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

I. K üçükaslan1; D. Kaya2; B. Emre3; H. Bollwein4; N. Özyurtlu1; S. B. Mülazimoğlu5; S. Aslan6

1Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Dicle University, Turkey; 2Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Harran University, Turkey; 3Clinic for Reproduction Medicine, Vetsuisse Faculty, University of Zuerich, Switzerland; 4Technopolis-Hemosoft IT and Training Services Inc., Hacettepe University, Turkey; 5Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Ankara University, Turkey

Key words
Cow, endometritis, echotexture, treatment

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% metacresolsulfophonic acid and formaldehyde) in cows which became pregnant after treatment. Material and methods: According to the severity of endometritis 21 cows were divided into three groups: E1 (slight, n = 7), E2 (moderate, n = 8), E3 (severe, n = 6). The control group (C, n = 11) consisted of cows without endometritis that did not receive an intrauterine medication. 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 days (d) 2, 3, 5 and 10. Results: At 0 h, GR was significantly lower in group E2 than in groups E1 and C (p < 0.05). There was an increase in GR values between 0 h and 10 d in group E2 and E3, but a decrease during the same time interval in group C (p < 0.05). In contrast, CON values of group E2 were lower (p < 0.05) at 0 h compared to other timepoints of examination and lower than in group C. HOM values were lower (p < 0.05) in groups E1, E2 and E3 than in group C on d 5 and d 10. HOM values were higher at 1 h compared to 6 h, d 2 and d 10 in group E3 (p < 0.05). By contrast to GR values, HOM values were higher in group C at d 6 and d 10 than they were in group E3. MGL values of group E2 were higher (p < 0.05) than in group C until d 10 and higher (p < 0.05) in group E3 than in group C at 6 h after treatment. In group E2 an increase of MGL values until d 2 was followed by a decrease (p < 0.05). Conclusion: 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.

Correspondence to
Ibrahim K üçükaslan
Dicle University
Faculty of Veterinary Medicine
Department of Obstetrics and Gynaecology
21280 Kampus/Diyarbakır
Turkey
Email: i.kucukaslan@gmail.com

Zusammenfassung
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 Endometriumbildern (B-Modus) die Echostrukturparameter Kontrast (CON), Gradient (GR), Homogenität (HOM) und mittlere Graustufe (MGL) von Bildern analysiert. Resultate: Am 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 am 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 Heilungsverlaufs nach Endometritisbehandlung dienen.

Bewertung von Echotexturparametern des Endometriums und zytologischen Zervizabefunden bei Kühen während und nach Behandlung einer Endometritis

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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 uterus infections postpartum. The measurement of the diameter of uterine horn and cervix uteri and the amount and type of accumulated fluid in 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). Ultrasonicographic 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 is 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 with 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 uterine infections (4, 18, 32). Recently cases of endometritis have been classified as clinical and subclinical endometritis based on 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 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 ranges between 10.0% and 18.0%, depending on the period (4, 18). In addition accumulation of fluid in 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$_{2a}$ are used more frequently (20, 22). Some studies have reported successful 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 an antiseptic solution and to present the significance of these parameters in the recovery period of endometritis in cows. In addition, the mechanism of occurrence of these changes especially in those 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.

Materials and methods

Animals and treatments

A total of 32 Holstein Friesian dairy cows in good general health condition aged 4.14 ± 0.58 years with an average 305-day milk production of 5358.92 ± 196.81 l and a mean body condition score (BCS, 1–5 scale) of 3.57 ± 0.55 were included in the study. Twenty-one of these cows had been diagnosed with endometritis during the postpartum examinations.

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 postpartum day. Three different groups were formed based on the severity of endometritis: group E1: 1st degree, slight endometritis (n = 7); group E2: 2nd degree, moderate endometritis (n = 8); group E3: 3rd degree, severe endometritis (n = 6). The control group (C, n = 11) comprised cow without endometritis that did not receive an intrauterine medication.

An intrauterine antiseptic solution (100 ml of 3% Lotagen®, metacresolsulphonic acid and formaldehyde) was administered for the treatment of endometritis. Endometrial cytological samples were collected from treated animals (groups E1, E2 and E3) before (0 h) and 10 days (d) after the infusion. In addition, 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. Thirteen non-pregnant cows were excluded from the study for the uniformity of the study and only pregnant cows were evaluated to reflect the differences between the recovery periods of endometritis cases.

In control animals all cervical cytological sampling, ultrasonography and echotexture analysis were performed in the same order, and AI was performed in the first estrus after the last examination.

Ultrasonographic examination

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 ultrasonographic examinations. In order to capture images, cross-sectional images were ob-
tained from the dorsal surface of the asymmetric uterine horn in groups E1, E2, E3 and bilateral uterine horn in group C as close as possible to the corpus uteri.

Cytological evaluation

After transrectal palpation and ultrasonographic examination 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 inserted up to 2–3 cm into the cervix. The same sampling procedure was repeated in the examination conducted 10 days after the administration of the antiseptic solution. The sampling procedure in control animals was identical.

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).

Echotexture analysis

The images frozen during ultrasonographic examination were loaded onto a digital image recorder (SSF-M20 Multimedia Player) and then transferred onto a computer. All ultrasonographic 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 x 10) were identified for analysis in each of the B mode ultrasonographic images (number of evaluated cows x 4 = analyzed ROI). In the selected ROI’s (▶Fig. 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. The gradient value is calculated according to the formula (6):

\[ \text{GrMean} = \frac{1}{N} \sum_{(x,y) \epsilon \text{ROI}} G(x,y) \]

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, defines either micro- or macrotextur 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

\[ \text{HOM} = \sum_{(i,j) \epsilon \text{ROI}} p(i,j)^2 \]

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

\[ \text{CON} = \sum_{(i,j) \epsilon \text{ROI}} (i,j)^2 \cdot p(i,j) \]

CON = contrast, i,j = the row (i) and column (j) index, p(i,j) = value in the section of the co-occurrence matrix

Mean Gray Level (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

\[ \mu_g = \frac{1}{N} \sum_{(x,y) \epsilon \text{ROI}} g_{xy} \]

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

Statistical analysis

The SPSS®14.01 (SPSS Inc., Chicago, Illinois, USA) package program was used for the statistical analysis. The One-Way ANOVA was used for the statistical analysis of the echotexture parameters.

Fig. 1 Cross sectional sonographic B-mode image of an uterine horn. The squares mark the ROI’s (Regions of Interest) selected for the analysis of echotexture parameters.

Abb. 1 Sonographischer Querschnitt eines Uterushorns im B-Mode. Die Quadrate markieren die für die Analyse der Echotexturparameter ausgewählten ROIs (Region of Interests).
test was utilized in the assessment of the differences between the groups in terms of the echotexture analyses on the control days (Dunnett’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 ± standard error of mean (SEM). p ≤ 0.05 was considered statistically significant.

Results

Comparison of PMN ratios of cytological samples

At 0 h (immediately before treatment) PMN ratio was highest in group E3 with 28.0% whereas it was lowest in group E1 with a rate of 11.8% (p < 0.01). When comparing groups E1, E2 and E3 both among each other and with group C, the PMN ratios were significantly different (p < 0.01) at 0 h and on d 10. However, no statistically significant difference (p < 0.05) was noted between group E1 and group C on d 10. When all of the groups are considered, the lowest percentages were found in group C. Moreover, a significant decrease (p < 0.001) in the PMN percentages was identified in animals with endometritis before and after treatment (▶Table 1).

Table 1 Comparison of PMN ratios (%) between the groups. Values are presented as mean ± SEM.

<table>
<thead>
<tr>
<th>Group</th>
<th>Hour 0</th>
<th>Day 10</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>11.80 ± 1.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.00 ± 1.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>E2</td>
<td>15.50 ± 1.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.41 ± 1.62&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>E3</td>
<td>28.00 ± 4.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.18 ± 3.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CG</td>
<td>6.08 ± 1.16&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.58 ± 1.3&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>p value</td>
<td>a:b:c:d &lt; 0.01</td>
<td>a:b &lt; 0.01</td>
<td></td>
</tr>
</tbody>
</table>

Evaluation of echotexture parameters

When the echotexture parameters were compared between group C and endometritis groups, GR was lower in group E2 than in groups E1 and C before treatment (0 h) (p < 0.05). By the time the GR levels of group E2 increased and became significantly greater than those of group C at d 5 and d 10 after treatment (p < 0.05). In group E3 a significant increase was determined in the GR levels at 6 h and it was higher than those of group C (p < 0.05). GR values of group E2 were lower before treatment than at the other examination timepoints contrary to the values of group C (p < 0.05) (▶Table 2).

The differences in the CON values were found to be significant between groups E2 and C at 0 h and between groups E3 and C at 10 d (p < 0.05). In addition, in group E2 CON values were lower at 0 h immediately prior to administration of the antiseptic solution than at the other examination timepoints (p < 0.05). In group C the CON levels were found to be significantly lower at the examination timepoint 1 h (p < 0.05) than they were at 0 h and at d 2 (▶Table 2).

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

The MGL values of group E2 were higher than in group C at almost all examination timepoints until d 10 (p < 0.05). After an

Table 2 Changes in gradient values during the study period. Different letters (a, b, c, d) in the columns and different numbers (1, 2, 3, 4) in the lines indicate the differences. Values are presented as mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Hour 0</th>
<th>Hour 1</th>
<th>Hour 6</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 10</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>15.53 ± 0.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.67 ± 0.77&lt;sup&gt;1&lt;/sup&gt;</td>
<td>15.63 ± 0.70&lt;sup&gt;1&lt;/sup&gt;</td>
<td>15.16 ± 0.71&lt;sup&gt;1&lt;/sup&gt;</td>
<td>14.10 ± 0.72&lt;sup&gt;2&lt;/sup&gt;</td>
<td>&lt; 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>12.39 ± 0.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.04 ± 0.79&lt;sup&gt;1&lt;/sup&gt;</td>
<td>16.41 ± 0.84&lt;sup&gt;2&lt;/sup&gt;</td>
<td>14.95 ± 0.55&lt;sup&gt;2&lt;sup&gt;1&lt;/sup&gt;,3&lt;/sup&gt;</td>
<td>16.73 ± 0.65&lt;sup&gt;2&lt;/sup&gt;</td>
<td>15.79 ± 0.65&lt;sup&gt;2&lt;/sup&gt;</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>E3</td>
<td>14.48 ± 0.93&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.28 ± 0.76&lt;sup&gt;1&lt;/sup&gt;</td>
<td>16.89 ± 0.58&lt;sup&gt;2&lt;/sup&gt;</td>
<td>15.25 ± 0.62&lt;sup&gt;1,4&lt;/sup&gt;</td>
<td>16.82 ± 0.98&lt;sup&gt;1&lt;/sup&gt;</td>
<td>17.04 ± 0.91&lt;sup&gt;2&lt;/sup&gt;</td>
<td>&lt; 0.05</td>
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</tr>
<tr>
<td>Control</td>
<td>15.77 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.40 ± 0.38&lt;sup&gt;1,2&lt;/sup&gt;</td>
<td>15.44 ± 0.40&lt;sup&gt;1&lt;/sup&gt;</td>
<td>15.29 ± 0.45</td>
<td>13.95 ± 0.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.67 ± 0.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt; 0.05</td>
<td></td>
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<tr>
<td>p value</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&gt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>

Tab. 1 Vergleich der PMN-Anteile (%) zwischen den Gruppen. Die Werte sind als Mittelwert ± SEM dargestellt.

Tab. 2 Änderungen der Gradientwerte im Studiенverlauf. Unterschiedliche Buchstaben (a, b, c, d) in den Spalten und verschiedene Zahlen (1, 2, 3, 4) in den Zeilen zeigen Unterschiede an. Die Werte sind als Mittelwert ± SEM dargestellt.
Table 3  Changes in contrast values during the study period. Different letters (a, b, c, d) in the columns and different numbers (1, 2, 3, 4) in the lines indicate the differences. Values are presented as mean ± SEM.

<table>
<thead>
<tr>
<th>Group</th>
<th>Hour 0</th>
<th>Hour 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 10</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>61.85 ± 6.39</td>
<td>57.29 ± 6.17</td>
<td>75.79 ± 10.54</td>
<td>62.75 ± 5.61</td>
<td>59.18 ± 5.88</td>
<td>69.10 ± 8.35</td>
<td>53.99 ± 5.14</td>
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<td>(n = 7)</td>
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<tr>
<td>E2</td>
<td>41.72 ± 4.63</td>
<td>65.14 ± 6.34</td>
<td>68.18 ± 6.62</td>
<td>69.63 ± 7.97</td>
<td>53.78 ± 3.29</td>
<td>70.01 ± 4.98</td>
<td>63.59 ± 5.66</td>
</tr>
<tr>
<td>(n = 8)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>E3</td>
<td>70.86 ± 13.29</td>
<td>59.35 ± 6.85</td>
<td>77.78 ± 9.97</td>
<td>71.88 ± 6.89</td>
<td>53.66 ± 4.59</td>
<td>70.27 ± 8.03</td>
<td>75.26 ± 9.03</td>
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<td>(n = 6)</td>
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<tr>
<td>Control</td>
<td>65.88 ± 2.56</td>
<td>51.46 ± 2.82</td>
<td>55.77 ± 3.28</td>
<td>61.40 ± 3.76</td>
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<td>68.82 ± 8.61</td>
<td>46.95 ± 3.55</td>
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<td>(n = 11)</td>
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p value  < 0.05  > 0.05  > 0.05  > 0.05  > 0.05  < 0.05

Table 4  Changes in homogeneity values during the study period. Different letters (a, b, c, d) in the columns and different numbers (1, 2, 3, 4) in the lines indicate the differences. Values (x10⁻²) are presented as mean ± SEM.

<table>
<thead>
<tr>
<th>Group</th>
<th>Hour 0</th>
<th>Hour 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 10</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>8.6 ± 0.6</td>
<td>8.1 ± 0.6</td>
<td>7.3 ± 0.3</td>
<td>8.1 ± 0.6</td>
<td>7.2 ± 0.6</td>
<td>7.6 ± 0.6</td>
<td>7.8 ± 0.4</td>
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<tr>
<td>(n = 7)</td>
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<tr>
<td>E2</td>
<td>9.6 ± 0.8</td>
<td>7.0 ± 0.5</td>
<td>7.4 ± 0.5</td>
<td>7.0 ± 0.5</td>
<td>7.0 ± 0.5</td>
<td>7.5 ± 0.5</td>
<td>7.5 ± 0.6</td>
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<td>(n = 8)</td>
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<tr>
<td>E3</td>
<td>7.0 ± 0.5</td>
<td>8.2 ± 0.4</td>
<td>6.7 ± 0.4</td>
<td>6.7 ± 0.3</td>
<td>6.9 ± 0.5</td>
<td>7.0 ± 0.5</td>
<td>6.6 ± 0.5</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.5 ± 0.2</td>
<td>9.8 ± 0.9</td>
<td>8.3 ± 0.2</td>
<td>7.8 ± 0.2</td>
<td>8.2 ± 0.3</td>
<td>9.8 ± 0.6</td>
<td>11.7 ± 0.9</td>
</tr>
<tr>
<td>(n = 11)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

p value  > 0.05  > 0.05  < 0.05  > 0.05  > 0.05  < 0.05  < 0.05

Table 5  Changes in Mean Gray Level values during the study period. Different letters (a, b, c, d) in the columns and different numbers (1, 2, 3, 4) in the lines indicate the differences. Values are presented as mean ± SEM.

<table>
<thead>
<tr>
<th>Group</th>
<th>Hour 0</th>
<th>Hour 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 10</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>73.06 ± 3.40</td>
<td>84.70 ± 4.49</td>
<td>94.09 ± 4.71</td>
<td>77.66 ± 3.27</td>
<td>80.70 ± 3.72</td>
<td>83.81 ± 3.56</td>
<td>70.12 ± 3.56</td>
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<tr>
<td>(n = 7)</td>
<td></td>
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<tr>
<td>E2</td>
<td>66.02 ± 3.26</td>
<td>90.41 ± 4.26</td>
<td>86.23 ± 4.21</td>
<td>94.59 ± 2.85</td>
<td>71.38 ± 2.89</td>
<td>89.90 ± 3.61</td>
<td>80.66 ± 3.03</td>
</tr>
<tr>
<td>(n = 8)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E3</td>
<td>75.62 ± 4.60</td>
<td>79.44 ± 3.49</td>
<td>95.14 ± 3.11</td>
<td>83.90 ± 4.35</td>
<td>75.56 ± 4.92</td>
<td>78.73 ± 2.94</td>
<td>87.75 ± 5.77</td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>76.69 ± 1.59</td>
<td>73.59 ± 1.99</td>
<td>75.64 ± 2.19</td>
<td>82.57 ± 2.02</td>
<td>77.52 ± 2.28</td>
<td>72.46 ± 2.29</td>
<td>75.35 ± 2.41</td>
</tr>
<tr>
<td>(n = 11)</td>
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</tr>
</tbody>
</table>

p value  < 0.05  < 0.05  < 0.05  > 0.05  < 0.05  > 0.05  > 0.05
increase until d 2 after treatment (p < 0.05) a decrease was recorded in the following examination period. In group E3 the MGL reached higher values at 6 h after treatment (p < 0.05) which also exceeded those in group C at this examination timepoint (p < 0.05) (Table 5).

**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 a 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 for evaluation of the uterus after treatment.

While the GR was not definitive for the diagnosis at 0 h, there were significant differences in the values of group E2 as compared to group 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 were lower in E3 endometritis than in group C in this study.

It was determined that the HOM values were significantly higher (p < 0.05) in group E3 than in group C at 6 h and on d 10 after treatment 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 homogeneity of pixels with an area of less than 0.01 mm$^2$ (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 6 h and 10 d 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 group E1 after treatment were not different from those obtained in group CG. 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). According to Kasimanickam et al. (18) endometritis can be determined at PMN rates of 10.0%–18.0% depending on the period. These researchers demonstrated that as the postpartum period grows longer, the PMN percentages decline towards 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 11.8% (E1) to 28.0% (E3) (p < 0.01) in the PMN ratios, in
Conclusion for practice

This study provides an insight to objective evaluation of pathological changes of the endometrium and contributes to an accurate diagnosis of pathological conditions of the endometrium. Echotexture analysis of B-mode images captured before and after treatment of endometritis gives more accurate results in the evaluation of the treatment success.

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 group CG and endometritis groups at 0 h (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 to diagnose endometritis and monitor the elimination of inflammatory cells after treatment.

The method presented in this paper may advance the definition of endometritis by using additional echotexture parameters such as the Mean Numeric Pixel Value (37) or endometrial biopsy samples together. The evaluation of the changes in the echotexture parameters due to other treatment methods (intratuterine antibiotic treatment, PGF_{2a} intramuscularly) could improve the scope of this study.

In conclusion, it is thought that echotexture parameters could be used as an auxillary method for the evaluation of the recovery period in addition to transrectal palpation, ultrasonography and vaginal cytology after intrauterine treatment of endometritis. It could be helpful especially by considering the echotexture values at 6 hours and 10 days after intrauterine treatment. 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 treatment. According to the results of the present study further studies comparing both pregnant and nonpregnant cows in larger numbers with additional echotexture parameters could be more useful for the definition and classification of endometritis.

Conflict of interest

The authors confirm that they do not have any conflict of interest. This study was supported by BIYEP (Project no: K120140 kod-7) and DÜBAP (Project no: 07-01–16). Part of the study was presented in the 13th Annual Conference of the European Society for Domestic Animal Reproduction (ESDAR). The authors thank Akbel Milk and Dairy Products Inc. for providing animals and facilities.

References


I. Küçükaslan et al.: Endometrial echotexture in cows with endometritis


