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

Mauricio Javier Giuliodori [a], Carole Delavaud [b], Yves Chilliard [b], Damasia Becú-Villalobos [c], Isabel Lacau-Mengido [c], Rodolfo Luzbel de la Sota [d,*]

[a] Cátedra de Fisiología, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina
[b] INRA, UR1213 Herbivores, F-63122 Saint Genès Champanelle, France
[c] Instituto de Biología y Medicina Experimental, Conicet, Ciudad Autónoma de Buenos Aires, Argentina
[d] Cátedra y Servicio de Reproducción Animal, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina

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.

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,

* Corresponding author at: Cátedra y Servicio de Reproducción, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. Calle 60 y 118. La Plata. B1900AVW, Argentina. Tel.: +54 221 4236663; fax: +54 221 4257980.
E-mail address: luzbel@fcv.unlp.edu.ar (R.L. de la Sota).

1871-1413/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
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

[Model 2]), DO_j is the effect of DO (j: 1 = no, 2 = yes), $(W^*DO)_{ij}$ is the interaction term, S_k is the random effect of the cow (k = 1,...,20), and e_{ijkl} is the random error term. The PROC MIXED of SAS 9.1 (SAS, 2003) was also used to assess the effect of DO on milk yield at 1st milk check (MY1). The third mixed model was defined as: $Y_{ij} = I + DO_i + S_j + e_{ij}$, where Y_{ij} is the observed value of MY1, I is model intercept, DO_j is the effect of DO (j: no vs. yes), S_j is the random effect of the cow (k = 1,...,20), and e_{ij} is the random error term. The covariance structure having the smallest Akaike's information criterion and Schwarz's Bayesian criterion was used (Littell et al., 2002). The PROC LOGISTIC of SAS 9.1 (SAS, 2003) was used to test the effect of BCS, NEFA, IGF-1 and leptin concentrations from week before and from week after parturition (Prepartum and postpartum models, respectively) on the likelihood for getting DO. The logistic models were defined as Logit $(\pi) = I + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4$ where π is the log of the odds of the event (i.e.: DO); I is the intercept; b_1 through b_4 are the parameters of the covariates X_1 through X_4 representing the values of BCS, NEFA, Leptin and IGF-1 on the week before and after parturition. Postpartum model also included the effect of BCS change during study period and of MY1 as covariates. Modeling was performed using manual backward elimination with an exclusion criteria set at $P>0.15$. The continuous variables that remained in the logistic models were evaluated with receiver operator characteristic (ROC) analysis to determine a critical threshold for predicting late cyclers by using SIGMAPLOT 10.0 (Systat, 2006) with a pre-test probability of 0.25 and a cost ratio of 1. The ROC curves analyze sensitivity vs. 1 − specificity. Sensitivity was the proportion of animals diagnosed as late cyclers that were above a given threshold, and specificity was the proportion of animals diagnosed as early cyclers that were below a given threshold (Greiner et al., 2000). The critical threshold was the point on the ROC curve that had the highest combined sensitivity and specificity. Interpretation of critical threshold was based on the area under the curve (AUC). Swets (1988) suggested that the test is non-informative if the AUC is 0.5; that it is accurate if AUC is 0.5–0.7; that it is very accurate if AUC is 0.7–0.9; that it is highly accurate if AUC is 0.9–1.0; and finally that it is perfect if AUC is 1. Likelihood ratio positive (LR+) was the probability that a test result at or above the threshold would be more likely to come from an animal later diagnosed with the event (i.e.: DO). The PROC PHREG of SAS 9.1 (SAS, 2003) was used to test the effect of BCS, NEFA, IGF-1 and leptin concentrations from the week before and from the week after parturition (Prepartum and postpartum models, respectively) on the hazard for ROP. The Cox's proportional hazard ratio model was defined as $\text{Log } h(t) = \text{Log } h_0(t) + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4$ where Log h(t) is the log of the hazard of the event (cycling) at time t; Log $h_0(t)$ is the log of baseline hazard when all the variables are zero (intercept); b_1 through b_4 are the parameters of the covariates X_1 through X_4 representing the values of BCS, NEFA, Leptin and IGF-1 on the week before and after parturition.

3. Results

Changes of metabolic indicators during study period (Model 1) are shown in left panel of Fig. 1. The ROP was observed on days 20.5 ± 1.7 and 42.0 ± 3.13 for normal ($N = 15$) and DO cows ($N = 5$), respectively. Week had a significant effect ($P<0.01$) on BCS, IGF-1, and NEFA, and it also had a linear effect ($P<0.01$) on BCS and NEFA. In addition, prepartum BCS was higher than postpartum BCS (3.19 ± 0.04 vs. 2.87 ± 0.02, $P<0.01$), and prepartum IGF-1 concentrations were higher than postpartum IGF-1 (31.67 ± 1.65 vs. 23.47 ± 0.98 nM, $P<0.01$). The DO cows had lower BCS, and higher NEFA concentrations than normal herdmates (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). Cows lost BCS during both prepartum and postpartum periods (total decrease approximately 0.6 of a unit of BCS). Plasma NEFA concentrations increased prepartum to peak values at parturition, and then decreased up to the end of study period. Plasma leptin concentrations were relatively constant over the study period. Plasma IGF-1 concentrations decreased prepartum and fluctuated postpartum. Changes of metabolic indicators around ROP (Model 2) are shown in right panel of Fig. 1. Week had a linear effect ($P<0.01$) on BCS and NEFA. DO cows had lower BCS than normal herdmates (2.59 ± 0.10 vs. 2.99 ± 0.06, $P<0.01$). On the week of ROP, DO cows had lower BCS than normal cows (2.60 ± 0.12 vs. 2.90 ± 0.07, $P<0.05$), but similar NEFA (0.34 ± 0.07 vs. 0.37 ± 0.05 mM, $P = 0.70$), leptin (0.17 ± 0.05 vs. 0.19 ± 0.03 nM, $P = 0.81$), and IGF-1 concentrations (21.30 ± 4.92 vs. 19.56 ± 3.11 nM, early vs. late Cycler, $P = 0.77$). The MY1 was similar for DO and normal cows (21.40 ± 2.47 vs. 20.92 ± 1.59 kg/d, $P = 0.87$).

In logistic regression analysis, NEFA concentration was the only predictor variable remaining in both models. In the prepartum model, the adjusted odds ratio (AOR) for NEFA was 1.004 (95% CI = 0.999–1.010, $P = 0.099$), while in the postpartum model the AOR was 1.005 (95% CI = 0.998–1.012, $P = 0.088$). Hence, the risk for DO increased 0.4% and 0.5% per every increasing μM of NEFA in prepartum and postpartum, respectively ([AOR − 1]*100 was used to transform AOR into percentage; Allison, 1995). Consequently, the risk for DO was increased 40–50% per every 100 μM (0.1 mM) of increase in NEFA concentration around parturition. The ROC curve analysis showed for prepartum and postpartum NEFA that AUCs were 0.85 and 0.80, cut-off values were 0.39 and 0.47 mM and LR + were approximately 3, so that DO cows were 3 times more likely to come from cows having NEFA concentrations above cut-off values (Table 1 and Fig. 2).

In Cox regression analysis, the only predictors remaining in the models were prepartum IGF-1 (Hazard Rate, HR = 1.022, 95% CI = 0.996–1.048, $P = 0.097$) and postpartum NEFA (HR = 0.990, 95% CI = 0.978–1.001, $P = 0.084$). Hence, the hazard for ROP increased 2.2% per unit of increase in IGF-1 (ng/ml) and decreased 1% per μM of increase in NEFA ([HR − 1]* 100 was used to transform HR into percentage; Allison, 1995).

4. Discussion

The present study focused on the assessment of metabolic indicators of EB around parturition and around time of ROP, and the relationship between those indicators and interval to ROP and risk for DO in grazing dairy cows. The main findings of this study were, firstly, that DO cows had lower BCS and higher NEFA around parturition than normal herdmates, and also they had lower BCS around time of ROP than normal cows; secondly, that risk for DO increased as NEFAs (pre- and

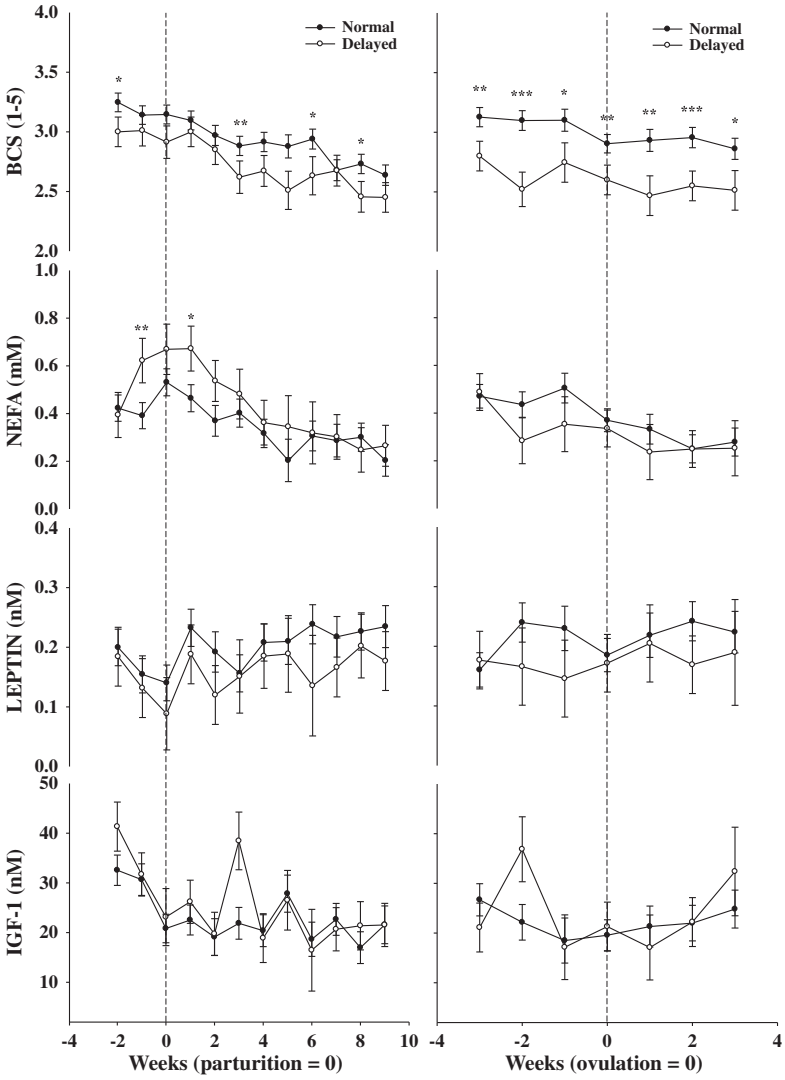

Fig. 1. LSM ± SEM values for BCS and for plasma NEFA, IGF1 and leptin concentrations from weeks related to parturition (left panel) and related to resumption of ovulation postpartum (ROP, right panel) in grazing dairy cows. $^{*}P<0.10$, $^{**}P<0.05$, and $^{***}P<0.01$.

Table 1
Receiver operator characteristic (ROC) curve analysis for metabolic indicators (prepartum and postpartum) used as predictor variables for delayed ovulation in grazing dairy cows.

	ROC curve analysis					
	ROC[a]	Cut-off[b]	Se[c]	Sp[d]	LR+[e]	*P*
NEFA prepartum	0.85 ± 0.1	0.39	1.00	0.67	3.00	0.039
NEFA postpartum	0.80 ± 0.1	0.47	1.00	0.64	2.75	0.089

Delayed ovulation (DO) was defined as a cow having her first postpartum progesterone rise (indicative of ovulation) on week ≥5.

[a] ROC: area under the curve of receiver operating characteristic curve (X ± SE).

[b] Cut-off: mM of NEFA.

[c] Se: sensitivity (proportion of cows diagnosed as DO that were above the NEFA threshold).

[d] Sp: specificity (proportion of cows did not diagnose as DO that were below the NEFA threshold).

[e] LR+: likelihood ratio positive. The area difference between the two NEFA tests (prepartum NEFA vs. postpartum NEFA) is 0.05 ± 0.17, Chi squared = 0.11, degrees of freedom = 1, $P = 0.735$.

post-partum) increased, and thirdly, that hazard for ROP increased as prepartum IGF-1 increased while that hazard decreased as postpartum NEFA increased. So, from the blood metabolites pattern of our cows, we suggest that NEFA may play a role linking EB and reproduction. In fact, a direct action of NEFA on hypothalamic energy centers has already been demonstrated in rats (Lam et al., 2005). The NEFAs enter the hypothalamic neurons by simple diffusion and are esterified to fatty acil-CoA, so that neuronal pool size of fatty acil-CoA is proportional to plasma NEFA concentration. Then, an increase in neuronal pool size can deactivate neural pathways that drive food intake, while a decrease in that pool size can activate pathways stimulating food intake (Lam et al., 2005). Therefore, according to plasma NEFA profile of our cows it could be expected that a similar mechanism linking NEFA and reproductive centers could be working. Consequently, NEFA concentrations above a certain level would be interpreted as a negative input by hypothalamic centers leading to a depression

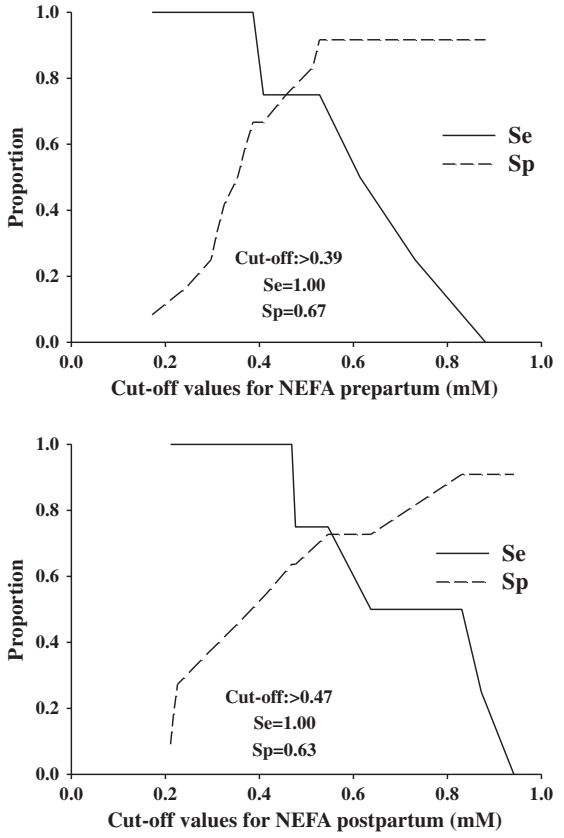

Fig. 2. Sensitivity (Se) and specificity (Sp) of plasma NEFA on the weeks before (top panel) and after parturition (bottom panel) as predictive test of delayed ovulation in grazing dairy cows.

on GnRH pulse generator and thus delaying ROP. That is, a NEFA threshold could exist, above which reproductive centers are inhibited leading to delayed ROP, and below which centers are released and therefore, ROP shortly after parturition is possible. According to ROC analysis and keeping in mind the small sample size, NEFA threshold would be approximately 0.39 mM for one week prepartum week approximately 0.47 mM for one week postpartum. The hypothesis of a NEFA threshold could be supported because DO cows and normal herdmates ovulated at similar NEFA (and also leptin and IGF-1) concentrations. So, it seems that NEFA should decrease below a critical level for ovulation to take place. That threshold could be reached shorter after parturition by cows having high BCS and low NEFA around parturition (indicative of better EB) than their herdmates. Therefore, cows having high NEFA around parturition (indicative of poor EB) would take longer interval to ROP and suffer from DO. To be cautious, it is possible that some of the effects attributed to NEFA could have been caused by insulin that regulates lipolysis (and NEFA concentration, Grummer, 1995) and was not measured in this study.

The positive effect found of prepartum IGF-1 on hazard for ROP could be explained by the stimulatory actions of IGF-1 at all levels of the hypothalamic-pituitary-gonadal axis demonstrated in vivo and in vitro (Daftary and Gore, 2005; Diskin et al., 2003). Particularly, on bovine theca cells in vitro, IGF-1 increased the number of LH-binding sites and enhanced LH-induced production of P4 (Stewart et al., 1995). Finally, Francisco et al. (2003), by using multiple regressions, found that IGF-1 is a good predictor of days to ROP and of plasma P4 concentration.

The difference in BCS and NEFA (and indirectly in EB) found in our cows may be caused by a different level of dry matter intake (DMI) and not by a difference in milk yield because normal and DO cows had similar milk production at the start of lactation. It is well known that EB is the main determiner of ROP by controlling GnRH pulse generator and thus LH pulsatility (Beam and Butler, 1997; Canfield et al., 1990; Canfield and Butler, 1990, 1991). It has been proposed that many metabolic hormones and metabolites could signal EB to hypothalamic centers (Chagas et al., 2007). BCS, a measure of size of fat store available for mobilization, and NEFA, a measure of fat mobilization, are among them (Grummer, 1995). So, it is clear that BCS (and IGF-1) decreases and NEFA increases when EB becomes negative for a long period (Cissé et al., 1991). Therefore, a higher BCS would reflect a better EB during previous periods, and a lower NEFA concentration, around parturition, would reflect a better EB at that time, and both facts would result in an earlier ROP as was observed in our cows. Current evidence suggests that cows should calve down in a BCS of 2.75–3 and not lose more than 0.5 of a unit of BCS between calving and first service (Overton and Waldron, 2004).

The interest in NEFA monitoring at cow-level as a management tool has been growing (Ospina et al., 2010a) and its application seems promissory since high NEFA levels peripartum were shown to be related to detrimental effects on both, milk production and reproductive performance (Ospina et al., 2010b). In the last decade, many direct interesting effects of NEFA have been demonstrated. To cite some, high NEFA concentrations have a toxic effect on granulosa cells in vitro decreasing proliferation and steroidogenesis (Vanholder et al., 2004) that could lead to a delay in ROP. In addition, high NEFA (and GH) concentrations could antagonize insulin action and create a state of insulin resistance decreasing the sensitivity of the ovary to LH and FSH (Lucy, 2007) leading also to a delay in ROP. Anyway, irrespective of the mechanism linking peripartum fat mobilization with hypothalamic centers controlling reproduction, monitoring of NEFA on the week before and after parturition could help to predict the probability of DO in grazing dairy cows and deserves further attention. In this sense, according to our data, the risk for DO increased 40–50% per every 100 μM (0.1 mM) of increase in NEFA concentration around parturition and DO cows are 3 times more likely to come from cows having NEFA concentrations above cut-off values. In line with this, Bossaert et al. (2008) found that the proportion of multiparous cows which ovulated by 40 d postpartum was double for cows having lower prepartum NEFA concentrations. In another work, cows that ovulate their first dominant follicle postpartum had lower NEFA (<0.4 mM) than anovulatory herdmates (Kawashima et al., 2007).

5. Conclusion

In conclusion, cows having DO have lower BCS and higher fat mobilization peripartum. As NEFA concentration increases the risk for DO also increases and the hazard for ROP decreases. Finally, the hazard for ROP increases as prepartum IGF-1 increases.

Acknowledgements

The study was financed by a grant given to De La Sota RL (Proyecto de Incentivos, Univ. Nacional de La Plata No.: V11/134).

References

Allison, P.D., 1995. Survival analysis using SAS: a practical guide. SAS Institute Inc., Cary, NC.

Armstrong, D.G., Gong, J.G., Webb, R., 2003. Interactions between nutrition and ovarian activity in cattle: physiological, cellular and molecular mechanisms. Reproduction 61, 1–12 Suppl.

Beam, S.W., Butler, W.R., 1997. Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol. Reprod. 56, 133–142.

Beam, S.W., Butler, W.R., 1998. Energy balance, metabolic hormones, and early postpartum follicular development in dairy cows fed prilled lipid. J. Dairy Sci. 81, 121–131.

Beam, S.W., Butler, W.R., 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. J. Reprod. Fertil. 54, 411–424 Suppl..

Becu-Villalobos, D., Garcia-Tornadu, I., Shroeder, G., Salado, E.E., Gagliostro, G., Delavaud, C., Chilliard, Y., Lacau-Mengido, I.M., 2007. Effect of fat supplementation on leptin, insulin-like growth factor I, growth hormone, and insulin in cattle. Can. J. Vet. Res. 70, 218–225.

Bossaert, P., Leroy, J.L.M.R., De Vliegher, S., Opsomer, G., 2008. Interrelations between glucose-induced insulin response, metabolic indicators, and time of first ovulation in high-yielding dairy cows. J. Dairy Sci. 91, 3363–3371.

Canfield, R.W., Butler, R.W., 1990. Energy balance and pulsatile luteinizing hormone secretion in early postpartum dairy cows. Dom. Anim. Endocrinol. 7, 323–330.

Canfield, R.W., Butler, R.W., 1991. Energy balance, first ovulation and the effects of naloxone on LH secretion in early postpartum dairy cows. J. Anim. Sci. 69, 740–746.

Canfield, R.W., Sniffen, C.J., Butler, R.W., 1990. Effects of excess degradable protein on postpartum reproduction and energy balance in dairy cattle. J. Dairy Sci. 73, 2342–2349.

Cavestany, D., Blanc, J.E., Kulcsar, M., Uriarte, G., Chilibroste, P., Meikle, A., Febel, H., Ferraris, A., Krall, E., 2005. Studies of the transition cow under a pasture-based milk production system: metabolic profiles. J. Vet. Med. A 52, 1–7.

Cavestany, D., Kulcsar, M., Crespi, D., Chilliard, Y., La Manna, A., Balogh, O., Keresztes, M., Delavaud, C., Huszenicza, G., Meikle, A., 2009a. Effect of prepartum energetic supplementation on productive and reproductive characteristics, and metabolic and hormonal profiles in dairy cows under grazing conditions. Reprod Dom. Anim. 44, 663–671.

Cavestany, D., Vinoles, C., Crowe, M.A., La Manna, A., Mendoza, A., 2009b. Effect of prepartum diet on postpartum ovarian activity in Holstein cows in a pasture-based dairy system. Anim. Reprod. Sci. 114, 1–13.

Chagas, L.M., Bass, J.J., Blache, D., Burke, C.R., Kay, J.K., Lindsay, D.R., Lucy, M.C., Martin, G.B., Meier, S., Rhodes, F.M., Roche, J.R., Thatcher, W.W., Webb, R., 2007. Invited review: new perspectives on the roles of nutrition and metabolic priorities in the subfertility of high-producing dairy cows. J. Dairy Sci. 90, 4022–4032.

Chilliard, Y., Bocquier, F., Doreau, M., 1998. Digestive and metabolic adaptations of ruminants to undernutrition, and consequences on reproduction. Reprod. Nutr. Dev. 38, 131–152.

Cissé, M., Chilliard, Y., Coxam, V., Davicco, M.J., Rémond, B., 1991. Slow release somatotropin in dairy heifers and cows fed two levels of energy concentrate. 2. Plasma hormones and metabolites. J. Dairy Sci. 74, 1382–1394.

Clark, C.E.F., Fulkerson, W.J., Nandra, K.S., Barchia, I., MacMillan, K.L., 2005. The use of indicators to assess the degree of mobilization of body reserves in dairy cows in early lactation on a pasture-based diet. Livest. Prod. Sci. 94, 199–211.

Daftary, S.S., Gore, A.C., 2005. IGF-1 in the brain as a regulator of reproductive neuroendocrine function. Exp. Biol. Med. 230, 292–306.

Darwash, A.O., Lamming, G.E., Woolliams, J.A., 1997. The phenotypic associationbetween the interval to postpartum ovulation and traditional measures offertility in dairy cattle. Anim. Sci. 65, 9–16.

De Vries, M.J., Veerkamp, R.F., 2000. Energy balance of dairy cattle in relation to milk production variables and fertility. J. Dairy Sci. 83, 62–69.

Delavaud, C., Ferlay, A., Faulconnier, Y., Bocquier, F., Kann, G., Chilliard, Y., 2002. Plasma leptin concentration in adult cattle: effects of breed, adiposity, feeding level, and meal intake. J. Anim. Sci. 80, 1317–1328.

Díaz-Torga, G.S., Mejía, M.E., González-Iglesias, A., Formía, N., Becú-Villalobos, D., Lacau-Mengido, I.M., 2001. Metabolic cues for puberty onset in free grazing Holstein heifers naturally infected with nematodes. Theriogenology 56, 111–122.

Diskin, M.G., Mackey, D.R., Roche, J.F., Sreenan, J.M., 2003. Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle. Anim. Reprod. Sci. 78, 345–370.

Edmonson, A.J., Lean, I.J., Weaver, L.D., Farve, T., Webster, G., 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72, 68–78.

Ferguson, J.D., Galligan, D.T., Thomsen, N., 1994. Principal descriptors of body condition score in Holstein cows. J. Dairy Sci. 77, 2695–2703.

Francisco, C.C., Spicer, L.J., Payton, M.E., 2003. Predicting cholesterol, progesterone, and days to ovulation using postpartum metabolic and endocrine measures. J. Dairy Sci. 86, 2852–2863.

Galvão, K., Frajblat, M., Butler, W., Brittin, S., Guard, C., Gilbert, R., 2010. Effect ofearly postpartum ovulation on fertility in dairy cows. Reprod. Domest. Anim. 45, 207–211.

Gautam, G., Nakao, T., Yamada, K., Yoshida, C., 2010. Defining delayed resumption of ovarian activity postpartum and its impact on subsequent reproductive performance in Holstein cows. Theriogenology 73, 180–189.

Greiner, M., Pfeiffer, D., Smith, R.D., 2000. Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. Prev. Vet. Med. 45, 23–41.

Grummer, R.R., 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. J. Anim. Sci. 73, 2820–2833.

Harrison, R.O., Ford, S.P., Young, J.W., Conley, A.J., Freeman, A.E., 1990. Increased milk production versus reproductive and energy status of high producing dairy cows. J. Dairy Sci. 73, 2749–2758.

Kawashima, C., Sakaguchi, M., Suzuke, T., Sasamoto, Y., Takahashi, Y., Matsui, M., Miyamoto, A., 2007. Metabolic profiles in ovulatory and anovulatory primiparous dairy cows during the first follicular wave postpartum. J. Reprod. Dev. 53, 113–120.

Lam, T.K.T., Schwartz, G.J., Rossetti, L., 2005. Hypothalamic sensing of fatty acids. Nat. Neurosci. 8, 579–584.

Littell, R.C., Stroup, W.W., Freund, R.J., 2002. SAS for Linear Models, 4th ed. SAS Institute Inc., Cary, NC.

Lucy, M.C., 2007. Fertility in high-producing dairy cows: reasons for decline and corrective strategies for sustainable improvement. Soc Reprod Fertil Suppl. 64, 237–254.

McCoy, M.A., Lennox, S.D., Mayne, C.S., McCaughey, W.J., Edgar, H.W., Catney, D.C., Verner, M., Mackey, D.R., Gordon, A.W., 2006. Milk progesterone profiles and their relationships with fertility, production and disease in dairy cows in Northern Ireland. Anim. Sci. 82, 213–222.

Meikle, A., Kulcsar, M., Chilliard, Y., Febel, H., Delavaud, C., Cavestany, D., Chilibroste, P., 2004. Effects of parity and body condition at parturition on endocrine and reproductive parameters of the cow. Reproduction 127, 727–737.

Ospina, P.A., Nydam, D.V., Stokol, T., Overton, T.R., 2010a. Evaluation of nonesterified fatty acids and beta-hydroxybutyrate in transition dairy cattle in the northeastern United States: critical thresholds for prediction of clinical diseases. J. Dairy Sci. 93, 546–554.

Ospina, P.A., Nydam, D.V., Stokol, T., Overton, T.R., 2010b. Associations of elevated nonesterified fatty acids and beta-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. J. Dairy Sci. 93, 1596–1603.

Overton, T.R., Waldron, M.R., 2004. Nutritional management of transition dairy cows: strategies to optimize metabolic health. J. Dairy Sci. 87, E105–E119.

SAS Institute, 2003. SAS and STAT User's Guide, Release 9.1. SAS Institute Inc., Cary, NC.

Senatore, E.M., Butler, W.R., Oltenacu, P.A., 1996. Relationship between energy balance and postpartum ovarian activity and fertility in first lactation dairy cows. J. Anim. Sci. 62, 17–23.

Stewart, R.E., Spicer, L.J., Hamilton, T.D., Keefer, B.E., 1995. Effects of insulin-like growth factor I and insulin on proliferation and on basal luteinizing hormone induced steroidogenesis of bovine thecal cells: involvement of glucose and receptors for insulin-like growth factor I and luteinizing hormone. J. Anim. Sci. 73, 3719–3731.

Swets, J.A., 1988. Measuring the accuracy of diagnostic systems. Science 240, 1285–1293.

Systat., 2006. Sigmaplot User's Guide, version 10.0. Systat Software, Inc., Chicago, IL.

Thatcher, W.W., Wilcox, C.J., 1973. Postpartum estrus as an indicator of reproductive status in the dairy cow. J. Dairy Sci. 56, 608–615.

Vanholder, T., Leroy, J.L.M.R., Van Soom, A., Opsomer, G., Maes, D., Coryn, M., de Kruif, A., 2004. Effect of non-esterified fatty acids on bovine granulosa cell steroidogenesis and proliferation in vitro. Anim. Reprod. Sci. 87, 33–44.

Wathes, D.C., Taylor, V.J., Cheng, Z., Mann, G.E., 2003. Follicle growth, corpus luteum function and their effects on embryo development in postpartum dairy cows. Reproduction 31, 219–237 Suppl.