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Early pregnancy diagnosis on days 18 to 21 postinsemination using high-resolution imaging in lactating dairy cows

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ABSTRACT

The aim was to assess the ability of corpus luteum (CL) and uterine ultrasound characteristics on d 18 to 21 to predict pregnancy status in lactating dairy cows. Ultrasound examinations were carried out on cows (n = 164) on d 18 to 21 following artificial insemination (AI). Images of the uterus and CL were captured using a Voluson *i* ultrasound device (General Electric Healthcare Systems, Vienna, Austria) equipped with a 12-MHz, multi frequency, linear array probe. Serum concentrations of progesterone were determined from blood samples collected at each ultrasound examination. Images of the CL were captured and stored for calculation of CL tissue area and echotexture. Images of the CL and associated blood flow area were captured and stored for analysis of luteal blood flow ratio. Longitudinal B-mode images of the uterine horns were stored for analysis of echotexture. Diagnosis of pregnancy was made at each ultrasound examination based on CL blood flow, CL size, and uterine echotexture. Pregnancy was confirmed by ultrasonography on d 30 after AI. The relationship between ultrasound measures and pregnancy outcome, as well as the accuracy of the pregnancy diagnosis made at each ultrasound examination was assessed. Progesterone concentrations and CL tissue area were greater in pregnant compared with nonpregnant cows on all days. The CL blood flow ratio was higher in pregnant compared with nonpregnant cows on d 20 and 21 after AI. Echotexture measures of the CL and uterus were not different between pregnant and nonpregnant cows on any day of examination. The best logistic regression model to predict pregnancy included scores for CL blood flow, CL size, and uterine echotexture on d 21 following AI. Accuracy of pregnancy diagnosis was highest on d 21, with sensitivity and specificity being 97.6 and 97.5%, respectively. Uterine echotexture scores were similar for pregnant and nonpregnant cows from d 18 to 20. On d 21, pregnant cows had higher uterine echotexture scores compared with nonpregnant cows. The logistic regression equation most likely to provide a correct pregnancy diagnosis in lactating dairy cows included the visual score for CL blood flow, CL size, and uterine echotexture on d 21 after AI. In support of this finding, the diagnostic accuracy for visual scores of CL blood flow, CL size, and uterine echotexture were also highest on d 21.

Key words: uterus, corpus luteum, ultrasound, dairy cow, early pregnancy

INTRODUCTION

Pregnancy diagnosis is a routine tool used in the reproductive management of dairy cows (Fricke, 2002) to identify both pregnant and nonpregnant cows (Pierson and Ginther, 1984; Kastelic et al., 1988). Since the 1950s, fertility in dairy cows has declined in association with intensive selection for increased milk production (Butler, 2003). Within the same timeframe, a decline in the duration and intensity of estrous activity has been observed (Lopez et al., 2004; Dobson et al., 2008). The result is poor estrous detection and higher culling rates (Stevenson et al., 2003). Currently, the average estrous detection rates in Ireland are approximately 70% (Mee, 2004; Dillon et al., 2006). Furthermore, the rate of early embryo loss in both moderate- and high-yielding dairy cows is estimated to be between 40 and 56%, respectively (Diskin and Morris, 2008). Over half of this loss is estimated to occur before d 16 after breeding (Dunne et al., 2000; Diskin and Morris, 2008), with some research suggesting greater loss occurring before d 8 in high-yielding cows (Sartori et al., 2010).

It is important that all eligible cows are inseminated soon after the end of the voluntary waiting period to maintain reproductive efficiency (Dewey et al., 2010). The early identification of nonpregnant cows has the potential to have a direct effect on the calving to conception interval (Stevenson et al., 2003). Furthermore, a high cost benefit has been associated with early pregnancy detection of dairy cows (Oltenacu et al., 1990). Blood tests are now available for pregnancy diagnosis through the detection of pregnancy-associated glycoproteins (Silva et al., 2007); however, the test has a 2-d

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wait period and requires cows to be approximately 25 to 30 d postinsemination for accurate results (Green et al., 2005, 2009). Rapid pregnancy-associated glycoproteins tests have revealed a high degree of accuracy in both cows and heifers that are at least 25 d post-AI (Silva et al., 2007; Green et al., 2009).

The process of pregnancy recognition and implantation involves major remodeling of the endometrium, in particular the endometrial glands (Gray et al., 2001; Spencer et al., 2007). Ultrasound may have the ability to monitor several uterine characteristics associated with increased endometrial glandular secretions, including the presence of edema (Gajewski et al., 1999) and intrauterine fluid (Pierson and Ginther, 1987; Kastelic et al., 1991). Ultrasound examinations of the uterus have noted a change in the amount of uterine fluid, along with the number of grey zones just before ovulation in cattle (Pierson and Ginther, 1987). Furthermore, changes in uterine echotexture from d 18 to 21 have been noted in cyclic animals (Kastelic et al., 1989).

Most commercial ultrasound machines operate between 5 and 7.5 MHz. Studies have not used probes more powerful than 7.5 MHz since research first began on the use of ultrasound for pregnancy diagnosis in commercial dairy herds (Kastelic et al., 1989, 1991; Pieterse et al., 1990; Romano et al., 2006). Ultrasound characteristics of the uterus and corpus luteum (**CL**) have been assessed as possible markers for early pregnancy diagnosis. In isolation, these markers do not appear to perform well (Kastelic et al., 1989; Badtram et al., 1991; Herzog et al., 2011). In practice, pregnancy diagnosis involves examination of the entire reproductive tract. Even when an embryonic heartbeat is detected, it is recommended that the ovaries be assessed for presence of a CL (López-Gatius and García-Ispierto, 2010).

The overall aim of the current study was to assess the ability of ultrasound measures of CL and uterine characteristics from d 18 to 21 following AI in dairy cows to predict pregnancy status. Specifically, we addressed questions of (1) whether changes in uterine and CL characteristics from d 18 to 21 were different in pregnant and nonpregnant dairy cows; (2) if image analysis data could predict pregnancy status in dairy cows using logistic regression; and (3) how accurate pregnancy can be predicted by a visual diagnosis using scores for CL size, CL blood flow, and uterine echotexture for pregnancy diagnosis.

MATERIALS AND METHODS

Animals and Treatments

All experimental procedures involving animals were licensed by the Department of Health and Children, Ireland. Protocols were in accord with the Cruelty to Animals Act (Ireland 1876) and the European Community Directive 86/609/EC and were sanctioned by the Institutional Animal Research Ethics Committee in University College Dublin.

Dairy cows (n = 164) of varying age, stage of lactation, and parity were AI following detected estrus. A total of 344 ultrasound examinations were carried out from d 18 to 21. Ultrasound examinations were carried out during one autumn (November to December 2012, n = 22 cows scanned) and one spring (May to June 2012, n = 142 cows scanned) breeding season. Jersey, Holstein-Jersey, and Holstein-Friesian cows were used. All cows were milked twice daily (0700 and 1600 h).

Cows in the autumn-calving group were housed indoors in a cubicle house with slatted passageways for the duration of the experiment. Cows were offered a diet that consisted of 50:50 maize silage (DM = 344 g/kg, CP = 76 g/kg, and ME = 11.8 MJ/kg of DM), grass silage (DM = 239 g/kg, CP =101 g/kg, and ME = 11.1 MJ/kg of DM) ad libitum, plus 8 kg of concentrates (DM = 883 g/kg, CP = 281 g/kg, and ME = 12.9 MJ/kg of DM) per day at milking. Cow in the spring-calving group were fed a pasture-based diet with a daily DM allowance of 14 kg supplemented with 8 kg of concentrate per day at milking.

BW and BCS

Body weight and BCS were recorded weekly. Body condition score was assessed by the same person on a scale of 1 to 5 (with 1 being extremely thin and 5 being extremely fat) with increments of 0.25 (Lowman et al., 1976). The mean ± SEM weights of cows in the pregnant and nonpregnant groups were 521.97 ± 11.39 and 517.42 ± 11.05 kg ($P = 0.77$), respectively. The mean ± SEM BCS of cows in the pregnant and nonpregnant groups were 2.76 ± 2.6 and 2.77 ± 2.75 ($P = 0.79$), respectively.

Progesterone Assay

Blood samples were collected by coccygeal venipuncture into Vacutainers (Becton Dickinson, Plymouth, UK) at each ultrasound examination for assay of progesterone (**P4**) concentrations. Following collection, blood samples were refrigerated (4°C) for 12 to 24 h before being centrifuged at 1,500 × g for 20 min at 4°C. Concentrations of P4 were measured using a commercially available solid-phase RIA (Coat-A-Count Progesterone, Diagnostic Products Corp., Los Angeles, CA) as previously described and validated (Forde et al., 2011). Intraassay CV were 12.6, 5.8, and 8.0% for serum pools containing low, medium, and high P4