Theriogenology 119 (2018) 225–232

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Optimization of diagnostic methods and criteria of endometritis for various postpartum days to evaluate infertility in dairy cows

Takeshi Koyama [a, *], Ryosuke Omori [b], Keisuke Koyama [a], Yoshitaka Matsui [a], Masahito Sugimoto [a]

[a] Hokkaido Research Organization, Dairy Research Center, Nakashibetsu, Hokkaido, 086-1153, Japan
[b] Division of Bioinformatics, Research Center for Zoonosis Control, Hokkaido University, Sapporo, Hokkaido, 001-0020, Japan

ARTICLE INFO

Article history:
Received 20 November 2017
Received in revised form
3 July 2018
Accepted 5 July 2018

Keywords:
Dairy cow
Endometritis
Diagnosis

ABSTRACT

Postpartum endometritis is the main cause of infertility in dairy cows, but there is a lack of critical diagnostic criteria. We aimed to 1) determine the optimal diagnostic method and criteria of endometritis for various postpartum days to evaluate infertility, and 2) assess the diagnostic accuracy of a combination of diagnostic methods. Holstein dairy cows (n = 441) from nine commercial dairy herds were examined at 42 ± 7 days postpartum by using 5 methods: 1) transrectal palpation measurement of the cervical diameter, 2) ultrasonographic measurement of the fluid in uterus (FIU) score, 3) vaginoscopic detection of external uterine orifice hyperemia, 4) vaginal discharge score (VDS), and 5) endometrial cytological percentage of polymorphonuclear leukocytes (PMN%). The clinical findings that were significant in the Pearson chisquare test and had the highest sum of sensitivity and specificity for infertility at 100, 125, 150, 175, and 200 days after parturition were determined the optimal criteria of endometritis. Logistic regression and the Cox proportional hazards model were used to compare the accuracies of the different diagnostic methods for infertility at various postpartum days. The combinations of methods which were significant in Pearson chisquare test and had the highest sum of sensitivity and specificity for infertility were proposed as the optimal combination for determination of endometritis status for various postpartum day. The optimal diagnostic criteria were PMN% ≥ 6.0, FIU ≥ 2 (continuous hyperechogenic or large amount of storage material), or VDS ≥ 2 (mucopurulent or worse vaginal discharge) for postpartum days 100 and 125; PMN% ≥ 8.0, FIU = 3 (large amount of storage material), VDS ≥ 2, or external uterine orifice hyperemia for day 150; PMN% ≥ 6.0, FIU = 3, VDS ≥ 2, or external uterine orifice hyperemia for day 175; and PMN% ≥ 5.0, FIU = 3, VDS ≥ 2, or external uterine orifice hyperemia for day 200. Only the results of endometrial cytology were related to infertility regardless of the postpartum days to evaluate infertility or statistical models. Compared with the sensitivity and specificity of a single diagnostic method, the sensitivity of a combination of methods improved but specificity decreased. We concluded that different diagnostic methods and criteria were required for postpartum days to evaluate infertility and diagnostic accuracy was improved by a combination of diagnostic methods rather than by a single method.

1. Introduction

Postpartum endometritis is one of the main causes of decreased reproductive performance in dairy cows [1]. Accurate diagnosis and appropriate treatment of endometritis is critical for improvement of reproductive performance. Postpartum endometritis of dairy cows is defined as inflammation limited to the endometrium occurring at least 21 days after parturition without systematic signs of illness [2]. Several methods of diagnosis for endometritis have been proposed: (1) transrectal palpation [3], (2) vaginoscopy [4,5], (3) transrectal ultrasonography [4,6], (4) endometrial cytology [4,6,7], and (5) biopsy [8]. Transrectal palpation measures the diameter of the cervical canal, and a cervical diameter >7.5 cm after postpartum day 20 has been defined as endometritis in cows [3]. Diagnosis by vaginoscopy is performed by measuring the contamination ratio of pus in the mucus or presence of worse vaginal

* Corresponding author.
E-mail address: koyama-takeshi@hro.or.jp (T. Koyama).

https://doi.org/10.1016/j.theriogenology.2018.07.002
0093-691X/

discharge [9]. Presence of mucopurulent or worse vaginal discharge has been used to identify cows with endometritis [7]. Detection of fluid in uterus (FIU) by transrectal ultrasonography has been used as a criterion of endometritis [4,6]. Diagnosis by endometrial cytology has been performed by uterine lavage [4,10] or cytobrush technique [4,6,7], and a specific percentage of polymorphonuclear leukocytes (PMN%) is the diagnostic criterion. Diagnosis by uterine biopsy is the histopathological assessment of endometrium tissue [11], and presence of endometrial inflammatory infiltrate is the criterion [12].

There is no critical bio-marker for diagnosis of endometritis, but most previous studies have used a finding of infertility [4,6,7]. However, dairy cow infertility is influenced by other important factors unrelated to uterine disease; e.g., heterogeneity of reproductive management among dairy farms. The number of days open varies depending on the reproductive management of each herd, so the pregnancy status on a specific postpartum day cannot measure infertility precisely.

In previous studies, endometrial cytology has been used as a reference test [4,6,7,13]. Diagnosis by endometrial cytology requires laboratory tests and skillful collection of samples. On the other hand, diagnoses by transrectal palpation, transrectal ultrasonography, and vaginoscopy are non-laboratory tests and are easier to perform practically. However, the accuracies of these diagnostic methods have not been sufficiently high to identify diseased animals [4]. If a combination of methods excluding endometrial cytology can improve diagnostic accuracy, it would be possible to diagnose endometritis in a clinical environment with high accuracy.

The primary purpose of this study was to determine the optimal diagnostic method and criteria of endometritis for different postpartum days to evaluate pregnancy status. A secondary study purpose was to assess the accuracy of a combination of diagnostic methods, excluding endometrial cytology.

2. Material and methods

2.1. Study farms

Holstein Friesian cows ($n = 465$) from nine commercial tie (4 herds) and free-stall (5 herds) dairy herds in east Hokkaido, Japan, that calved between May 2012 and August 2014 were enrolled in this study. Herd sizes ranged from 68 to 119 milking cows. All cows were milked twice daily. In 7 of 9 herds, artificial insemination (AI) was performed after a voluntary waiting period (VWP), and the VWPs ranged from 40 to 60 days. In the other 2 herds, AI was performed regardless of the postpartum day if estrus was detected. None of the tested cows were treated specifically for endometritis during the 200 postpartum days. Prostaglandin F2α, or an analog, and GnRH products were routinely used within these herds for the purposes of estrus induction and timed AI.

2.2. Uterine examinations

Examination of the uterus was performed at 42 ± 7 days after parturition. Cows were enrolled only once during the study duration. Each herd was visited once a week by the same researcher and veterinarian to collect data.

The examinations of the uterus were performed in the order of (1) transrectal palpation measurement of the cervical diameter, (2) transrectal ultrasonographic measurement of the fluid in uterus (FIU) score, (3) vaginoscopic detection of hyperemia in the external uterine orifice, (4) measurement of the vaginal discharge score (VDS), and (5) endometrial cytology measurement of the percentage of polymorphonuclear leukocytes (PMN%).

Prior to the examinations, we lightly scraped feces from the rectum. Consequently, the cervical canal was palpated through the rectum, and the cervical diameter was classified as <5 cm, 5–7.4 cm, and ≥7.5 cm.

After transrectal palpation, the ovary structure and presence of FIU were observed by transrectal ultrasonography (HS-101V, Honda Electronics, Aichi, Japan) in all cows. Observation of the uterus and ovary was performed in the order of the uterine body, uterine horn, and ovary. If FIU was detected in the uterine body or horn, it was scored as follows: 0, no fluid or echo-free; 1, small amount of discontinuous hyperechogenic (thin <1 mm); 2, continuous hyperechogenic (thin ≥1 mm); and 3, large amount of storage material that looked like a snowstorm. The ovarian structure was investigated for diameter and number of follicle and corpus luteum. Follicular cysts was defined as fluid-filled structure >25 mm diameter in the absence of a corpus luteum.

Prior to the vaginoscopy, the vulva was cleaned with a paper towel and disinfected in 75% alcohol. Next, the stainless steel vaginoscope with a length of 30 cm (Hujihira, Tokyo, Japna) was disinfected in 75% alcohol and then inserted into the vagina far enough to enable visualization of the external uterine orifice. The external uterine orifice was observed for hyperemia, swelling, and opening of the external uterine orifice. The hyperemia of the external uterine orifice was classified as follows: 0; not present, 1; present (Fig. 1). The vaginal discharge was visually examined and classified according to a previous study method [9], with modification, as the VDS score: 0, no or clear mucus; 1, clear mucus containing flecks of white pus; 2, exudate containing ≤50% white or cream pus; 3, exudate containing >50% white cream or bloody pus; and 4, foul smelling discharge.

After the vaginoscopy, endometrial cell samples were collected by using a cytobrush (Honest supper blush, Honest Medical, Tokyo, Japan) technique modified for use in cows [14]. The cytobrush was cut to 7 cm in length and threaded onto a brass hollow pipe 50 cm in length, 2 mm in diameter, and with a wall thickness of 0.5 mm and then placed in a brass hollow pipe 50 cm in length, 4 mm in diameter, and with a wall thickness of 0.5 mm. This instrument was placed in a sanitary plastic tube (Fujihira, Tokyo, Japan) from which a 1.0×0.2 cm portion approximately 1.0 cm from the tip to the length was cut away, and then a sanitary sleeve (A.I/E.T Sanitary sheaths, IMV TECHNOLOGIES, France) was added to protect from vaginal contamination. All the instruments were disposable. The sanitary sleeve was punctured in front of the external uterine orifice, and the instrument was inserted into the uterine body through the cervical canal. The outside brass hollow pipe was pulled to expose the cytobrush. The cytobrush was rolled onto the uterine body wall, pulled back into the outside brass hollow pipe, and removed from the vagina. The cytobrush was rolled onto a clean glass slide and dried in air. All slides were stained with modified Wright-Giemsa satin (Diff-Quik; Sysmex, Kobe, Japan). To obtain the PMN%, each slide was examined at 400 × magnification to perform a differential cell count of 200 cells (endometrial cells and PMNs) by a single observer to provide a quantitative assessment of endometrial inflammation.

2.3. Data collection

The seasons of calving (spring: March–May; summer: June–August; autumn: September–November; winter: December–February) and parity (first or second and greater) for all cows were collected by using on-farm data-recording forms, and the AI and cull date for all cows were collected from the Hokkaido Dairy Milk Recording and Testing Association (http://www.hmrt.or.jp/). The pregnancy statuses of the tested cows were followed up until 200 days after parturition or until the date of culling (if ≤ 200 days).