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Characterization of luteal dynamics in lactating Holstein cows for 32 days after synchronization of ovulation and timed artificial insemination

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ABSTRACT

Approximately 20 to 30% of cows diagnosed not pregnant 32 d after timed artificial insemination (TAI) lack a corpus luteum (CL), and cows submitted to a resynchronization protocol in the absence of a CL have about 10% fewer pregnancies per AI (P/AI) than cows with a CL. An understanding of luteal dynamics after synchronization of ovulation and TAI may help refine strategies for reinseminating cows failing to conceive. Lactating Holstein cows (n = 141) were synchronized for first TAI using a Double-Ovsynch protocol. Thrice weekly from 4 to 32 d after TAI, blood samples were collected for evaluation of plasma progesterone (P4) concentrations, and CL diameter was measured using transrectal ultrasonography. Pregnancy status was determined using transrectal ultrasonography 32 d after TAI. Nonsynchronized cows (n = 4) were removed from the study. For cows diagnosed pregnant 32 d after TAI (n = 57), P4 increased from 4 to 15 d and then remained constant until 32 d after TAI, whereas CL volume increased from 4 to 11 d and then remained constant until 32 d after TAI. For cows diagnosed not pregnant 32 d after TAI (n = 80), P4 profiles were evaluated using statistical cluster analysis based on the day after TAI that P4 decreased to <1 ng/mL, resulting in 5 clusters: (1) CL regression 15 d after TAI (1.3%), (2) CL regression 18 to 22 d after TAI (55.0%), (3) CL regression 25 to 27 d after TAI (17.5%), (4) CL regression 29 to 32 d after TAI (5.0%), and (5) CL maintained until 32 d after TAI (21.3%). Plasma pregnancy-associated glycoprotein (PAG) levels at 25 and 32 d after TAI differed among clusters and were below the cut-off value of the assay for the classification of cows as not pregnant for cows in clusters 2, 3, and 4, whereas more than half of the cows in cluster 5 had increased plasma PAG levels. We conclude that at least half of the nonpregnant cows that maintained their CL until 32 d after TAI were initially pregnant but underwent early pregnancy loss based on increased plasma PAG levels at 25 and 32 d after TAI.
Key words: corpus luteum, progesterone, pregnancy loss, pregnancy-associated glycoproteins

INTRODUCTION

The use of fertility programs for synchronization of ovulation and timed AI (**TAI**) has increased service rate and pregnancies per AI (**P/AI**) in high-producing lactating dairy cows at first service (Fricke et al., 2015; Santos et al., 2016b). Nonetheless, 50 to 70% of cows fail to conceive after first service (Brusveen et al., 2009; Carvalho et al., 2014; Stevenson et al., 2014). Because of poor detection and expression of estrus in high-producing dairy cows (Lopez et al., 2004), coupling a nonpregnancy diagnosis with a management strategy to rapidly resubmit cows to AI increases reproductive efficiency by decreasing the interval between AI services (Fricke, 2002; Fricke et al., 2003, 2016).

A common reproductive management strategy to decrease the interval between AI services is weekly submission of nonpregnant cows to an Ovsynch protocol for resynchronization of ovulation (**Resynch**) and TAI (Caraviello et al., 2006; Fricke et al., 2014; Santos et al., 2016a). A key factor limiting fertility during the Ovsynch protocol is the response to each of the sequential hormonal treatments (Giordano et al., 2012b; Carvalho et al., 2015b; Fricke et al., 2015). In this regard, initiation of an Ovsynch protocol during early diestrus (d 5–9) results in more P/AI than initiation of an Ovsynch protocol at other stages of the estrous cycle (Vasconcelos et al., 1999). Lactating dairy cows have an estrous cycle length of approximately 23 d (Sartori et al., 2004); therefore, initiation of a Resynch protocol 32 d after AI (i.e., d 9 of the estrous cycle) should result in more P/AI than initiation of a Resynch protocol

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25 d (i.e., d 2 of the estrous cycle) or 39 d (i.e., d 16 of the estrous cycle) after AI. Several studies have compared initiation of a Resynch protocol at different intervals after AI and reported no differences in P/AI for cows initiating a Resynch protocol 25 versus 32 d after insemination (Fricke et al., 2003; Silva et al., 2009; Bruno et al., 2014) or 32 versus 39 d after insemination (Bilby et al., 2013; Lopes et al., 2013). A consistent and intriguing observation in all of these studies is that the proportion of cows without a corpus luteum (**CL**) or with low progesterone (**P4**) concentrations at initiation of a Resynch protocol does not differ between cows initiating the Resynch protocol at 25 versus 32 d (Fricke et al., 2003; Silva et al., 2009; Bruno et al., 2014) or at 32 versus 39 d (Bilby et al., 2013; Lopes et al., 2013) after insemination and ranges from 20 to 30% of the cows diagnosed not pregnant.

An analysis of 42,252 cows in 159 herds across England and Wales reported that the distribution of interservice intervals in lactating dairy cows is longer than the expected 18 to 24 d, with a significant proportion of cows having extended interservice intervals (Remnant et al., 2015). Longer interservice intervals, particularly in high-producing dairy cows, may result from successful conception and maintenance of the CL followed by early pregnancy loss and luteal regression, thereby delaying return to estrus (Diskin et al., 2011). Pregnancy-associated glycoproteins (**PAG**) belong to a large family of aspartic proteinases expressed by the placenta of domestic ruminants including cows, ewes, and goats (Haugejorden et al., 2006; Wallace et al., 2015), and assays for detecting PAG levels in blood and milk have been developed and commercialized to determine pregnancy status in cattle (Ricci et al., 2015). Further, circulating PAG levels are dramatically affected by pregnancy loss early during pregnancy and have been used to define pregnancy loss in dairy cows (Giordano et al., 2012; Ricci et al., 2015).

A better understanding of luteal dynamics after synchronization of ovulation and TAI may help refine strategies for reinseminating cows failing to conceive. Thus, the objective of our study was to evaluate P4 concentrations and luteal dynamics from 4 to 32 d after TAI and PAG levels at 25 and 32 d after TAI. Our hypothesis was that luteal regression after TAI among cows diagnosed nonpregnant would not be synchronous and that some cows would have extended luteal phases and increased plasma PAG levels.

MATERIALS AND METHODS

All animal handling procedures were approved by the Animal Care and Use Committee of the College of Agricultural and Life Sciences at the University of Wisconsin–Madison.

Synchronization of Ovulation and Timed AI

Cows were housed at the University of Wisconsin–Madison Dairy Cattle Research Center (Arlington, WI) in loose housing with headlocks. Cows were fed ad libitum a TMR formulated to meet or exceed NRC (2001) requirements for high-producing dairy cows. Primiparous cows were housed separately from multiparous cows. The rolling herd average was 13,880 kg and average daily milk production was 41.6 kg/cow per day with 3.8% fat and 3.2% protein for the herd during the study period.

Lactating Holstein cows (n = 141; 41 primiparous and 100 multiparous) from 53 ± 3 DIM were synchronized for first TAI using a Double-Ovsynch protocol (Souza et al., 2008). Prostaglandin $F_{2\alpha}$ (25 mg/dose of dinoprost tromethamine; Lutalyse) and GnRH (100 μg/dose of gonadorelin hydrochloride; Factrel) were from Zoetis (Madison, NJ). Briefly, cows received the first GnRH treatment of the Presynch portion of the Double-Ovsynch protocol at 53 ± 3 DIM, followed by treatment with $PGF_{2\alpha}$ 7 d later and a GnRH treatment 72 h after $PGF_{2\alpha}$. Seven days later, cows received an Ovsynch-56 protocol (GnRH at 70 ± 3 DIM, $PGF_{2\alpha}$ 7 d later, GnRH 56 h after $PGF_{2\alpha}$, and AI 16–20 h later), and all cows received a TAI at 80 ± 3 DIM. Three experienced AI technicians performed all inseminations using sires with high genetic merit and proven fertility.

Blood Sampling, P4, and PAG Assays

Blood samples were collected thrice weekly (Monday, Wednesday, and Friday) from 4 to 32 d after TAI (Figure 1) for analysis of plasma P4 concentrations and at 25 and 32 d after TAI for evaluation of plasma PAG levels. Samples were collected by venipuncture of the median coccygeal artery or vein into 10-mL evacuated plasma collection tubes (Vacutainer; BD, Franklin Lakes, NJ). Blood samples were immediately placed on ice and were centrifuged (1,600 × *g*; 4°C) for 20 min, and plasma was harvested and stored at −20°C in 2-mL Safe-Lock tubes (Eppendorf AG, Hamburg, Germany).

Plasma P4 concentrations were assayed using a solid-phase, no-extraction RIA (Coat-a-Count; Diagnostic Products Corp., Los Angeles, CA). The average sensitivity for the 3 assays was 0.027 ng/mL. The average intra-assay coefficient of variation was 5.61%, and the interassay coefficient of variation was 6.91% based on a quality control sample (2.50 ng/mL of P4) that was replicated within each assay.