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Endometrial echotexture variables in postpartum cows with subclinical endometritis

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ABSTRACT

The aim of this study was to evaluate endometrial echotexture changes on ultrasonographic digital images during subclinical endometritis using a computer-assisted image analysis program. Endometrial samples were collected from 140 Brown Swiss cows (days in milk = 35 ± 3) using a cytobrush method and classified as having a non-inflamed uterus ($n = 66$) and uterus with acute ($n = 42$), subacute ($n = 21$), and chronic ($n = 11$) inflammations. The mean cellular infiltration density was 0%, 31 ± 5%, 37 ± 6%, and 16 ± 8% for cows with non-inflamed uterus and cows with acute, subacute, and chronic uterine inflammations ($P < 0.0001$). As the cell infiltration density increased, both cervical diameter and mean gray level did not change. There were a liner decrease in homogeneity and a linear increase in contrast in response to increased cellular infiltration density. The sensitivity and specificity were 79.73% and 46.97% for the homogeneity value and 59.46% and 69.70% for the contrast value, respectively. In conclusion, monitoring endometrial echotexture alterations, especially homogeneity and contrast, changed depending on the cellular density and inflammation status and may be potential diagnostic markers for subclinical endometritis in cows.

1. Introduction

Real time B-mode ultrasonography has been one of the most commonly used diagnostic tools in farm animal reproduction since it was first used in 1980 in mares (Palmer and Driancourt, 1980), which later became available for cows (Pierson and Ginther, 1984; Reeves et al., 1984). Ultrasonography is an important routine tool for use in veterinary medicine to assess physiological and pathological changes in the reproductive system, which mainly covers detection of pregnancy at early stages, follicular dynamics in ovaries, and prediction of ovulation time as well as diagnosis of ovarian cysts, infections and tumors (Ginther, 2014). Contrast-enhanced and color Doppler ultrasonography, multi-dimensional ultrasonography, and computer-assisted image analysis (CAIA) are some of the advancements in ultrasonic imaging available to veterinary medicine (Nakamura et al., 2009; Morel et al., 2010; Cengiz et al., 2014).

Computer-assisted image analysis, a computer algorithm, is termed "echotexture analysis" and used in human (Chen et al., 2008) and veterinary (Arashiro et al., 2010) medicine. A two-dimensional ultrasonographic image is a matrix of square picture elements (pixels), which vary in grayscale values ranging from 0 (absolute black) to 255 (absolute White; Singh et al., 1997). This

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methodology permits a quantitative assessment of the density of each pixel present on an image. Thus, minor alterations on digital images of the tissues that cannot be distinguished by the human eye can be detected in numerical values (Siqueira et al., 2009), eliminating subjectivity in visual evaluation. The mean grey level (MGL), homogeneity (HOM), and contrast (CON) are major variables of the digital images to make inferences about tissue echotexture (Kucukaslan et al., 2014).

Endometritis is an inflammation, which is limited to endometrium and occurs at least 21 days after calving without exhibiting systemic signs of the condition (Gilbert et al., 2005). Clinical endometritis can be diagnosed according to symptoms such as abnormal vaginal discharge (a fetid watery red-brown or mucoprulent), uterine and cervical enlargement, whereas subclinical cases need specific examinations employing ultrasonography, cytology, or biopsy (Sheldon et al., 2006; Barlund et al., 2008). Although real time ultrasonography has been extensively used in animal reproduction (Ginther, 2014), it is inadequate to differentiate healthy and subclinical uterine inflammations (Barlund et al., 2008). In ultrasonography, increases in fluid accumulation and endometrial thickness are typical findings (Pierson and Ginther, 1987; Kähn, 2004). However, sensitivity and specificity of diagnosing subclinical endometritis by using ultrasonography is lower than cytology examinations. Endometrial cytology is thus preferred for the diagnosis, although it is an invasive procedure (Barlund et al., 2008). This experiment was conducted to evaluate variations in echotexture variables during subclinical endometritis using the CAIA method, a quantitative and objective assessment.

2. Materials and methods

2.1. Cows

The study was conducted on 140 (parity 1, $n = 22$; parity 2, $n = 37$; parity 3, $n = 29$; and parity ≥ 4, $n = 52$) Brown Swiss cows housed in Atatürk University Research Farm (39°54'E; 41°13'N; altitude of 1980 m). The cows that had no clinical and systemic signs of metritis (e.g., abnormal vaginal discharge, apathy, anorexia, and fever) in early postpartum (d < 21) and that had no clinical endometritis diagnosis (e.g., abnormal vaginal discharge in vaginoscopy and enlarged uterine horn in rectal palpation) on d 35 ± 3 postpartum were used in the study (Sheldon et al., 2006).

2.2. Endometrial sampling

The endometrial samples were collected using endocervical brushes (Plasti-med®, Istanbul, Turkey) as described by Kasimanickam et al. (2004). The brush was joined to another brush for passage through the cervix. A stainless steel catheter, which was placed into a plastic artificial insemination cover was used as the sampling instrument. The instrument was placed in a clean rectal palpation sleeve for protection from the vaginal and perianal contamination. Briefly, after cleaning the external vulvar area, the instrument was placed into the vagina. When the instrument reached to the external orificium of the cervix, the sleeve was punctured and the instrument was advanced through the cervix. The steel catheter was then retracted, while advancing the cytobrush through the plastic cover until it reached the endometrium. The uterus was slightly massaged while the cytobrush was twisting two or three times in a clockwise direction. Retraction of the cytobrush into the plastic cover was subsequently performed and the instrument was removed from the uterus. The smear was expelled onto a glass slide and fixed with methanol about 5 min before staining. The same researcher performed all endometrial samplings.

2.3. Cytological examination and classification of endometritis

The fixed samples were stained using Giemsa and examined under light microscopy. Postpartum subclinical endometritis was diagnosed based on the density of inflammatory cells (>5%) with the cytological examination (Oral et al., 2009). The samples were also subjected to the cytopathological classification based on presence of polymorpho-nuclear cells (PMN) and lymphocytes (LYM) to define inflammatory status (Schlafer and Miller, 2007). The samples with ≥5% PMN, 5% PMN + LYM, and 5% LYM were considered acute, sub-acute, and chronic, respectively.

2.4. Handling of digital images and measuring of cervical diameter

Digital images of the endometrium were collected by transrectal ultrasonography using a 7.5 MHz linear-array transducer connected to a portable B-mode ultrasonic scanner (Agroscan AL®, Noveko International Inc., Angoulême, France) as described previously (Kähn, 2004). The same machine setting standardized [i.e., Mode (B), depth (8 cm), MHz (7.5), focus zone, B-gain, B-brightness] was used throughout the study. To improve accuracy and prevision, at least three circular cross-sections of the uterus of each cow were recorded. The images from the point of larger curvature of uterus were recorded using a digital recorder (PMP-100®, Sigmatek, France). In addition to endometrial images, cervical diameters were also measured by the transrectal ultrasonography. The same researcher performed ultrasonography throughout the study to eliminate person-to-person variation.

2.5. Echotexture analysis

The outline of the echotexture analyses of the endometrium was previously described Cengiz et al. (2014). Briefly, the images saved in the non-compressed bitmap file format were analyzed using a custom-developed software (BS200 Pro® Image Processing and Analysis Software, BAB, Ankara, Turkey). Four regions of interests (ROI, 20×20 pixels) were identified on each image for MGL, HOM, and CON variables. The ROI areas were selected only for endometrial layer to avoid results being confounded by unintended evaluation of the myometrium and luminal fluid (Fig. 1).