erhöht ($p < 0,05$). Im Gegensatz zu dem GR-Werte waren HOM-Werte bei den CG-Kühen 6 Stunden an den Zeitpunkten 6h und 10d höher als bei den E3-Kühen. Die MGL Werte der E2-Kühe waren höher als bei den CG-Tieren bis zum Tag 10 ($p < 0,05$) und die MGL-Werte der E3-Kühe waren zum Zeitpunkt 6h höher ($p < 0,05$) als die entsprechenden Werte der Gruppe. Bei den E2-Tieren stiegen die MGL-Werte bis zum 2. Tag an und fielen danach wieder ab ($p < 0,05$). Schlussfolgerung und klinische Relevanz: Die an sonographischen B-Bildern berechneten Echostrukturparameter spiegeln die Veränderungen im Endometrium wider und können herangezogen werden, um den Heilungsverlauf nach einer Endometritisbehandlung zu beurteilen.

Schlüsselwörter: Kuh, Endometritis, Echotextur, Behandlung

1. Introduction

There have been technological developments in the use of transrectal real time ultrasonography for understanding the reproductive biology in cattle. Real time ultrasonography can be used for the determination of postpartum uterus infections. The measurement of the diameter of uterine horn and cervix uteri and the amount and type of accumulated fluid into the uterus should be considered in the evaluation of the presence and severity of inflammatory changes of the uterine wall (2, 18). Although it is difficult to determine the pathological changes by using ultrasound in conditions without any fluid accumulation, it is an important method for the determination of endometritis (4, 17). Aslan et al. (2) reported that it is possible to determine the pathological changes of the uterus by performing systematic examinations during the involution period. Today, computer-assisted image analysis instead of conventional subjective image analysis provides a more objective assessment of the tissue to be examined (3, 16). Ultrasonographic images are composed of pixels that are represented numerically (0-255) in the shades of gray according to their brightness intensity (27, 33, 35). In the assessment of the tissue echotexture, a mathematical matrix is generated by using these numerical values, and two main variables are analyzed: the mean pixel value and the pixel heterogeneity (34). This assessment method particularly used for the evaluation of morphological changes in the endometrium and the corpus luteum during the estrus cycle (15, 30, 35). Such investigations of endometritis in cows are limited, although association of echotexture parameters to inflammation as well as the changes in the endometrium during the estrus cycle have been reported previously (29). Several methods

exist for the diagnosis and classification of uterus infections (4, 18, 32). Recently cases of endometritis have been classified as clinical and subclinical endometritis based on the clinical findings (22, 32). Clinical endometritis is characterized by the presence of purulent or mucopurulent vaginal discharge with a >7.5 cm cervical diameter of the uterus in transrectal palpation after days 21 or 26 postpartum (22, 32). Subclinical endometritis is defined by the evaluation of the PMN cells in the uterine cytology samples whose proportion range between 10.0%–18.0%, depending on the period (4, 18). In addition fluid accumulation into the uterus due to endometritis can be determined by transrectal ultrasonography (4, 18, 25).

Although there are several alternatives for the treatment of endometritis, use of intrauterine antiseptics, intrauterine antibiotics and PGF $_{2\alpha}$ are more frequent (20, 22). Some studies have reported succesful treatment and fertility rates after intrauterine administration of Lotagen® (metacresolsulphonic acid and formaldehyde), which is an antiseptic and astringent solution that does not cause several changes in the unaltered endometrium, rather has only an astringent and regenerative effect on the pathologically changed tissue when prepared in appropriate proportions (i.e. $<3\%$) (8, 14, 40).

The aim of this study was to evaluate the changes in the echotexture parameters after the intrauterine administration of antiseptic solution and to present the significance of these parameters in the recovery period of endometritis in cows. In addition in this study the mechanism of occurrence of these changes especially in the cows that become pregnant after intrauterine treatment, were investigated as well as whether the echotexture parameters should be used as an axillary method for the clinical classification of endometritis.

2. Materials and methods

2.1 Animals and treatments

A total of 32 Holstein Friesian dairy cows in good general health condition of age 4.14 ± 0.58 year, with an average milk production of 5358.92 ± 196.81 Lt in 305 days and a mean body condition (BCS, 1–5 scale) of 3.57 ± 0.55 were evaluated, of which 21 cows which determined to have endometritis during the postpartum examinations were included in the study.

Endometritis was classified in three categories as follows: 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) based on the classifications described earlier (7, 32) which were identified during the routine transrectal palpation and vaginal examinations conducted starting from the 45th of postpartum day. Four different groups were formed based on the severity of endometritis. **Group I:** 1st degree endometritis (E1, slight, n=7), **Group II:** 2nd degree (E2, moderate, n=8), **Group III:** 3rd degree (E3, severe, n=6) and **Group IV:** control group without endometritis (no intrauterine administration) (CG, n=11).

An intrauterine 100ml antiseptic solution (3% Lotagen®: metacresolsulphonic acid and formaldehyde) was administered for the treatment of endometritis. Endometrial cytological samples were collected from treated animals (GI, GII and GIII) and CG before (0h) and 10 days after the infusion. In addition, an ultrasonography was performed during the examinations at different time periods (at hours 0, 1 and 6 and on day 2, 3, 5 and 10) and the echotexture analyses in the captured images were performed on the same days. All of the animals were inseminated by artificial insemination (AI) in the first estrus after the last examination. Non-pregnant cows were culled out for the uniformity of the study and only pregnant cows were evaluated to reflect the differences between the recovery periods of endometritis cases. All cervical cytological sampling, ultrasonography and echotexture analysis were performed in the same order in CG, and AI was performed in the first estrus after the last examination.

2.2 *Ultrasonography*

A real-time B mode portable ultrasonography device equipped with a 5 MHz linear rectal probe (Agroscan L, E.C.M Company, Angoulême-France) was used in the ultrasonography examinations. In order to capture images, cross-sectional images were obtained from the dorsal surface of the asymmetric uterine horn in groups E1, E2, E3 and bilateral uterine horn in CG as close as possible to the corpus uteri.

2.3 *Cytological evaluation*

After the transrectal palpation, followed by an ultrasonography examination to obtain the ultrasonographic images of the uterus, a sample was collected from the cervix uteri using a sterile cotton swab (1, 38). The vulva of each cow was cleaned and a speculum was inserted through the vagina. Next, a sterile cotton swab (15 cm length) held with a cervical forceps (45 cm length) was used for cytological sampling. The sampling was performed by inserting the

swab up to 2–3 cm into the cervix. The same sampling procedure was repeated in the examination conducted 10 day after the administration of the antiseptic solution. The sampling procedure in CG was same as others.

The cytological preparations of the samples were fixed with 50% ether + 50% ethanol and then stained by Papanicolaou staining method. Then, 100 cells (including epithelial cells, lymphocytes, macrophages and neutrophils) were counted out at 400x magnification under a light microscope (Olympus CX21FS1, Olympus Optical Co., Tokyo, Japan) for the assessment of PMN cell ratios (19, 25).

2.4 Echotexture analysis

The images frozen during the ultrasonography examination were loaded onto a digital image recorder (SSF-M20 Multimedia Player) and then transferred onto a computer. All the ultrasonography images were captured using the same image settings. The images were evaluated in terms of the echotexture parameters by using the BS200 Pro® Image Processing and Analysis Software (BAB Software, Ankara, Turkey). Four Regions of Interest (**ROI**) of at least 100 pixels (10×10) were identified for analysis in each of the B mode ultrasonography images (Number of evaluated cows × 4 = Analyzed ROI). In the selected ROI's (Figure 1), the following parameters were used for the echotexture analysis. Gradient (**GR**): Variations in grey values of neighbor pixels, defines the microtexture of the sample. When the gradient value is 0, the image is totally homogeneous. The gradient value is calculated according to the below formula (6).

$$\text{GrMean} = \frac{1}{N} * \sum_{(x,y) \in \text{ROI}} G(x,y)$$

GrMean: Mean Gradient Value, N : total number of pixels in a ROI, $G(x,y)$: Gradient in the section (x,y), x,y = the row (x) and column (y) index.

Homogeneity (**HOM**): Uniformity of grey value combination of neighbor pixels in defined matrix, and defines either micro- or macrotextr of the sample. HOM values range between 0 and 1. The formula for the calculation of HOM was described by Delorme and Zuna (6) and Raeth et al.(28) as

$$\text{HOM} = \sum_{(i,j)} p(i,j)^2$$

HOM= Homogeneity, i,j = the row (i) and column (j) index, $p(i,j)$ = Value in the section of the co-occurrence matrix.

Contrast (**CON**): A measurement of the number of the large grey-level differences present in the ROI indicates. Gives information about the macrotexture of the image and is described by Lefebvre et al. (23) as

$$CON = \sum_{(i,j) \in ROI} (i, j)^2 * p(i, j)$$

CON= Contrast, i, j = the row (i) and column (j) index, $p(i, j)$ = Value in the section of the co-occurrence matrix.

(**MGL**): Arithmetical average grey level of all pixels in the picture, defines the brightness of the image. Values for MGL range between 0 and 255 and are described by Raeth et al.(28) as

$$\mu_g = \frac{1}{N} * \sum_{(x,y)} g_{xy}$$

μ_g is the mean grey level (values: 0–255); N : Size of the ROI as pixels; x, y the row (x) and column (y) index and g_{xy} is the grey level in pixel.

2.5 Statistical analysis

The SPSS®14.01 (SPSS Inc., Chicago, Illinois, USA) package program was used for the statistical analysis. The One-Way ANOVA test was utilized in the assessment of the differences between the groups in terms of the echotexture analyses on the control days (Dunett's T3), and the General Linear Model repeated measurement (Repeated Measures Define Factor) test was used to analyze time-dependent variations of the echotexture parameters in each group. The Kolmogorov–Smirnov test was conducted to evaluate whether the distribution of the groups was homogenous. All of the values are represented as mean $\pm$ standard error of mean (SEM). $p \leq 0.05$ considered statistically significant.

3. Results

3.1. Comparison of PMN ratios of Cytological Samples.

PMN ratio was the highest at 0h (immediately before the application) in E3 endometritis at 28.00% whereas it was the lowest in E1 endometritis with a rate of 11.8% ($p < 0.01$). When the E1, E2 and E3 cases of endometritis were compared both among each other and with the CG, the PMN ratios were significantly different ($p < 0.01$) at 0h and on 10d. However, no statistically significant difference ($p < 0.05$) was noted between the cases of E1 endometritis and the CG on 10d. When all of the groups are considered, the lowest

percentages were found in the CG. Moreover, a significant decrease ($p<0.001$) in the PMN percentages was identified in animals with endometritis before and after treatment (Table 1).

3.2. Evaluation of Echotexture Parameters.

When the echotexture parameters were compared between CG and endometritis groups, GR was lower in E2 group than E1 and CG before the treatment (h0) ($p<0.05$). By the time the GR levels of E2 increased and became significantly greater than those of the CG on the 5h and 10d after treatment ($p<0.05$). In E3 group a significant increase were determined in the GR levels at 6h and it was higher than those of CG ($p<0.05$). Also GR values of E2 group were lower before the treatment than at the other examination time periods contrary to the values of CG ($p<0.05$) (Table 2).

The differences in the CON values were found to be significant between E2 and CG at 0h and between E3 and CG at 10d ($p<0.05$). In addition in E2 group the CON values were lower at 0h immediately prior to the administration of antiseptic solution than in the other examination time periods ($p<0.05$). In CG the CON levels were found to be significantly lower just after the treatment at the 1h ($p<0.05$) than they were at 0h and the 6h (Table 3).

The HOM values remained higher in CG on days 5 and 10 after the treatment than in the other groups ($p<0.05$). On the other hand in E2 endometritis a significant decrease ($p<0.05$) in the HOM values was determined until 5d after the treatment. Cases of E3 endometritis demonstrated higher values at 1h after the treatment than at 6h, and on 2d and 10d ($p<0.05$). Unlike GR values, the HOM values were determined to be higher in CG than in E3 at 6h and 10d after the treatment ($p<0.05$) and higher values also were obtained immediately after the treatment at 1h and 6h than at 0h and on 2d (Table 4).

The MGL values of E2 were almost higher in all examinations than in CG until 10d ($p<0.05$). The MGL levels reached higher levels in 6h after treatment again in E3 ($p<0.05$) and were also higher than those in CG at this examination time period ($p<0.05$). Then, the MGL values increased until 2d after the treatment ($p<0.05$) and decreased during the examination period in E2 (Table 5).

4. Discussion

Real-time ultrasonography is an important method for the determination of postpartum uterus infections (4, 17). However, it is difficult to determine the pathological

changes in the uterine wall by ultrasound in conditions without any fluid accumulation. Currently; computer-assisted image analysis provides more objective assessment of the tissue being examined compared to conventional subjective assessment of images (3, 16) This method is especially used for the evaluation of the morphological changes in the endometrium and the corpus luteum during the estrus cycle (15, 30, 35). Furthermore, although similar investigations of endometritis in cows are limited, it has been reported that the echotexture parameters correspond to inflammation and that changes occur in the endometrium during the estrus cycle (29).

The present study was not designed for the treatment of endometritis. It was only designed to investigate whether it is possible to determine the endometrial changes and whether the echotexture parameters should be used as an axillary method for the clinical classification of endometritis or by using the echotexture parameters after the therapy.

In this study while the GR were not definitive for the diagnosis at 0h, there were significant differences in the values of E2 as compared with the values of CG during the study period.

As an echotexture parameter, GR describes the changes in the local grey pixels and the mean GR value is 0 for completely homogenous tissues (5, 10, 12). In addition to hyperemia and congestion in endometritis cases, desquamation and necrosis have been observed in superficial cells (24). It is obvious that the echotexture parameters respond in different ways and at different times to the aforementioned changes in the endometrium and to the variation in these changes due to the severity of the endometritis.

Homogeneity shows the level at which the pixel pairs in the image have the same (uniform) structure (10). When the HOM values are high there are few grey value combinations in the image, but all the value combinations are equally distributed. In other words, in situations where the numbers of grey areas are very low, the HOM values increase (28). It is thought that an increase in grey combinations and the disruption in the uniform distribution of these combinations is due to the development of asymmetry in E3 endometritis and the usual presence of mucopurulent discharge has been reported (7, 9). This explanation may serve as the basis for the fact that the HOM values are lower in E3 endometritis than in CG in this study.

It was determined that the HOM values were significantly higher ($p<0.05$) in E3 than in CG at the 6h and on the 10d after the administration because the HOM values have an inverse

relationship with the GR values (21, 30). The changes in homogeneity obtained with the computer-assisted software were contrary to the evaluation of fluctuations in endometrial homogeneity observed visually via the human eye (26) Computer-assisted analysis compares the echogeneity of pixels with an area of less than 0.01mm^2 (30).

The echotexture parameters reflect the physical status of the ovary (growing, static or regressing) in a study that determined the functional status of the ovary with the computer-assisted echotexture analysis (mean pixel value and heterogeneity) of the ultrasonographic images (36). The results obtained in this study showed that the echotexture parameters (HOM, GR, MGL) of the ultrasonographic images evaluated during the study period reflected the endometrial changes between 6h and 10d after treatment. Determination of the endometrial changes in this study may offer the possibility of an objective evaluation of pathological changes in the endometrium and thereby contribute to a more accurate diagnosis of the endometrial pathological conditions.

Antiseptic solution acts rapidly after being administered and causes moderate changes in the endometrium and stroma. It has been demonstrated that at least 1 week is required for the regeneration of the degenerative changes occurring in the endometrium until 3 days after treatment (31). The fact that the antiseptic solution does not cause many changes to the unaltered endometrium and exhibits only an astringent effect (40) explains why the values obtained in E1 were not different from those obtained in CG after the treatment.

Histological changes such as at the beginning of regenerative changes in the cubic surface epithelium on the 6th day and the presence of inflammatory changes in the endometrium on the 3rd day after the administration and later (31) also support these findings.

The evaluation of endometritis cases with a different system according to high-power field (hpf) demonstrated a linear increase in the number of neutrophil granulocytes with 1–2 neutrophil/hpf in low degree cases of endometritis, 3–7 neutrophil/hpf in medium degree cases, and 8 neutrophil/hpf in high degree cases (13). Ahmadi et al. (1) reported that the cervical cytology can be used for the diagnosis of endometritis in cows.

Several cytological methods have been reported by different authors for the diagnosis of endometritis (1, 18, 19). Kasimanickam et al. (18) determined that endometritis could be determined at PMN of 10.0%–18.0% depending on the period. These researchers demonstrated that as the postpartum period grows longer, the PMN percentages declines

toward 10.0%. Some studies indicated PMN percentage of 5% (1, 11, 25). However, the PMN cells in these studies were either derived from the lavage (1, 11) or from the endometritis cases until the 180th postpartum day (25). Yavari et. al (39) compared the results of endometrial and cervical cytology for the classification of different degrees of endometritis in cows and reported no significant differences between them. In the same study PMN percentages were higher in E3 endometritis cases than in E1 and E2 endometritis cases. We also found an increase from 11.80% (E1) to 28% (E3) ($p<0.01$) in the PMN ratios, in concordance with the reported evaluation of the PMN ratios with cytobrush by Kasimanickam et al. (18). Furthermore, the fact that there were significant differences between CG and endometritis groups at 0h ($p<0.01$) as well as significant decreases among E1, E2 and E3 in the PMN cell percentages on the 10th day after treatment ($p<0.001$) shows that cervical cytology is an important method that can also be used for the diagnosis of endometritis and the elimination of inflammatory cells after the treatment.

The significant decreases ($p<0.001$) in the PMN cell percentages on the 10d after treatment in E1, E2 and E3 show that cervical cytology is an important method that can be used to diagnose endometritis and monitor the elimination of inflammatory cells after treatment.

Several different echotexture parameters exist and a study considers additional parameters such as the Mean Numeric Pixel Value (37) and endometrial biopsy samples may advance the definition of the endometritis by using the method given in this paper. The changes in the echotexture parameters due to the other treatment methods (intrauterine antibiotic treatment, PGF2 α ; i.m.) could improve the scope of this study.

In conclusion, it is thought that evaluation of the changes in the echotexture parameters after intrauterine treatment of endometritis could be used as an axillary method for the evaluation of the recovery period as well as transrectal palpation, ultrasonography and vaginal cytology. It could be possible especially by considering the echotexture values at 6 hour and 10 day after the intrauterine administration. In addition, cervical cytology can be used as an additional method for both the diagnosis of endometritis and in the investigation of inflammatory changes in the uterus after the treatment. The presented parameters of the recovered animals of this study exhibited with another study comparing both pregnant and nonpregnant cows in larger numbers and echotexture parameters could be more useful for the definition and classification of endometritis.

Conclusion for practice: This study provides an insight to objective evaluation of pathological changes of the endometrium and contributes to accurate diagnosis of endometrial pathological conditions. It is obvious to get more accurate results in the evaluation of the success of the treatment by echotexture analysis of the B-mode images captured before and after the treatment of endometritis.

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Figure and Table Legends:

Figure 1: Cross sectional sonographic B-mode image of an uterine horn.

The squares are the **ROI**'s (Region of Interest) selected for the analysis of echotexture parameters.

Abbildung 1: Querschnitt eines Uterushorns im sonographischen B Modus.

Die Quadrate stellen die für die Analyse der Echotexturparameter ausgewählte **ROIs** (Region of Interest) dar.

472

473 **Table 1:** Comparison of PMN ratios between the groups.

474 Values are represented as mean $\pm$ SEM.

475 **Tabelle 1:** Vergleich von PMN-Verhältnisse zwischen den Gruppen.

476 Die Werte sind als Mittelwert $\pm$ SEM dargestellt.

477

478 **Table 2:** Changes in Gradient values during the study period

479 Different letters (a,b,c,d) in the columns and different numbers (1,2,3,4) in the lines indicate
480 the differences. Values are represented as mean $\pm$ SEM.

481 **Tabelle 2:** Änderungen der Gradientwerte während der Studienprozess.

482 Unterschiedliche Buchstaben (a, b, c, d) in den Spalten und verschiedene Zahlen (1,2,3,4) in
483 den Zeilen zeigen die Unterschiede. Die Werte sind als Mittelwert $\pm$ SEM dargestellt.

484 **Table 3:** Changes in Contrast values during the study period.

485 Different letters (a,b,c,d) in the columns and different numbers (1,2,3,4) in the lines indicate
486 the differences. Values are represented as mean $\pm$ SEM.

487 **Tabelle 3:** Änderungen der Kontrastwerte während der Studienprozess.

488 Unterschiedliche Buchstaben (a, b, c, d) in den Spalten und verschiedene Zahlen (1,2,3,4) in
489 den Zeilen zeigen die Unterschiede. Die Werte sind als Mittelwert $\pm$ SEM dargestellt.

490 **Table 4:** Changes in Homogeneity values during the study period.

491 Different letters (a,b,c,d) in the columns and different numbers (1,2,3,4) in the lines indicate
492 the differences. Values are represented as mean $\pm$ SEM.

493 **Tabelle 4:** Änderungen der Homogenitätswerte während der Studienperiode.

494 Unterschiedliche Buchstaben (a, b, c, d) in den Spalten und verschiedene Zahlen (1,2,3,4) in
495 den Zeilen zeigen die Unterschiede. Die Werte sind als Mittelwert $\pm$ SEM dargestellt.

496 **Table 5:** Changes in Mean Gray Level values during the study period.

497 Different letters (a,b,c,d) in the columns and different numbers (1,2,3,4) in the lines indicate
498 the differences. Values are represented as mean $\pm$ SEM.

499 **Tabelle 5:** Änderungen der Graustufenwerte während der Studienperiode.

500 Unterschiedliche Buchstaben (a, b, c, d) in den Spalten und verschiedene Zahlen (1,2,3,4) in
501 den Zeilen zeigen die Unterschiede. Die Werte sind als Mittelwert $\pm$ SEM dargestellt.