J. Dairy Sci. 96:1–12
http://dx.doi.org/10.3168/jds.2013-6814

Color Doppler flow imaging for the early detection of nonpregnant cattle at 20 days after timed artificial insemination

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ABSTRACT

The objective was to determine the accuracy of a pregnancy test for predicting nonpregnant cattle based on the evaluation of corpus luteum (CL) blood flow at 20 d (CLBF-d20) after timed artificial insemination (TAI). Crossbred Holstein-Gir dairy heifers (n = 209) and lactating cows (n = 317) were synchronized for TAI using the following protocol: intravaginal implant (1.0 g of progesterone) and 2 mg of estradiol benzoate i.m. on d −10, implant removal and 0.526 mg of sodium cloprostenol i.m. on d −2, 1 mg of estradiol benzoate i.m. on d −1, and TAI on d 0. On d 20, animals underwent grayscale ultrasonography (US) to locate the CL and color flow Doppler to evaluate CLBF-d20 using a portable ultrasound equipped with a 7.5-MHz rectal transducer. Based only on a visual, subjective CLBF evaluation, the animals were classified as pregnant or not pregnant. On d 30 to 35, blinded from results of the previous diagnosis, the same operator performed a final pregnancy diagnosis using US to visualize the fetal heartbeat (gold standard; US-d30). A second evaluator also analyzed the CLBF-d20 in the same animals by watching 7-s recorded videos. Blood samples were collected from a subset of 171 females to determine, by RIA, plasma progesterone (P_4) concentrations, which indicate CL function. The final pregnancy outcome (US-d30) was retrospectively compared with the CLBF-d20 diagnoses and then classified either as correct or incorrect. The number of true positive, true negative, false positive, and false negative decisions were inserted into a 2 × 2 decision matrix. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the CLBF-d20 test were calculated using specific equations. Binomial variables (pregnancy rate and proportions) were analyzed using Fisher's exact test for the effect of parity and to compare between evaluators and tests (CLBF-d20 vs. plasma P_4). The kappa values were calculated to quantify the agreement between CLBF-d20 and the gold standard (US-d30) and between evaluators. The performance parameters of CLBF-d20 test were as follows: sensitivity = 99.0%, specificity = 53.7%, positive predictive value = 65.1%, negative predictive value = 98.5%, and accuracy = 74.8%. False negatives represented only 0.4% of the exams. No differences existed in these parameters between evaluators (no. 1 vs. no. 2) and tests (CLBF-d20 vs. plasma P_4). Moreover, a high level of agreement was observed between evaluators (0.91). In conclusion, visual evaluation of CLBF-d20 represents a quick, reliable, and consistent diagnostic test that enables the early detection of nonpregnant cattle.
Key words: corpus luteum, blood flow, luteolysis, pregnancy

INTRODUCTION

Pregnancy diagnosis has long been a routine activity in the management of cattle reproduction (Cowie, 1948; cited by Fricke and Lamb, 2005). Its primary purpose is to detect, as early as possible, animals that have failed to conceive, determine the cause of pregnancy failure, and to determine whether to rebreed (Fricke and Lamb, 2005) or cull such animals. The early diagnosis and rapid rebreeding of nonpregnant animals reduces interinsemination intervals (Stevenson, 2005) and is undoubtedly part of the strategy used to improve reproductive performance (Fricke, 2002). Thus, it may be especially advantageous if protocols for timed AI (**TAI**) are used (Fricke et al., 2003).

There is a long history of attempts to diagnose pregnancy in cattle at an early stage (Ghannam and Sorensen, 1967; Ludwick and Rader, 1968). Nevertheless, practical and reliable methods to generate a definitive diagnosis using ultrasonography at early stages of pregnancy (<25 d) remain of limited accuracy (**ACC**; based

Received March 14, 2013.
Accepted July 1, 2013.
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on predictive values) or may even be unavailable currently. Although previous reports have demonstrated that it is possible to successfully diagnose pregnancy as early as <20 d after breeding or AI using conventional grayscale ultrasonography (**US**; Kastelic et al., 1988, 1989, 1991; Pieterse et al., 1990), the reliabilities and accuracies of scans at early stages (d 21 to 25) are quite low (Pieterse et al., 1990; Quintela et al., 2012) most likely due to the inability to clearly visualize the embryo, which is commonly found close to the uterine wall during that stage of pregnancy (Quintela et al., 2012). Additionally, the possibility of confusing intrauterine estrus mucus with pregnancy remains (Pierson and Ginther, 1984; Kastelic and Ginther, 1989; Pieterse et al., 1990; Kastelic et al., 1991; Quintela et al., 2012). Consequently, pregnancy diagnosis by scanning the uterus to visualize the embryonic vesicle is of limited ACC, shows a relatively high incidence of misdiagnosis, and is not typically adopted in practice before 26 to 28 d of gestation (Pieterse et al., 1990; Romano et al., 2006; Quintela et al., 2012). The incorporation of new technologies in addition to the grayscale B-mode US, such as Doppler ultrasound, enables a more detailed assessment of the uterus, ovarian follicles, and corpora lutea. Color-flow mode (**CFM**) permits visualization of blood flow within tissues and structures based on the principles of the Doppler effect (Singh et al., 2003; Ginther, 2007; Matsui and Miyamoto, 2009) and indirectly enables inferences to be made on the functional status of the tissue (Herzog et al., 2010).

The establishment and maintenance of pregnancy in cattle is dependent on the presence of a functional, active corpus luteum (**CL**) and the production of a sufficient level of progesterone (**P_4**; Mann and Lamming, 1999; Lucy, 2001; Parr et al., 2012). During luteolysis, the loss of luteal function (P_4 secretion) is associated with a progressive decrease in blood flow in response to $PGF_{2\alpha}$ (Niswender et al., 2000; Schams and Berisha, 2004). Morphological changes caused by luteal regression, however, only become evident later (Niswender et al., 1994; Siqueira et al., 2009). Thus, the usefulness of conventional B-mode US in evaluating CL function during the time of luteolysis is limited by the temporal asynchrony between functional and morphological regression (Kastelic et al., 1990; Assey et al., 1993; Siqueira et al., 2009). The use of color Doppler ultrasound can overcome these limitations because it enables the real-time assessment of CL blood flow (**CLBF**), which reflects the functionality of the gland indirectly, particularly by the end of the estrous cycle (Miyamoto et al., 2006; Herzog et al., 2010).

Interestingly, although it has been suggested that color Doppler flow imaging could be useful for a more accurate early diagnosis of pregnancy in cattle (Quintela et al., 2012), particularly if performed at 19 to 21 d after AI (Matsui and Miyamoto, 2009), results on the use of CLBF evaluations to determine pregnancy status are controversial. Recent studies reported that the evaluation of CLBF results alone was insufficient for the early diagnosis of pregnancy in bovine embryo transfer recipients due to low specificity (**Sp**) and sensitivity (**Se**; Utt et al., 2009) and was not a reliable diagnostic method for pregnancy in lactating dairy cows due to a high variation between animals (Herzog et al., 2011). However, this apparent inconsistency of CLBF may be due to differences in Doppler ultrasound settings, in the criteria used for data analysis, or in the time points used for CLBF evaluations relative to AI. A study of the performance of a CLBF-based pregnancy diagnosis test in a large TAI program, in which a great number of cows are inseminated and checked for pregnancy at a later time point may be an optimal strategy by which to address these questions on the potential for the use of color Doppler imaging in the routine reproductive management on dairy farms.

Thus, the objective of this study was to determine the ACC of a pregnancy diagnostic test, based only on the visual evaluation of CLBF using color Doppler flow imaging, on predicting nonpregnant animals at 20 d after TAI. We hypothesized that a single, subjective CLBF evaluation using color Doppler imaging after the expected luteolysis (d 20; **CLBF-d20**) would accurately detect the majority of animals that failed to conceive, thus enabling their early resynchronization.

MATERIALS AND METHODS

Animals, Experimental Design, and Estrus Synchronization

Holstein-Gir crossbred nulliparous heifers (20 to 22 mo and >330 kg of BW; n = 209) and multiparous lactating dairy cows (>3 yr old; n = 317), which were primarily Holstein (blood share of 77.0 ± 18.1%), with a mean BCS of 2.75 ± 0.56 (range: 2.0 to 4.0; scale 1 to 5; Edmonson et al., 1989) were enrolled in this study and subjected to 5 rounds of TAI. Lactating cows were >45 d DIM and their lactation records indicated an average production of 4,125 ± 641 kg of milk over a 305-d period, which was estimated using the highest milk yield during one of the previous lactations from each cow in the study. Cattle were maintained using a mixed land-based production system at the Embrapa Dairy Cattle Research Center located in Coronel Pacheco, Minas Gerais, Brazil. Animals were maintained on a pasture overnight and fed maize silage and grain ration as supplements during the day with ad libitum access to water, salt, and mineral mixture.