



Concentrations of nonesterified fatty acids and glucose in blood of periparturient dairy cows are indicative of pregnancy success at first insemination

H. A. Garverick,^{*1} M. N. Harris,* R. Vogel-Bluel,* J. D. Sampson,* J. Bader,* W. R. Lamberson,* J. N. Spain,* M. C. Lucy,* and R. S. Youngquist†

^{*}Department of Animal Sciences, and

[†]Department of Veterinary Medicine and Surgery, University of Missouri, Columbia 65211

ABSTRACT

Greater blood concentrations of nonesterified fatty acids (NEFA) and lesser blood concentrations of glucose are indicative of the normal process of nutrient partitioning that occurs in early postpartum dairy cows. The objective was to determine the relationship between blood NEFA and glucose concentrations and subsequent conception at first insemination in postpartum dairy cows. Holstein ($n = 148$) and Guernsey ($n = 8$) dairy cows were blood sampled at approximately d 10, 7, and 3 prepartum, on the day of calving and 3, 7, 14, and 21 d postpartum for measurement of NEFA and glucose concentrations. Serum and plasma were harvested and used for measurement of NEFA and glucose concentrations, respectively. Cows were given a presynchronization treatment (2 injections of PGF_{2α} 14 d apart) with the second PGF_{2α} injection occurring 14 d before the initiation of the timed AI (TAI) protocol. Blood for determination of progesterone concentrations was collected at each presynchronization injection and at the initiation of the TAI protocol that was used for first insemination (74 ± 7 d postpartum). Cows were considered noncycling if serum progesterone concentrations at the 2 presynchronization PGF_{2α} injections (d 37 and 51 ± 7 postpartum) and at the initiation of the TAI protocol (d 65 ± 7 postpartum) were ≤ 1 ng/mL, and there was no indication of ovulation or presence of a corpus luteum by ultrasound examination at the initiation of the TAI protocol. Pregnancy was determined at 33 d and again at 61 d after first insemination by using ultrasound. Across all days, serum NEFA and plasma glucose concentrations were not different between cows that ovulated before the initiation of the TAI program (cycling) compared with those that did not ovulate (noncycling). Serum NEFA concentrations,

however, were less and plasma glucose concentrations were greater during the early postpartum period for cows that subsequently became pregnant at first insemination compared with those that failed to become pregnant. Logistic regressions were used to predict the probability of pregnancy based on NEFA and glucose concentrations from individual days. The prediction with the greatest likelihood ratio was for d 3 postpartum NEFA and glucose concentrations. Nutritional status during the early postpartum period (within 1 wk after calving), as indicated by blood NEFA and glucose concentrations, may affect subsequent fertility by a mechanism that is independent from interval to first ovulation.

Key words: nonesterified fatty acid, glucose, periparturient, fertility

INTRODUCTION

Pregnancy per AI (**P/AI**) in lactating dairy cows has decreased relative to those recorded more than 50 years ago (Butler and Smith, 1989; Stevenson et al., 2008). Changes in the physiology and management of dairy cattle seem to contribute to the decrease in fertility (Royal et al., 2002; Chagas et al., 2007). A tremendous demand exists for nutrients during lactation and the associated severity of negative energy balance during early lactation has been one mechanistic explanation for lower fertility in dairy cows (Beam and Butler, 1999; Butler, 2000). Cows with greater NEFA are presumably mobilizing more adipose tissue to support milk production and are losing more body condition compared with cows with lesser NEFA (Beever, 2006). Cows with NEFA and BHBA concentrations in blood above a critical threshold can be predicted to have a greater incidence of diseases such as ketosis and displaced abomasum (Ospina et al., 2010a). These diseases were associated with reduced postpartum reproductive performance (Ospina et al., 2010c). In addition to fat mobilization in early postpartum cows,

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¹Corresponding author: garverickh@missouri.edu

liver gluconeogenesis increases to provide glucose for milk lactose synthesis (Bauman and Currie, 1980). The large demand for glucose may decrease the amount of glucose available to other tissues in the body, including those that are involved in postpartum reproduction (Wathes et al., 2011; Green et al., 2012).

Follicles that are growing shortly after calving reach ovulatory size when cows are 60 to 90 d postpartum (approximate time of first insemination; Webb et al., 2003). The integrity of follicles and their resident oocytes may be compromised in postpartum cows because extreme concentrations of circulating hormones and metabolites can potentially affect their function (Britt, 1994; Leroy et al., 2008a,b). At the same time, the uterus is undergoing involution and repair, a process that is controlled in part by immune cells that are suppressed in postpartum cows (LeBlanc, 2010). Cows that develop metritis have lesser DMI during the prepartum and postpartum periods, and metritis is a risk factor for infertility in dairy cows (Gumen et al., 2011). Nutritional events that occur during the periparturient and postpartum periods, therefore, can alter ovarian, oviductal, and uterine function and potentially affect subsequent fertility in the postpartum cow. We hypothesized that cows that failed to cycle or failed to conceive to first insemination would have postpartum blood NEFA and glucose concentrations that indicated a greater severity of negative energy balance. The objective of this study, therefore, was to assess blood NEFA and glucose relative to the cycling and first service pregnancy status of the cow.

MATERIALS AND METHODS

Animals and Feeding

The experimental procedures were approved by the University of Missouri Animal Care and Use Committee (reference no. 3899). Holstein ($n = 148$) and Guernsey ($n = 8$) cows at the University of Missouri Foremost Dairy Research Center (Columbia) were used in the study. The cows were first or greater parity and were deemed suitable for postpartum breeding by the examining veterinarian (R. S. Youngquist). Data were collected during a 2-yr period from December 1 through June 20 of each year. The Foremost farm consists of approximately 225 cows with an annual rolling herd average in excess of 10,000 kg of milk. Daily milk weights were available for 70 cows during the first year of the study. Body condition scores (1 = thin to 5 = fat; Wildman et al., 1982) were determined at parturition and at first breeding postpartum. Cows were housed in a freestall barn with access to an exercise lot be-

fore calving and fed a TMR that was formulated for late pregnant, nonlactating cows (dry cow diet). After calving, cows were housed in a freestall barn and fed a lactating cow TMR. The TMR was formulated to meet or exceed NRC (2001) recommendations. Feed was provided twice daily; once at approximately 0800 h and a second time at 1600 h. After calving, cows were milked twice daily at 0400 and 1600 h. The experiment began 10 d before expected calving and continued until a second pregnancy exam at 61 d after first AI.

Blood Collection Schedule and Assays for NEFA and Glucose

Blood samples were collected on d 10, 7, and 3 before expected calving (and every 3 d until calving), on the day of calving and on d 3, 7, 14, and 21 after calving by puncture of the medial caudal coccygeal vein or artery. For plasma (glucose determination), blood was collected into EDTA-coated tubes, cooled on ice, driven to the laboratory, centrifuged at $1500 \times g$, and plasma was stored frozen at -20°C (total time 2 to 4 h from collection to freezing). Blood glucose concentrations were measured for cows on the first year of the study ($n = 89$). For serum (NEFA determination), blood was collected into noncoated tubes, allowed to clot overnight, and processed for -20°C storage using the procedures described for plasma. Plasma glucose concentrations were determined enzymatically with the glucose oxidase method (Thermo Fisher Scientific, Waltham, MA). Absorbance was quantified using a Beckman DU-65 spectrophotometer (Beckman Instruments, Brea, CA). Serum NEFA concentrations were determined using a NEFA C kit (Wako Diagnostics, Richmond, VA). Colorimetric development was quantified on a Tecan rainbow plate reader (Tecan US Inc., Durham, NC).

Timed AI Program

All cows were enrolled in a Presynch Ovsynch (or Presynch Cosynch) protocol. At 37 ± 7 d postpartum, cows were injected with $\text{PGF}_{2\alpha}$ (Lutalyse; 25 mg of dinoprost tromethamine, i.m.; Pfizer Animal Health, New York, NY) and a second injection of $\text{PGF}_{2\alpha}$ was given 14 d later (51 ± 7 d postpartum). Fourteen days later (65 ± 7 d postpartum) the Ovsynch-Cosynch protocol was initiated. Cows were given the first injection of GnRH (100 μg , OvaCyst; IVX Animal Health Inc., St. Joseph, MO) followed 7 d later with another injection of $\text{PGF}_{2\alpha}$ (72 ± 7 d postpartum). Noncycling cows (absence of a corpus luteum or no indication of ovulation by transrectal ultrasonography (Aloka 500V with a 5.0 MHz

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