Livestock Science 141 (2011) 123–128

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

High NEFA concentrations around parturition are associated with delayed ovulations in grazing dairy cows

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ARTICLE INFO

Article history:
Received 10 March 2011
Received in revised form 12 May 2011
Accepted 17 May 2011

Keywords:
NEFA
Metabolic status
Peripartum
Resumption of ovulation
Delayed ovulation
Grazing dairy cows

ABSTRACT

The objectives of this study were to assess indicators of metabolic status of grazing dairy cows around parturition, and the relationship between these indicators with the resumption of ovulations postpartum (ROP). Holstein multiparous cows ($N=20$) grazing on improved pastures and supplemented with concentrates were body condition scored and tail bled weekly from wk −2 through +9 related to parturition. Plasma samples were analyzed for non-esterified fatty acids (NEFA), leptin, insulin-like growth factor-1 (IGF-1) and progesterone (P_4). Data were analyzed with mixed models, logistic regression, with receiver operator characteristic (ROC), and Cox regression analysis. Cows having Delayed Ovulation ([DO], ROP on week ≥5) had lower BCS, and higher NEFAs than cows having a normal ROP around parturition (BCS: 2.73 ± 0.08 vs. 2.94 ± 0.05, $P<0.05$, and NEFA: 0.43 ± 0.04 vs. 0.35 ± 0.02 mM, $P<0.10$, respectively). Also, DO cows had lower BCS than normal herdmates (2.59 ± 0.10 vs. 2.99 ± 0.06, $P<0.01$) around time of ROP, but they had similar NEFA, leptin and IGF-1. The risk for DO increased as NEFA increased (0.4% and 0.5% per every increasing mM of NEFA in prepartum and postpartum, respectively). The ROC curve showed that NEFA (prepartum and postpartum) had areas of 0.85 and 0.80, and cut-off values of 0.39 and 0.47 mM. Finally, hazard for ROP increased as prepartum IGF-1 increased, and it decreased as postpartum NEFA increased. In conclusion, cows with lower BCS and higher prepartum and postpartum NEFA had higher odds for getting DO than herdmates with greater BCS and lower NEFA concentrations.
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1. Introduction

During postpartum period, resumption of ovulation (ROP) in dairy cows is essential to achieve the goal of calving interval <380 days. The fact that, the sooner a cow starts to cycle postpartum, the higher is the fertility achieved during the breeding period has long been recognized (Thatcher and Wilcox, 1973). In this sense, longer intervals to ROP are associated with reduced fertility due to prolonged calving intervals (McCoy et al., 2006). Cows having their ROP by 21 days have higher hazard for pregnancy than herdmates having their ROP by 35–42 days (Galvão et al., 2010). Thus, every day delay in the interval to ROP is linked to a delay of 0.41 days in the interval to conception (Darwash et al., 1997). Recently, Delayed Ovulation (DO) has been defined based on the measurement of its impact on reproductive performance (Gautam et al., 2010). According to them, a ROP after 35 days was significantly associated with lower risk for pregnancy and longer calving to pregnancy intervals. The preoptic–hypothalamic continuum is involved in nutritional sensing,

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doi:10.1016/j.livsci.2011.05.007

control of appetite, and control of secretion of gonadotropins (GnRH pulse generator, Chagas et al., 2007). The main factor to modulate the GnRH pulse generator is energy balance (EB, Beam and Butler, 1997, 1998; Canfield et al., 1990; Canfield and Butler, 1990, 1991). The ROP occurs approximately 19 d after EB nadir (Beam and Butler, 1999), and the lower the EB nadir the longer is the interval between parturition and ROP (de Vries and Veerkamp, 2000). As EB is difficult to assess under farm conditions, some indirect estimators have been tested (Clark et al., 2005). Among them, body condition score (BCS) is considered a long term (static) EB estimator; whereas, metabolic hormones like insulin, insulin-like growth factor-1 (IGF-1), leptin, and metabolites such as non-esterified fatty acids (NEFA), beta-hydroxybutyrate, and glucose are considered short term (dynamic) EB estimators (Chilliard et al., 1998). All these indicators of EB have also been postulated as possible signals linking EB to GnRH pulse generator and thus, to ROP (Beam and Butler, 1999; Diskin et al., 2003; Wathes et al., 2003), but despite of all the progress made on this field in the last decades the physiological pathways for that link remain unknown (Armstrong et al., 2003; Chagas et al., 2007). Dairy cows under pastoral conditions have been reported to lose weight and BCS during dry period and also to have high prepartum NEFA concentrations (Cavestany et al., 2005, 2009a,b; Meikle et al., 2004). Therefore, it is expected that grazing dairy cows with lower BCS, higher fat mobilization (i.e.: higher NEFA concentrations), and lower IGF-1 concentrations may suffer from a longer interval to ROP and DO. The objectives of this study were to assess: 1) metabolic indicators of EB around parturition and around time of ROP as possible signals to reproductive centers in grazing dairy cows, 2) the relationship between these indicators on the week before and after parturition with the likelihood for having Delayed Ovulation (DO), and 3) the ability of these indicators to predict risk for having DO.

2. Materials and methods

2.1. Animals & treatments

The study was conducted in the herd of the experimental dairy farm of the National University of La Plata located in Buenos Aires Province (35°36′ S, 60°32′ W, Argentina). Multiparous Holstein cows ($N=20$) calving between February 15th and April 15th 2007 were enrolled in the study. Dry cows were grazing on improved pastures (mixture of legumes and grasses) and were given 3 kg/d of corn silage at 1600 h. Lactating dairy cows were rotationally grazed on improved pastures, and were given 6 kg/d of corn silage after afternoon milking and 4 kg of a commercial concentrate (17% crude protein, and 1.7 Mcal net energy of lactation/kg) equally distributed twice daily in the milking parlor (0400 and 1600 h).

2.2. Sampling

Weekly scoring and sampling were performed from 2 wk prepartum through 9 wk postpartum. Cows were body condition scored (5-point scale, Edmonson et al., 1989 modified by Ferguson et al., 1994) and tail bled between 1400 and 1500 h during the prepartum period and between 1600 and 1800 h during the postpartum period (5–10 min before afternoon milking). Blood samples were collected in 10 ml polystyrene vials containing 20 mg Na_2EDTA and kept in ice bath during sampling. Plasma was harvested within 2 h post sampling and stored at −20 °C until analysis for metabolites and hormones. Milk yield at first monthly milk check (MY1) was obtained from dairy records. ROP was checked by weekly blood P_4 determination. A concentration higher than 3.18 nM (1 ng/ml) was considered as indicator of corpus luteum presence and thus of ROP (Harrison et al., 1990; Senatore et al., 1996). Diagnostic criterion: cows having their ROP on week ≥5 were considered as having DO (Gautam et al., 2010).

2.3. Laboratory analysis

Blood plasma samples were analyzed for NEFA with a commercial kit (NEFA-HR(2), Wako Chemicals, Richmond, VA 23237, USA). Intra- and inter- assay coefficients of variation were 5.7 and 7.8% respectively. The P_4 radioimmunoassay (RIA) was performed with an antibody provided by Dr. GD Niswender, and a labeled hormone (Progesterone [1,2,6,7 3 H(N)], Dupont NEN, Boston, Massachussets, USA). Assay sensitivity was 0.16 pmol/tube, and intra- and inter-assay coefficients of variance were 7.5 and 11.9%, respectively (Díaz-Torga et al., 2001). The IGF-1 RIA (Díaz-Torga et al., 2001) was performed with the IGF-1 antibody (UB2-495, Hormone Distribution Program, NIDDK) after acid-ethanol extraction. Minimum detectable concentration was 0.33 nM. Intra- and inter-assay coefficients of variation were 7.2 and 9.1%. Leptin RIA was performed by a double antibody method with ovine-specific antiserum (Delavaud et al., 2002) and recombinant bovine leptin (DS Labs, Webster, Texas, USA) iodinated in our laboratory (Becu-Villalobos et al., 2007). The minimum detectable concentration of leptin was 0.02 nM and intra- and inter -assay coefficients of variation were 6.7 and 9.0% respectively.

2.3.1. Statistical analysis

Data are shown as LSM ± SEM unless otherwise stated. Statistical significance was set at $P<0.05$, and a trend for significance was set at $P<0.10$. Only effects with $P<0.1$ were discussed. Cows having their ROP during postpartum week ≥5 were classified as DO. The cow was the experimental unit. The PROC MIXED of SAS 9.1 (SAS, 2003) was used to assess changes of metabolic indicators during study period (Model 1) and around time of ROP (Model 2). Response variables (BCS, NEFA, leptin, IGF-1) were analyzed as repeated measures (week related to parturition in Model 1 or week related to ROP in Model 2) with cow as a random effect and week, DO (yes vs. no) and their interaction as fixed effects. The slice statement was used to test the effect of DO for each level of week. A polynomial contrast was used to test the linear, quadratic and cubic effect of week. Another contrast was used to compare prepartum vs. postpartum values in model 1. The two mixed models for repeated measures were defined as: $Y_{ijk} = I + W_i + DO_j + (W^*DO)_{ij} + S_k + e_{ijk}$, where Y_{ijk} is the observed value of studied parameter, I is model intercept, W_i is the effect of time (week related to parturition: −2,…,9 [Model 1], and week related to ovulation: −3,…,3

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