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Pregnancy prediction on the day of embryo transfer (Day 7) and Day 14 by measuring luteal blood flow in dairy cows

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ABSTRACT

This study aimed to assess the suitability of luteal blood flow analyses measured by color Doppler ultrasonography (CDUS), to predict pregnancy at pre- and post-embryo transfer (ET) in dairy cows, and to compare with the established criterion like luteal size and plasma progesterone (P_4) concentrations. Lactating Holstein cows (n = 65) with spontaneous (n = 34) or synchronized estrus (n = 31) were examined. Cows with a CL greater than or equal to 20 mm in diameter (n = 58) received embryo transfer on Day 7 (Day 0 = estrus). Brightness mode images were captured for calculation of the CL area, luteal cavity area, and dominant follicle area on Days 3, 5, 7, and 14. Color Doppler ultrasonography examinations were conducted to determine the blood flow area (BFA) within the CL at the maximum diameter and the time-averaged maximum velocity (TAMV) of the base of the spiral artery on the same days. Plasma P_4 concentrations were determined from blood samples collected at each ultrasound examination. Pregnancy was diagnosed by an ultrasound on Day 30. There was no significant difference in the proportion of cows received embryo (91.2% vs. 87.1%, P = 0.70) and pregnancy rate (58.1% vs. 59.3%, P = 1.00) between the spontaneous estrus and synchronized groups. The BFA values of the pregnant group (n = 34) were approximately 1.42 and 1.54 times higher than those of the nonpregnant group (n = 24) on Days 7 (0.54 ± 0.04 cm^2 vs. 0.38 ± 0.02 cm^2; $P < 0.01$) and 14 (0.80 ± 0.23 cm^2 vs. 0.52 ± 0.22 cm^2; $P < 0.01$), respectively. The TAMV of the pregnant group was approximately 1.45 times higher than that of the nonpregnant group on Day 14 (57.8 ± 3.5 cm/s vs. 40.0 ± 3.3 cm/s; $P < 0.01$). However, no differences were found in the CL area, CL tissue area, dominant follicle area, and plasma P_4 concentrations among these groups. In addition, the best logistic regression model to predict pregnancy included scores for BFA on Day 7, BFA and TAMV on Day 14. Setting the cutoff value of BFA at 0.43 cm^2 yielded the highest sensitivity (79.4%) and specificity (75.0%) on Day 7, indicating the effectiveness of using BFA data for predicting pregnancy on Day 7. Furthermore, setting the cutoff value at one obtained from a sample with BFA 0.63 cm^2 and TAMV 50.60 cm/s yielded the highest sensitivity (85.3%) and specificity (91.7%) on Day 14. In conclusion, the evaluations of BFA on Day 7, and paired BFA and TAMV on Day 14 represent reliable predictors of pregnancy in the cow.

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1. Introduction

Bovine embryo transfer is a powerful tool for the expansion of progenies from the specific donor, and it is rapidly spreading not only for the expansion but genetic improvement of cattle [1,2]. Moreover, this technique has additional merits for improving the pregnancy rate in repeat breeders and avoiding heat stress during the first few days of embryogenesis [3]. In Japan, because the economic value of Japanese black beef (JB) calves is now 2 to 5 times higher than that of Holstein or JB–Holstein crossbred calves, many dairy farmers desire to produce JB calves by transferring JB embryo to Holstein cows. Currently, this trend is further facilitated by the popularization of sex-sorted semen so as to produce inheritor heifers steadily. Therefore, in terms of economic outcome, a profit and loss point of embryo transfer technology is mainly depending on the conception rate in the recipient cows.

To establish the conception in bovine embryo transfer (ET), the presence of a functional CL and sufficient production of progesterone (P_4) are required. Both of these factors are essential for the establishment and maintenance of gestation in cows [4–6]. However, the procedure based on plasma and/or milk P_4 assay is not suitable for on-farm diagnosis because of the relatively high costs for P_4 assay and the time lag between the blood collection and result availability. Therefore, other methods are usually preferred to predict CL function in the recipient, e.g., CL size measured by transrectal palpation, or ultrasonography is commonly used for the prediction because CL size correlates with plasma P_4 concentrations, and ultrasonographic measurement of the CL is an alternative procedure for plasma P_4 assay [7,8]. Therefore, we have always selected recipient cows on the basis of CL size measured by transrectal palpation or ultrasonography in the farm.

However, although fresh embryos are being transferred, the conception rate of bovine ET has not been improved over the last decades and is still not more than 50% [4,9]. Thus, to select conceptive recipients before the transfer, it is desired to establish a novel predictive determinant that is more accurate and easier to use than CL morphology and P_4 assay.

The CL is one of the most highly vascularized organs and receives the greatest blood flow per tissue volume in the body [10]. The blood vascular bed of developing bovine CL is supplied by spiral artery directly branching from the ovarian artery [11]. After ovulation, blood vessels from the theca interna invade into the cavity of the relevant follicle and form a network, which supplies the luteal cells. This neovascularization is necessary for the delivery of luteal steroids to the general circulation and for the provision of the circulating substrate, a low-density lipoprotein, which is used by the luteal cells for P_4 biosynthesis [12].

Color Doppler ultrasonography (CDUS) provides new information regarding blood flow in addition to the cross section of organs obtained by brightness (B)-mode ultrasonography and has been applied to clinical diagnoses in horses and cattle since the 1990s [13,14]. Color Doppler ultrasonography, which uses the frequency shift caused by the Doppler effect, is a useful and noninvasive technique for evaluating ovarian vascular function, enabling visual observation of blood flow [14,15]. In particular, changes in the blood flow within the follicle wall before ovulation and in the CL during its development and regression have been studied [16–18]. Color Doppler ultrasonography is a reliable procedure for evaluating luteal vascularity necessary for maintaining luteal function [14,16,19]. No correlation between the CL size and plasma P_4 concentrations has been reported during the luteolysis because functional regression of CL precedes its morphologic degeneration [7]. However, several reports indicate that the blood flow area (BFA) and/or blood flow velocity in a CL are closely associated with plasma P_4 concentrations throughout the estrous cycle, including the luteolytic phase. Therefore, CDUS is a useful procedure for assessing CL function [14,18,19]. Moreover, it has been reported that the assessment of luteal blood flow enables early detection of nonpregnant cattle 20 days after artificial insemination [20].

Overall, the present study aimed to assess the applicability of luteal blood flow data obtained by using CDUS for predicting conception before and after ET in dairy cows. Specifically, we addressed questions regarding: (1) whether changes in the morphology of CL and luteal blood flow are different in pregnant and nonpregnant cows; (2) whether vascular image analysis can predict conception at pre- and post-ET using logistic regression; and (3) how conception can be accurately predicted by luteal blood flow compared with CL size and P_4 concentrations.

2. Materials and methods

2.1. Farm and animals

The present study was conducted in a commercial dairy farm located in Miyagi Prefecture, Japan. Lactating Holstein-Friesian cows (n = 72; 13 primiparous and 59 multiparous) were housed in free stall facilities, fed complete rations (a mixture of forage and concentrate), and allowed free access to water and mineralized salt. All cows were milked twice daily, and daily milk production was 28.29 ± 2.42 kg/day (mean ± standard error of the mean). All cows were transferred with embryos recovered from JB throughout the year. Sixty-five cows (4.21 ± 0.27 year old, 2.50 ± 0.23 lactation number) were used in the present study. These cows had normal estrous cycles and were greater than or equal to 50 days postpartum. Body condition scores (BCS) were recorded on Day 0 (estrus = Day 0) by the same person using a scale of 1 to 5 in increments of 0.25 [21]. The mean BCS of the cows was 3.50 ± 0.08. The experimental design of this study was approved by the ethical review board of the Miyagi Prefectural Federated Agricultural Mutual Aid Association.

2.2. Estrus synchronization

The estrus of cows in the spontaneous estrus group (n = 34) was detected without synchronization. Cows in the synchronized group (n = 31) were treated with the following protocol for fixed-time ET according to previous research [20]: 2 mg of estradiol benzoate