Short communication: Prepartum plasma insulin-like growth factor-I concentrations based on day of insemination are lower in cows developing postpartum diseases

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ABSTRACT

Because periparturient production diseases are prevalent in dairy cows, early recognition is crucial. Several studies reported metabolic variables as risk predictors for subsequent diseases. To improve on-farm testing and application of those methods, the sampling procedure should take into account variation in gestation length. Furthermore, additional variables indicating cows at risk of any production disease should be sought. Therefore, the objective was to characterize differences between cows with and without postpartum production disease (retained fetal membranes, ketosis, hypocalcemia, abomasal displacement, metritis, mastitis) by prepartum measurement of serum nonesterified fatty acid (NEFA) and plasma insulin-like growth factor (IGF)-I concentrations relative to the artificial insemination (AI) that established pregnancy. Blood was collected from 41 Holstein Friesian cows on 235 to 241, 242 to 248, 249 to 255, 256 to 262, 263 to 269, 270 to 276, 277 to 283, and 284 to 290 d after AI. Health status was assessed daily for 3 wk after calving; 25 cows (66%) had at least one production disease. Cows developing postpartum diseases had higher mean serum NEFA concentrations (450 ± 26 $\mu\text{mol/L}$; mean $\pm$ SE) and lower plasma IGF-I concentrations (78 ± 6 ng/mL) prepartum compared with healthy cows (259 ± 19 $\mu\text{mol/L}$ and 117 ± 8 ng/mL, respectively). In conclusion, because of substantial variation among cows in gestation length, blood samples should be collected and studies performed on risk prediction relative to AI rather than expected date of calving. As the somatotrophic axis is one of the key regulators of metabolic adaptation for onset of lactation, IGF-I might be a useful variable to differentiate between cows susceptible to production diseases and cows

that are able to adapt adequately within the transition period and remain healthy.

Key words: production disease, gestation length, transition period, insulin-like growth factor-I

Short Communication

Successful transition from late pregnancy to early lactation in dairy cows is crucial for optimal health, productivity, and fertility (Grant and Albright, 1995; Drackley, 1999). If cows predisposed to develop postpartum diseases could be reliably identified well in advance of clinical disease, preventive strategies could be implemented. Blood NEFA and BHBA concentrations were suggested as reliable prepartum predictors for postpartum diseases (LeBlanc et al., 2005; Duffield, 2007; Ospina et al., 2010); dairy cows with elevated (>0.7 mmol/L) serum NEFA concentrations during the last week before calving had a 5-fold increased risk for postpartum ketosis (Duffield, 2000).

Epidemiological studies to calculate thresholds and time points for postpartum production diseases in dairy cattle have used blood sampling relative to the predicted day of calving. Despite the physiological basis of this approach, gestation length varies among individual cows, making it difficult to accurately predict day of calving. To improve on-farm testing and application of those methods, the sampling procedure should include variation in gestation length.

It is not clear whether better indicators than NEFA and BHBA exist for predicting production diseases that are not primarily a metabolic disturbance (e.g., hypocalcemia, mastitis). Because IGF-I is described as a sensitive signal between metabolism, negative energy balance, and reproduction (Königsson et al., 2008), it may have value as a marker of production diseases.

The objectives were to (1) characterize the distribution of length of gestation in dairy cows, and (2) determine whether prepartum plasma NEFA and IGF-I

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concentrations differ between cows that remain healthy during the subsequent transition period and those that develop production diseases. The hypotheses tested were that (1) date of AI (that established pregnancy) could be used to identify differences in cows with postpartum diseases, and (2) IGF-I concentrations before calving differ between healthy cows and those developing production diseases.

To characterize gestation length, 43,273 calvings between January 1, 2006, and June 30, 2009, were evaluated retrospectively. These data originated from 21 representative dairy herds, with an average of 781 cows (range: 220 to 2,410), in Mecklenburg-Vorpommern, Germany.

The main study involved 41 Holstein-Friesian cows at the Hannover University Farm. At 8 time points during gestation (relative to AI), blood samples were taken from each cow. Furthermore, all cows were monitored for postpartum diseases. These cows were used in accordance with German legislation on animal welfare (AZ 33.9-42502-04-08/1592).

The cows were dried off approximately 240 d after AI, grouped in a freestall housing system fitted with a slatted floor, and fed a partly mixed ration plus 1 kg of concentrate thrice daily. Calving occurred in single-cow, straw-bedded maternity pens. No dystocia occurred and obstetrical assistance (7 of 59 calvings) did not exceed moderate traction (1 person).

Starting 235 d after the AI that established pregnancy, blood sampling was performed between 235 and 241, 242 and 248, 249 and 255, 256 and 262, 263 and 269, 270 and 276, 277 and 283, and 284 and 290 d after AI. Blood samples ($n = 238$) were collected by jugular venipuncture into serum tubes and into tubes containing EDTA. The EDTA-plasma samples for hormone analyses were cooled within 30 min and subsequently centrifuged ($2,000 \times g$, 4°C , 10 min). Serum and plasma were kept frozen (-20°C) until analyzed.

Cows were clinically examined according to a fixed routine schedule (Rosenberger et al., 1991) as soon as at least one of the following occurred: (1) milk yield was reduced by 20% compared with average milk yield of the last 3 d; (2) <70% of the computed concentrate intake was consumed; or (3) farm personnel became aware of an impaired health status.

Based on clinical examinations, ketosis (**K**), left displacement of abomasum (**DA**), hypocalcemia, retained fetal membranes (**RFM**), clinical metritis, and mastitis were defined as follows. Cows were classified as clinically ketotic if concentrate intake, milk yield, or both, were reduced; a nitroprusside test for ketone bodies in urine was clearly positive (+++); and other diseases were not diagnosed (except DA). Diagnosis of DA was done by percussion and swinging auscultation in the

left flank. Cows were considered clinically hypocalcemic if one or more of the following symptoms were found: tremor, cool body surface, unstable gait, ataxia, and recumbency. An essential condition for diagnosis of hypocalcemia was a significant improvement of the clinical status immediately after i.v. infusion of calcium (10 g of calcium as calcium borogluconate 25%; CP Pharma, Burgdorf, Germany). A diagnosis of RFM was made if fetal membranes were not successfully expelled within 12 h after calving. Clinical metritis was defined as an abnormal vaginal discharge in association with a flaccid uterus with fluctuation as detected by transrectal palpation and verified by transrectal ultrasonography. Clinical mastitis was defined by an elevated cell count detected by a California Mastitis Test (+++), fibrin or clots in the milk, and a disturbance of general condition in the absence of other diagnoses.

Retrospectively, cows that remained clinically healthy for 21 d after calving were assigned to one group (healthy, **H**; $n = 16$). In 12 cows, RFM-metritis was diagnosed, 10 cows developed hypocalcemia, 7 cows had mastitis, and 6 cows had K-DA throughout the transition period. Sixty-four percent of the diseased cows had 1 disease, 16% had 2, and 20% had 3 production diseases within 3 wk after calving. One cow was found dead 3 d after calving; although no specific cause of death was apparent at necropsy, she was assigned to group production diseases (**PD**). All animals with a PD during the first 3 wk postpartum were assigned to the group PD ($n = 25$).

Serum NEFA concentrations were determined by using an automated clinical chemistry analyzer (ABX Pentra 400, Horiba, Montpellier, France) using colorimetric enzymatic reactions for NEFA concentration

Figure 1. Distribution of gestation length in 43,273 primiparous and pluriparous Holstein-Friesian cows from 21 herds.

(the intraassay CV was 6.2%). Total plasma IGF-I was analyzed using an IGF-I-coated tube immunoradiometric assay (DSL-5600, Diagnostic Systems Laboratories Inc., Webster, TX). Separation of IGF-I from its binding proteins was done with an acid-ethanol extraction procedure, and IGF-I concentrations were determined with a 2-site immunoradiometric assay. The intra- and interassay CV were 3.5 and 8.5%, respectively.

Statistical analyses were performed using SAS (SAS OnlineDoc 9.2., SAS Institute Inc., Cary, NC); $P < 0.05$ was considered significant for all analyses and comparisons. To calculate differences in IGF-I and NEFA concentrations between healthy and diseased cows prepartum, data were analyzed using the MIXED model procedure, with health, time, and their interactions as fixed effects, and cow nested in health as a random effect. Time was a repeated-measures effect to account for potential correlations and different variances among periods. Various variance-covariance matrices were fitted and the one with the lowest corrected Akaike's information criterion value was used for the final analy-

sis. For these calculations, data were log-transformed. Normal probability plots created by the UNIVARIATE procedure were used to check the residuals for normality and potential outliers. Three obvious outliers were removed before performing the final analysis; the resulting residuals were normally distributed.

To test differences for specific periods between groups tested, the Shapiro-Wilk test was used to determine if distributions were normal for the following intervals (d): 235 to 241: $n = 18$; 242 to 248: $n = 15$; 249 to 255: $n = 22$; 256 to 262: $n = 35$; 263 to 269: $n = 38$; 270 to 276: $n = 37$; 277 to 283: $n = 38$; and 284 to 290: $n = 35$. As many data sets of IGF-I and NEFA concentrations were not normally distributed, the nonparametric Wilcoxon rank sum test (PROC NPAR1WAY) was performed. The Friedman 2-way ANOVA was used to determine the effect of day after AI on repeated measurements (PROC FREQ). To analyze the influence of time by group interaction, a combination of Wilcoxon signed rank test (PROC UNIVARIATE) and Wilcoxon rank sum test (PROC NPAR1WAY) was used.

Figure 2. Mean ($\pm$ SEM) plasma NEFA and IGF-I concentrations in clinically healthy (H; $n = 16$) Holstein-Friesian cows, those with at least one production disease (PD; $n = 25$), and those that developed ketosis, abomasal displacement, or both (K-DA; $n = 6$) versus cows with production diseases except ketosis (PDEK; $n = 19$) between 235 d after AI and calving. Asterisks indicate differences ($P < 0.05$) between groups.

In the retrospective analysis, the mean ($\pm$ SD) gestation length was 280.5 ± 4.8 d (range 265 to 295; $n = 43,273$); 9.8 and 14.1% of all calvings occurred after a gestation length of <275 or >285 d, respectively (Figure 1).

In 41 cows, gestation length was 281.2 ± 4.3 d. Recent studies regarding indicators of dairy cows at risk for postpartum diseases reported thresholds for blood samples collected at specific intervals relative to calving (Carson et al., 2007; Duffield, 2007; Ospina et al., 2010). In a second step, LeBlanc et al. (2005) excluded all cows calving >286 d after AI and their respective analyses' data for risk assessment. Such cows and their results were still included in our study. In addition, the recommendation to collect blood at a specific date after AI or even a specific week postpartum would be easier to implement in the field than referring to the date of expected calving (because approximately 20% of all cows calve either <275 d or >285 d of pregnancy. Mean prepartum serum NEFA concentrations (259 ± 19 $\mu\text{mol/L}$; mean $\pm$ SE) were lower and plasma IGF-I concentrations were higher (117 ± 8 ng/mL) in cows that remained healthy postpartum compared with those developing any postpartum disease (450 ± 26 $\mu\text{mol/L}$, $P = 0.0006$; and 78 ± 6 ng/mL, $P = 0.0022$, respectively). The respective periods were 249 to 269 d after AI for differences in serum NEFA. Furthermore, cows developing exclusively K-DA after parturition had higher NEFA concentrations between 256 and 276 d after AI compared with cows with production diseases except ketosis (Figure 2).

Plasma IGF-I concentrations were significantly higher between 242 and 248 d after AI in H cows postpartum compared with PD cows after calving. Interestingly, IGF-I concentrations peaked between 242 and 248 d after AI in H cows. This result for IGF-I concentrations in H cows supports other studies (Cavestany et al., 2009; our unpublished data). Differences in prepartum IGF-I concentrations between PD and H cows did not persist as long as differences in serum NEFA concentrations. Moreover, because of the small number of observations for IGF-I between 242 and 248 d after AI, further studies are indicated in more cows to verify this conclusion. Additionally, the date of drying off should be recorded in further studies, because it would be a convenient time point for sampling, as all cows are usually handled

for mammary treatment or other practices at drying off and this could be the physiological basis for such an IGF-I peak. Unfortunately, the dates of drying off were not available for the current study.

Several key questions need to be addressed to validate biomarkers such as IGF-I for their applicability as risk variables. In the present study, IGF-I concentrations differed between PD and H cows, and the study protocol allowed such a difference to be demonstrated. Because of the limited number of cows used ($n = 41$), a reliable risk assessment was not possible.

In conclusion, prepartum blood sampling relative to AI was applicable to demonstrate lower serum NEFA and higher plasma IGF-I concentrations in cows remaining healthy compared with those affected by postpartum diseases.

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