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The Effect of Subclinical Ketosis in Early Lactation on Reproductive Performance of Postpartum Dairy Cows

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ABSTRACT

Data generated from 796 Holstein cows enrolled in a clinical trial to investigate the health effect of a monensin controlled release capsule were analyzed to investigate the association between circulating serum β-hydroxybutyrate (BHBA) concentration in the periparturient period and subsequent reproductive performance. Overall, accounting for both repeated measures within cow and clustering at the herd level, non-pregnant cows after first insemination tended to have increased circulating BHBA concentrations from 3 wk before calving to 9 wk after calving relative to pregnant cows. Including the interaction between the week of sample collection and pregnancy outcome, non-pregnant cows had higher circulating BHBA concentrations in the second week after calving than cows diagnosed pregnant after first artificial insemination. Within individual weeks, cows with circulating BHBA concentrations ≥1,000 μmol/L in the first week postpartum were less likely to be diagnosed pregnant after first insemination. In the second week postpartum, the cows with circulating BHBA concentrations ≥1,400 μmol/L were significantly less likely to be pregnant after first artificial insemination. A dose response relationship was found when a comparison of the probability of pregnancy after first insemination and duration of elevated circulating ketone bodies was investigated. The probability of pregnancy was reduced by 20% in cows diagnosed subclinically ketotic in either the first or second week postpartum. Nevertheless, cows above the subclinical ketosis threshold in both the first and second week postpartum were 50% less likely to be pregnant after first insemination. Similarly, the median time to pregnancy increased in cows experiencing elevated BHBA concentrations in either (124 d) or both (130 d) the first and second week postpartum relative to cows never experiencing elevated BHBA concentrations (108 d). To further investigate this, the effect of elevated circulating BHBA was permitted to vary with time. The effect decreased with time, such that the daily probability of pregnancy increased similar to nonsubclinically ketotic cows by approximately 160 d in milk. From this analysis, both the relative circulating concentration of BHBA and the duration of elevated circulating BHBA were negatively associated with the probability of pregnancy at first service.
Key words: subclinical ketosis, β-hydroxybutyrate, time to pregnancy

INTRODUCTION

The concept of homeorhesis or prioritization of energy distribution to meet specific physiological demands was refined in 1980 (Bauman and Currie, 1980). Partitioning of available energy to milk production early in lactation, at the expense of reproduction, has formed a conceptual framework to address the effect of negative energy balance on future reproductive performance, as measured by the time to establishment of luteal activity, probability of pregnancy after first AI [pregnancy risk (**PR**)/AI], and time to conception.

Cows experiencing delayed onset of luteal activity postpartum typically have lower DMI, produce less milk, and subsequently lose more body condition early in lactation (Staples et al., 1990). At the whole-animal level, the magnitude and duration of negative energy balance are influenced by feed intake and milk production. At the organ and molecular level, variation in energy utilization efficiency, ranging from rumen function to the ability of the liver to repartition mobilized fat, affect energy balance status.

Previous research has reported an association between calculated negative energy balance and reproductive traits, including days to first luteal activity, first service conception risk, days from calving to AI, and days from calving to conception (Villa-Godoy et al., 1988; Staples et al., 1990; Reist et al., 2003b). The physiological state of energy deficiency impairs hypothalamic responsiveness to circulating estradiol-17β, resulting in reduced GnRH pulse frequency and con-

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comitant reduction in LH required for follicular deviation and eventual ovulation (Jolly et al., 1995; Dawuda et al., 2002; Butler, 2003).

Energy balance is dependent on many factors. Various metabolic and endocrine blood and milk measures such as NEFA, ketone bodies, glucose, insulin, milk fat, or fat:protein ratio are indirect measures of energy balance. Circulating ketones increase when the maximal ability of the liver to oxidize NEFA and store triacylglycerol is exceeded. Associations between elevated circulating ketone concentration and periparturient uterine disease were described by Reist et al. (2003a); however, investigation of thresholds was based on the distribution of circulating ketone concentrations or anecdotal experience rather than the associated change in the risk of disease (Duffield et al., 1998). Similarly, the effect of postpartum circulating ketone concentration on PR/AI, time to commencement of luteal activity, and time to pregnancy has been described later in lactation with variable results (Andersson and Emanuelson, 1984; Cook et al., 2001). A circulating BHBA concentration threshold immediately postpartum, defined by demonstrable reduction in the PR/AI, has not been validated.

The objectives of this retrospective analysis were to investigate relationships between the magnitude and duration of increased serum BHBA measured at –3 wk and in each of the first, second, third, sixth, and ninth weeks postpartum and the PR/AI, the time from calving to first AI, and time from calving to pregnancy.

MATERIALS AND METHODS

Data were collected as part of a clinical trial to determine the effect of prepartum administration of monensin as a controlled release capsule (**CRC**) on postpartum health, energy balance, and reproductive performance (Duffield et al., 1998, 1999). Briefly, 25 Holstein herds around Guelph, Ontario, Canada, that were enrolled in milk recording through Ontario DHI were studied from March 1995 to December 1995. Herd size ranged from 25 to 160 lactating animals, with 305-d rolling herd average for milk production from 7,000 to 10,000 kg. Five herds were fed a TMR, and the remaining herds had a component feeding system. A technician visited each farm weekly at a consistent time of day. At each weekly visit, the technician administered the randomly assigned treatment (monensin CRC or placebo); recorded animal data including lactation, season of calving (winter: December through February; spring: March through May; summer: June through August; fall: September through November), and BCS (Ferguson et al., 1994; ≤2.5 thin; ≥2.75 to ≤3.5 fair; ≥3.75 fat); and collected blood samples. Each blood sample was collected from a coccygeal blood vessel into a 10-mL vacuum tube (Monoject red stopper blood collection tubes; Sherwood Medical, St. Louis, MO). Blood was collected at the time of monensin CRC or placebo oral administration 3 wk before calving and at wk 1, 2, 3, 6, and 9 postpartum. Blood samples were stored, on ice, in an insulated cooler from time of collection until sample processing occurred. Within 5 h postcollection, blood samples were centrifuged at $733 \times g$ for 10 min. The samples of serum were submitted to the Clinical Pathology Laboratory (Department of Pathobiology, Ontario Veterinary College) for the measurement of Ca, P, total protein, urea, glucose, and BHBA concentrations and determination of aspartate aminotransferase activity using an automated analyzer (Dacos 2 analyzer; Coulter Electronics, Hialeah, FL).

Peripartum disease information including dystocia (veterinary-assisted parturition), retained placenta (failure to pass the fetal membranes within 24 h), milk fever (veterinary diagnosed), metritis (inflammation of the uterus <15 DIM), clinical ketosis, displaced abomasum (left or right displacement diagnosed by a veterinarian), respiratory illness, and lameness was captured in on-farm data sheets, veterinary records, and on-farm computer record systems (Duffield et al., 1999). Reproductive data, including DIM at first insemination, conception date, number of inseminations, herd removal date, and pregnancy status at removal, were recorded using the same methods. There was minimal use of ovulation synchronization protocols. Pregnancy diagnosis was performed at least 40 d after insemination by rectal palpation. Five herds had first lactation animals excluded, because they had been exposed to a monensin premix within 4 wk of parturition.

Data Management and Statistical Analysis

Statistical analysis was performed using Intercooled Stata 9.1 (StataCorp LP, College Station, TX) and SAS (SAS Inst. Inc., Cary, NC). Descriptive statistics were used to characterize animals diagnosed pregnant after first insemination, relative to animals diagnosed not pregnant. Data were collected as part of a clinical trial; therefore, treatment remained in all analyses.

The shape of the BHBA curve between weeks was assessed using PROC MIXED in SAS (SAS Institute, 2004), accounting for the random effect of herd and repeated measures of cows within herd. After transforming the BHBA data into the natural logarithm scale, variables offered to the model included treatment, parity, periparturient disease, the pregnancy status diagnosed after first AI, and all 2-way interactions. Residual analysis was performed at the cow level. The distribution of residuals was symmetrical with long