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Using Doppler ultrasonography on day 34 of pregnancy to predict pregnancy loss in lactating dairy cattle

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ABSTRACT

The objective of this experiment was to determine whether uterine or ovarian vascular dynamics could be used to identify cows at risk for pregnancy loss. Our hypothesis was that cows that subsequently lose their pregnancy will have decreased corpus luteal (CL) perfusion, or an increased resistance index (RI; reduced blood flow), or both, at d 34 of pregnancy. Day 34 was chosen because it is a common time for dairy cattle to be checked for pregnancy. This experiment was performed in 2 replicates from November 2011 to April 2012 (n = 69) and from November 2012 to April 2013 (n = 53). Cows were bred via timed artificial insemination using Ovsynch-56 and checked for pregnancy on d 32 after artificial insemination. At d 34, cows confirmed pregnant were examined via transrectal Doppler ultrasonography. Blood samples collected via coccygeal vein were used to measure circulating plasma progesterone concentrations. Diameter of the corpus luteum and crown-rump length were measured. Color power Doppler ultrasonography was used to determine vascular perfusion to the CL, and RI was measured for the uterine arteries just after branching from the umbilical artery. Records were later examined to identify pregnancy status of cows after reconfirmation. Abortion rate did not differ between replicates (11.6% in replicate 1, 9.4% in replicate 2). Mean crown-rump length of embryos that were carried to term was greater on d 34 than that in cows that aborted (14.23 ± 0.27 vs. 13.21 ± 0.53 mm). Circulating progesterone concentration at d 34 was greater for cows that carried pregnancies to term than for those that aborted (9.1 ± 0.7 vs. 7.5 ± 1.0 ng/mL). The final logistic regression model consisted of crown-rump length, progesterone concentration, and RI of the uterine artery contralateral to pregnancy. Decreased crown-rump length and progesterone concentration tended to be associated with increased odds ratio for pregnancy loss, whereas CL perfusion and uterine blood flow were not associated with increased odds ratio of pregnancy loss. In conclusion, examining CL perfusion and RI of the uterine arteries on d 34 of pregnancy does not offer a method to identify lactating Dairy cattle at risk for pregnancy loss after d 34.
Key words: dairy cattle, pregnancy loss, Doppler ultrasonography

INTRODUCTION

Dairy producers face several challenges that affect financial returns, one of which is reproductive efficiency (Meadows et al., 2005). Methods have been developed to maximize the number of cows and heifers bred (Johnson and Funston, 2013; Wiltbank and Pursley, 2014); however, early and late embryonic and fetal losses remain a challenge. An estimated 60% of pregnancies are lost after conception in lactating dairy cows (Santos et al., 2004), and an estimated 12% of pregnancies fail after d 42 (Santos et al., 2004). Developing methods to identify cows at risk for pregnancy loss can provide opportunities to develop intervention strategies that will mitigate these losses.

Various indicators of pregnancy status may be used to predict pregnancy loss in cattle. Ultrasound-detected embryonic and fetal abnormalities are strong predictors of impending pregnancy failure in dairy cattle (Gábor et al., 2016). Also, circulating concentrations of pregnancy-associated glycoproteins may be used to predict pregnancy outcomes in cattle (López-Gatius et al., 2007; Thompson et al., 2010). Indicators of corpus luteum (**CL**) function may also predict pregnancy maintenance or loss in cattle. Size of the CL does not predict pregnancy maintenance or loss, but a positive association exists between the presence of 2 CL and pregnancy maintenance (López-Gatius et al., 2002; Starbuck et al., 2004). Also, circulating progesterone concentration is a predictor of pregnancy loss (Starbuck et al., 2004; López-Gatius et al., 2007). There is

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a positive correlation between CL blood flow, as evaluated via Doppler ultrasonography, and progesterone concentrations in cyclic and pregnant cattle examined up to d 40 of pregnancy (Utt et al., 2009; Herzog et al., 2010). It is not known whether CL blood flow can be used to predict pregnancy outcomes in cattle. However, such an association exists in humans. A positive correlation exists between CL blood perfusion index and circulating progesterone concentrations in women during the first 12 wk of pregnancy (Guerriero et al., 1999), and there is an increase in resistance index (**RI**) to the CL (i.e., reduced blood perfusion) in women with a threatened miscarriage between 6 and 12 wk of gestation compared with women with a normal pregnancy (Salim et al., 1994).

Resistance index is used in human obstetrics to evaluate pregnancies. It is used for similar purposes in livestock and, especially, large domesticated animals (Ginther, 2007). Differences in RI of the uterine artery ipsilateral to the conceptus pregnancy compared with the contralateral uterine artery are noted in pregnant cattle as early as 60 d of pregnancy in cows (Bollwein et al., 2002). Vascular changes in the endometrium have been examined using color Doppler ultrasonography, and differences in the amount of perfusion of the uterine endometrium in pregnant heifers compared with nonpregnant heifers are evident as early as d 16 of pregnancy (Silva and Ginther, 2009). There is a paucity of data regarding early changes in uterine blood flow and pregnancy maintenance in cattle, but increased impedance of the uterine artery (i.e., reduced blood flow rate) in women has been associated with recurrent pregnancy loss (Ferreira et al., 2007).

The objective of this project was to discern whether uterine or ovarian vascular dynamics would identify cows at risk for pregnancy loss. The hypothesis was that cows that abort after d 34 would have decreased CL perfusion or increased RI (suggestive of decreased uterine blood flow), or both, at d 34 of pregnancy.

MATERIALS AND METHODS

Experimental Design

This experiment was performed in 2 replicates from November 2011 to April 2012 (n = 69) and from November 2012 to April 2013 (n = 53) at the University of Florida Dairy Unit (Hague). First-service lactating Holstein cows (2 to 12 yr of age, mean = 2.2 yr) received timed AI using the Ovsynch-56 protocol. Pregnancy was diagnosed at d 32 post-AI by transrectal ultrasound. The presence of an embryo with a heartbeat was the criterion used to determine pregnancy. Cows diagnosed pregnant were re-examined by transrectal palpation of uterine contents at 46, 75, and 210 d of gestation to reconfirm pregnancy and to identify abortion. Records were later examined to identify cows that aborted and approximately when the abortion occurred, as well as DIM, milk yield, BCS, and BW.

Ultrasonography

At d 34, cows confirmed pregnant were examined via transrectal Doppler ultrasonography (Micromaxx; Sonosite, Bothell, WA) using a 5- to 10-MHz broadband 52-mm linear array (Micromaxx; Sonosite). Crown-rump length and diameter (average of height and width) of the CL were measured. Images of vascular perfusion to the CL were completed with color power Doppler ultrasonography at the maximum diameter of the CL, with the probe oriented from the caudal to cranial ovarian poles and recorded to digital video. The percent of the CL being perfused with blood was measured using digital videos by comparing the CL area containing blood flow versus the total CL area, as previously described by Kelley et al. (2009). The RI was measured for the uterine artery ipsilateral and contralateral to the uterine horn containing a pregnancy (Bollwein et al., 2000).

Blood Collection and Progesterone Analysis

Blood samples were collected on d 34 via coccygeal venipuncture into blood collection tubes containing EDTA (Vacutainer, 10 mL; Becton, Dickinson and Company, Franklin Lakes, NJ). Samples were placed on ice until plasma was isolated by centrifugation (5,000 $\times$ *g* for 15 min at 4°C). Plasma was frozen at −20°C until progesterone concentration was determined using Coat-A-Count solid-phase I^{125} RIA kits (DPC Diagnostic Products Inc., Los Angeles, CA; Cooke et al., 2009). Intra- and interassay coefficients of variation were 2.73% and 3.95%, respectively, and assay sensitivity was 0.1 ng/mL.

Statistical Analyses

Abortion rates between replicates were compared using the SAS FREQ (version 9.4; SAS Institute Inc., Cary, NC) procedure involving a Chi-squared statistic. Continuous data were normally distributed as assessed by a Kolmogorov-Smirnov test. Initially, RI, CL perfusion, CL diameter, crown-rump length, and plasma progesterone concentrations were analyzed as dependent variables using the SAS MIXED procedure. Independent variables used in the model were DIM, milk yield, parity (primiparous vs. multiparous), BCS, BW, pregnancy loss, and pregnancy loss × parity. Replicate was modeled as a covariate. A backward elimination