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Short communication

Cyclic changes in endometrial echotexture of cows using a computer-assisted program for the analysis of first- and second-order grey level statistics of B-Mode ultrasound images

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Abstract

The aim of this study was to test the suitability of a computer-assisted echotexture analysis program for analysing first- and second-order grey level statistics of grey levels of B-Mode ultrasound images to examine morphologic changes in the endometrium during oestrous cycle in cows. Four Simmental cows were examined for two consecutive oestrous cycles. Echotexture of the endometrium was assessed by mean grey level (MGL) and homogeneity (HOM) of digitised B-Mode images of the uterine body and both uterine horns. As there were no differences ($P > 0.05$) in MGL and HOM, respectively, between the images of the uterine body and the uterine horns, the mean values of all endometrial images were used for subsequent analyses of MGL and HOM. The factor 'day of oestrous cycle' showed a highly significant ($P < 0.0001$) effect and the factor 'cow' showed a significant ($P < 0.05$) effect on MGL and HOM. No differences ($P > 0.05$) in both echotexture parameters were measured between oestrous cycles within cows. MGL was negatively related to HOM ($r = -0.66$; $P < 0.0001$). Low MGL and high HOM occurred on Day 0 (= ovulation) and between Days −3 and −1. While MGL was consistently ($P > 0.05$) low between Days −3 and −1, significant changes of HOM with maximum levels on Day −2 ($P < 0.05$) were observed during this time. MGL was consistently ($P > 0.05$) high between Days 4 and 13 while HOM was consistently ($P > 0.05$) low between Days 2 and 13. From Day −3 to Day −1 ($r = 0.48$; $P < 0.05$) plasma oestrogen levels were correlated with

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HOM, but not with MGL ($P < 0.05$). The results of this study show that changes in endometrial morphology of cows can be measured using a computer-assisted texture analysis program.

Keywords: Cow; Oestrous cycle; Echotexture analysis; Endometrium; Oestrogens

1. Introduction

Since B-Mode sonography has been introduced, the possibility of monitoring morphologic changes in the bovine reproductive tract via a non-invasive technique has become readily available (Pierson and Ginther, 1984; Reeves et al., 1984). While most ultrasound devices allow two-dimensional measurements of the genital organs, up to now echotexture of the sonographic images is usually determined by subjective visual evaluation of the examiner.

In some of the more recent studies computer-assisted software have been used for the objective analysis of the echotexture of B-Mode images (Hirning et al., 1989; Bader et al., 1994; Bleck et al., 1994; Kastelic et al., 2001). For example, using such programs it was possible to investigate morphologic variations in echotexture of the corpus luteum during the oestrous cycle in cows (Singh et al., 1997; Tom et al., 1998).

However, most programs enabled only measurements of the brightness of ultrasound images by measuring the grey levels of the ultrasound images (Singh et al., 1997; Tom et al., 1998). In human medicine refined computer-assisted texture analysis programs were developed, which allowed not only the determination of light intensity, but also of spatial arrangement of pixels of digitised images by using parameters of second order statistics (Räth et al., 1985; Hirning et al., 1989; Zuna, 1991, pp. 135-144; Bader et al., 1994; Bleck et al., 1994, 2000; Delorme and Zuna, 1995; Lieback et al., 1996; Kastelic et al., 2001). These programs provided new, objective information about the morphology of scanned organs (Räth et al., 1985; Hirning et al., 1989; Bader et al., 1994; Bleck et al., 1994; Lieback et al., 1996; Kastelic et al., 2001).

The aim of the present study was to examine changes in endometrial echostructure throughout the oestrous cycle in cows using second order as well as first order statistics of grey levels of B-Mode ultrasound images using a refined computer-assisted echotexture analysis program and to relate the changes to sex steroid levels in plasma.

2. Materials and methods

Four uniparous non-lactating Simmental cows with a mean oestrous cycle length of 21.4 days (range, 19–25 days) were examined as described below. Their mean age was 3.5 year (range, 3–4 years).

Each cow underwent transrectal sonographic B-Mode examinations over two oestrous cycles. The examinations were conducted by the same operator. Time of ovulation was determined from daily ultrasound examinations. The last day on which the dominant follicle was visible was designated as Day −1, while the first day on which the ovulatory follicle was not seen was designated Day 0. Echotexture examinations were carried out on Days 0 (ovulation), 1, 2, 4, 7, 10, 13 and from Day 16 on daily until Day 0 of the next oestrous cycle. To enable comparison of parameters between oestrous cycles with different cycle lengths, the measurement data from Day 0 to Day 13 and from Day −4 to Day −1 were used for analyses.

In scanning the animals, the transducer was moved rectally over the dorsal surface of the reproductive tract. During each examination five different B-Mode images of the uterus were

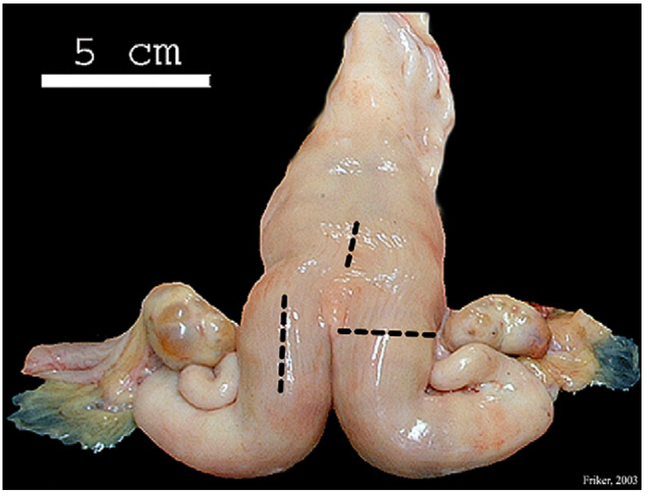

Fig. 1. Anatomical model of the bovine uterus. Black lines mark the locations where the B-Mode images of the uterus were taken. One cross- and one long-sectional image of each uterine horn and one long-sectional image of the cranial end of the cervix ($n = 5$ images) were taken.

taken: one cross- and one long-sectional image of each uterine horn approximately within 2 cm of the cranial end of the uterine body and one long-sectional image approximately within 1.5 cm of the cranial end of the cervix (Fig. 1).

All sonographic examinations were performed with a conventional imaging system using a 7.0 MHz microconvex probe (SSH 140A, Toshiba, Tokyo, Japan). The ultrasound scanner produced an image with a resolution of 1024 by 768 pixels with each pixel having 1 of 256 possible shades of grey. Standardised machine settings (i.e. depth, echo-amplification, persistence, pre- and post-processing) were not changed throughout the entire study. A digital still recorder (DKR 700, Sony, Tokyo, Japan) was used for digitisation of the analogous B-Mode images. Analysis of echotexture was accomplished using an IBM-compatible personal computer and a customized program specifically designed for analysis of ultrasound images (PEPE v1.0, German Cancer Research Centre, Heidelberg, Germany).

Digitized images of the endometrium were divided into four equal quadrants (Fig. 2). In each of these quadrants a quadratic region-of-interest (ROI) with a size of at least 1000 pixels was placed. The ROI encompassed only endometrial tissue, avoiding myometrial tissue and intrauterine fluid if present. Echotexture parameters mean grey level (MGL) and homogeneity (HOM) of the ROI were calculated as described in detail by Räth et al. (1985) or Delorme and Zuna (1995). Mean values of all four ROI were used for further evaluations. The parameter MGL expresses the grey values without taking their spatial arrangement into account. It is used to describe the total brightness of the image. Values for MGL range between 0 and 255.

Mean grey level (MGL) is defined (Räth et al., 1985) as

$$\mu_g = \frac{1}{N}\sum_{(n,m)} g_{nm}$$

where μ_g is the mean grey level (values: 0–255); N the number of Pixel; n, m the row (n) and column (m) index and g_{nm} is the grey level in pixel (n, m).

The higher the MGL-value the brighter the sonographic image and vice versa.

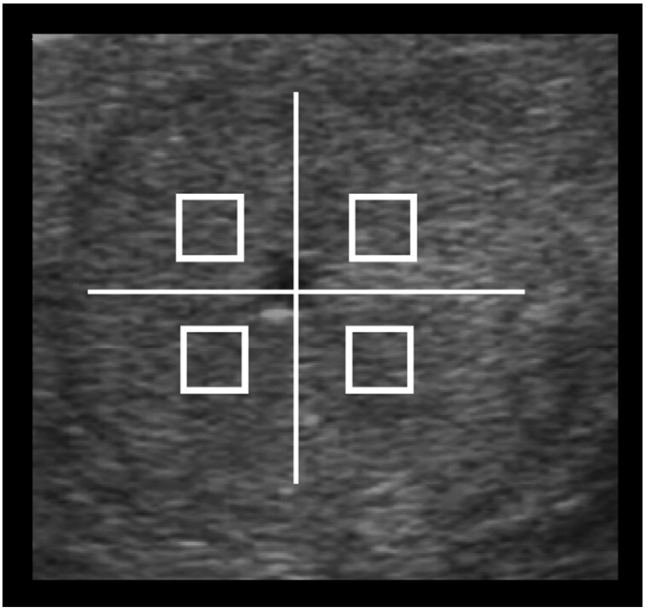

Fig. 2. Image of a uterine horn detailing analysis technique for a cross-sectional B-Mode image of the bovine endometrium. The image was divided into four equal quadrants. Echotexture parameters, mean grey level and homogeneity were measured by placing quadratic regions of interest covering at least 1000 pixels into each quadrant. The present image shows ultrasonographic texture characteristic of the uterine horn at the time of oestrus with a mean grey level value of 93 and a homogeneity value of 11.7.

The parameter HOM reflects the spatial arrangement of grey levels. Pixel pairs are assigned to each other on the basis of a specific, predefined displacement vector. A two-dimensional frequency histogram characterizing the occurrence of individual pixel pairs is called co-occurence matrix. Evaluating this matrix statistically the parameter HOM is calculated. HOM measures the uniformity of pixel pairs and its values range between 0 and 1.

Homogeneity (HOM) is defined (Räth et al., 1985; Delorme and Zuna, 1995) as

$$\mathrm{HOM} = \sum\nolimits_{(n,m)} p_{nm}^2$$

where HOM is the homogeneity; n, m the row (n) and column (m) index and p_{nm} is the record of co-occurrence matrix for cluster (n, m).

The higher the values for HOM the higher the uniformity of the image and vice versa. Because the values of HOM are very low, they are multiplied with 10^3 for a better illustration and reported as such in the results.

Steroid hormones were estimated by enzyme immunoassays as published earlier (Prakash et al., 1987; Meyer et al., 1997). In brief, progesterone (P) was measured directly in 5 μL plasma (antigen: progesterone-7α-carboxy-ethylthioether-BSA; enzyme: progesterone-6β-hemisuc-cinate-HRP). All intra- and inter-assay variations were less than 12%. For analysis of total estrogens (TE) an antibody (antigen: estradiol-17β-hemisuccinate-BSA) reacting almost equally well with estradiol-17β (100%), estrone (100%) and estradiol-17α (66%) was combined with estradiol-17β-hemisuccinate-HRP. After hydrolysis of conjugates and extraction of the unconjugated estrogens the residue of 8 μL plasma per well was analysed. The intra- and inter-assay variations were less than 12%. The suitability of using TE for the reliable monitoring of estradiol-17β activity is documented by Meyer et al. (1997).

Statistical analyses were carried out using Statview 5.0 and SPSS 11.0 statistical software packages (SAS Institute, Cary, North Carolina, USA). Different parameters were compared using the Spearman's correlation coefficient. Friedman test and estimation of the variance component were used to determine the effect of oestrous cycle and day of cycle on the parameters MGL and HOM. In addition, Fisher's protected LSD and Wilcoxon Signed Rank test were conducted post hoc to test for differences in measurements between cows, oestrous cycles and days of the oestrous cycle in the echotexture of the endometrium. Intra-observer reproducibility of MGL and HOM measurement results were assessed using the intra-class correlation coefficient (Intra-CC).

3. Results

There were no differences ($P > 0.05$) in MGL and HOM, respectively, between the five B-Mode images taken from the uterine body and both uterine horns during each examination. Thus, mean values of MGL and HOM of these images were used for further evaluations.

MGL as well as HOM showed similar ($P > 0.05$) changes during all oestrous cycles (Fig. 4). MGL was negatively correlated with HOM ($r = -0.66$; $P < 0.0001$). MGL showed basal values on Day 0 (mean ± s.e.: 94 ± 1.8) and between Day −3 and −1 (Fig. 4; an example for an image during this time period is given in Fig. 2). Maximum MGL values were measured between Days 4 and 13 (105 ± 1.5), whereby the values did not differ significantly from each other during this time ($P > 0.05$) (Fig. 4; an example for an image during dioestrus is given in Fig. 3). HOM was high on Day 0 (11.8 ± 0.36) and decreased to low values on Day 2 ($P < 0.05$) (Fig. 4). Between Day 2 (9.8 ± 0.30) and Day 13 constant basal HOM levels were measured. After Day 13 HOM

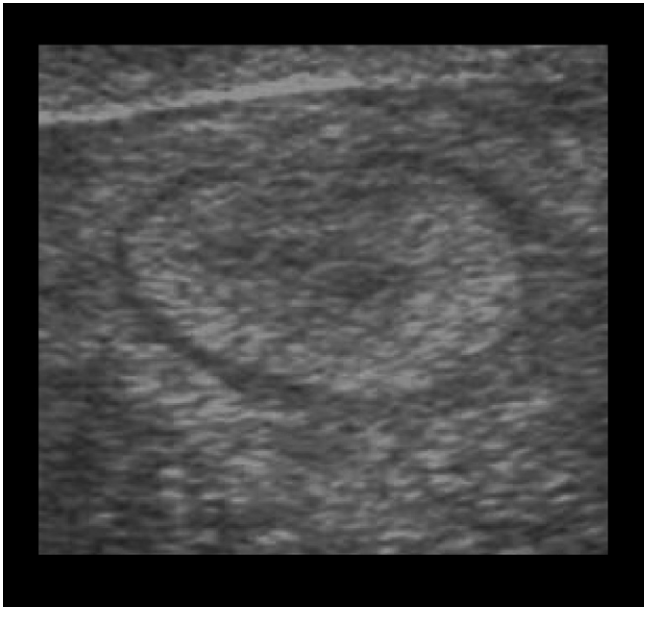

Fig. 3. Image of uterine horn showing ultrasonographic texture characteristic at dioestrus with a mean grey level value of 109 and a homogeneity value of 10.3.

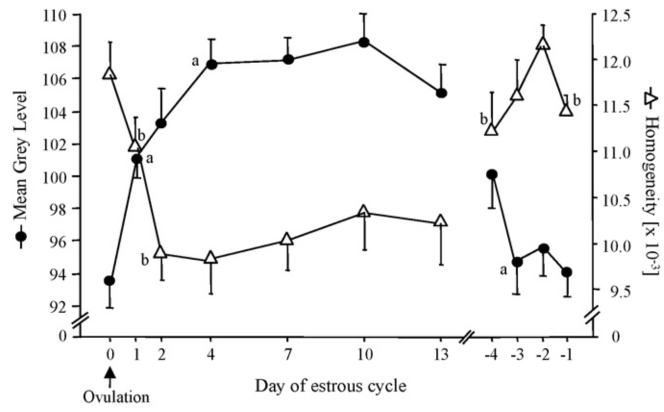

Fig. 4. Mean grey level and homogeneity of the endometrium over time. Values are means ± s.e. of four cows over two oestrous cycles. Values with letters (a and b) differ significantly from corresponding values of previous measurements ($P < 0.05$). Because the values of HOM were very low, they were multiplied with 10^3 for better illustration.

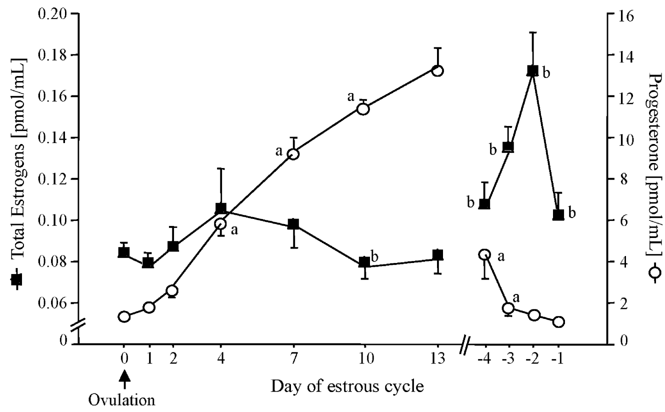

Fig. 5. Plasma concentration of oestrogen and progesterone. Values are means ± s.e. of four cows over two oestrous cycles. Values with letters (a and b) differ significantly from corresponding values of previous measurements ($P < 0.05$).

increased ($P < 0.05$) to a maximum on Day −2, followed by a moderate, but significant decline ($P < 0.05$) on Day −1 (Fig. 4).

The factor ‘day of oestrous cycle’ showed a highly significant ($P < 0.0001$) effect and the factor ‘cow’ a significant ($P < 0.05$) effect on MGL and HOM. No differences ($P > 0.05$) in both echotexture parameters were measured between oestrous cycles within cows.

While no relationship occurred between MGL and TE ($P > 0.05$), HOM was positively correlated with TE between Days −4 and −1 (Figs. 4 and 5; $r = 0.48$; $P < 0.05$). MGL and HOM were correlated to progesterone between Days 0 and −4 (Figs. 4 and 5; MGL: $r = 0.48$; $P < 0.05$; HOM: $r = -0.27$; $P < 0.05$).

4. Discussion

The results of this study show an inverse relationship between the changes in endometrial echogeneity and homogeneity during the oestrous cycle. This phenomenon is obviously due to changes in degree of oedema of the endometrium. During oedema formation, fluid in the intracellular space increases (Klinge, 1959; Veznik et al., 1979; Ohtani et al., 1993). Since fluid is hypoechoic compared to other tissues, the overall echogeneity of the endometrium decreases at ultrasound. Ginther (1998) referred to results from an unpublished study, which suggests that the size of hypoechoic areas increases secondary to endometrial oedema. This phenomenon would also explain increased values in homogeneity due to a high number of pixels with similar grey scale being near together. A decline in endometrial oedema is accompanied with a reduction in intracellular fluid (Vollmerhaus, 1957); which leads to an increase in echogeneity and at the same time to a decrease in homogeneity of the endometrium (Ginther, 1998).

The changes in homogeneity obtained with the computer-assisted software are contrary to evaluation of fluctuations in endometrial homogeneity observed visually via the human eye (Pierson and Ginther, 1987). The reason for this is most likely the divergence in the definition of the term "homogeneity" in computer-assisted analysis on the one side and in the subjective evaluation of ultrasonographic images on the other side. Computer-assisted analysis compares the echogeneity of pixels with an area of less than 0.01 mm^2. A person, however, analyses the grey scale distribution of larger areas, usually several square millimetres. Due to oedema formation during oestrus, endometrial folds engorge and the sonographic image appears to be more heterogeneous for the eye of the examining person (Pierson and Ginther, 1987), while on the other hand computer-assisted analysis of these images will result in relatively high values for homogeneity. Inverse circumstances occur during dioestrus.

The cyclic changes of the mean grey level obtained in this study are similar to those described by Pierson and Ginther (1987) when determining endometrial echogeneity with a simpler technique. While the mean grey level remained constant during the last few days prior to ovulation, significant changes in homogeneity were noticed during this time period. Therefore, only computer-assisted determination of homogeneity seems to be a suitable parameter to detect endometrial changes during pro-oestrus. These fluctuations of homogeneity were related to changes in plasma estrogen concentrations. As it is well known that estrogens induce endometrial oedema (Vollmerhaus, 1958; Liebich, 1993, pp. 255–272), this observation further supports the fact that homogeneity is mainly influenced by the amount of intracellular fluid accumulation. Other investigators (Pierson and Ginther, 1987) have suggested the use of ultrasonographic parameters as indicators for the effect of estrogens on the bovine uterus but were unable to support this hypothesis further due to lack of oestrogen level determinations.

No association has been found between homogeneity and plasma oestrogen levels after ovulation. At this time oestrogen levels decreased rapidly, while homogeneity only gradually diminished. In histological studies of the endometrium it was demonstrated that the decrease of endometrial oedema takes much longer than the decrease in oestrogen levels (Vollmerhaus, 1958). According to Ginther (1998) the decrease in intracellular fluid is based on resorption and loss of fluid into the uterine lumen. Compared to changes in plasma oestrogen levels these mechanisms require more time, thus explaining the missing relationship between homogeneity and plasma oestrogen levels after ovulation.

There were significant relationships between progesterone levels and echogeneity as well as homogeneity during met- and dioestrus. However, when comparing the cyclic changes of progesterone and echotexture parameters, no dependencies were detected. While the echogeneity and homogeneity remained at constant levels between Day 4 and Day 13, progesterone increased during this stage of the oestrous cycle. Therefore, the obtained correlation values presumably are due to the fact that progesterone levels are inversely proportional to oestrogen levels, the latter having a direct influence on echogeneity as mentioned before.

Quantitative echotexture analysis complements, but does not replace subjective analysis of B-Mode images of bovine endometrium. It offers the opportunity to obtain more detailed information after the transrectal examination of the genital tract in cows. For example, it may enable a more precise determination of the stage of oestrous cycle. Furthermore, it may be a helpful tool in the diagnosis of a subclinical endometritis in cows. To confirm these attitudes of the authors, a higher number of cycling cows and cows with pathological alterations of the uterus have to be examined.

In conclusion, the results demonstrate that cyclic changes in the degree of endometrial oedema can be differentiated using a computer-assisted software program for the analysis of first- and second-order grey level statistics of B-Mode ultrasound images. This method offers the possibility for an objective evaluation of physiological and pathological changes of the endometrium and could be a contribution to a more accurate diagnosis in fertility management of cows.

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