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Body condition loss and increased serum levels of nonesterified fatty acids enhance progesterone levels at estrus and reduce estrous activity and insemination rates in postpartum dairy cows

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ABSTRACT

Data from 96 Holstein Friesian cows on a commercial dairy farm were used to investigate whether body condition and serum levels of nonesterified fatty acids (NEFAs) postpartum (pp) affect progesterone (P4) levels, estrous activity, and fertility in dairy cows. The examination period started 14 days before the expected calving date and ended either when a cow was inseminated or at a maximum of 90 days pp. Body condition score (BCS; 1–5 scale) and backfat thickness (BFT) were determined every 2 weeks. Blood for analysis of NEFA and P4 concentrations was sampled weekly during the first 35 days pp and then every 48 hours until an ovulation was observed. Transrectal ultrasonography of the ovaries started at 21 days pp and was performed after blood sampling. If cows were not inseminated because of silent ovulation, sampling and ultrasonography continued on Days 7, 14, and 18 after ovulation and again every 48 hours until the next ovulation. Estrous activity was continuously measured with the Heatime estrus detection system. Pregnancy controls were performed ultrasonographically 28 and 42 days after AI. Cows with increased NEFA levels at 28 days pp had an increased risk of maintaining minimum P4 levels above 0.4 ng/mL at first recognized estrus ($P = 0.03$). Higher NEFA levels at Day 7 were associated with lower probability for a cow to have elevated P4 levels ($\geq$2 ng/mL) by Day 35 pp, indicating delayed commencement of luteal activity (C-LA). Estrous activity was not influenced ($P > 0.10$) by minimum P4 concentrations at estrus, but more animals with C-LA until Day 35 pp showed estrous activity compared to cows without C-LA throughout this period ($P = 0.006$). Estrous activity was lower in cows with a low BCS 14 days pp ($P = 0.02$) and with a low BFT 42 days pp ($P = 0.03$). Moreover, the probability to exhibit estrus was reduced with higher NEFA levels at 21 days pp ($P = 0.01$). Eighty-five cows were inseminated and 37 (44%) got pregnant after insemination. Higher NEFA levels, especially 14, 28, and 42 days pp, were associated with lower probability to inseminate a cow. Regarding the cows that were inseminated, no influence of BCS, BFT, or NEFA levels on pregnancy per artificial insemination was found. In conclusion, cows with higher loss of body condition and elevated NEFA concentrations had higher P4 concentrations during estrus, a reduced estrous activity and insemination rate, but no depression in pregnancy per artificial insemination.

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1. Introduction

Over the past decades, fertility has decreased in dairy cows, whereas milk production has increased [1]. Nowadays, economic efficiency in dairy farms is mainly determined by fertility management [2]. Besides aspects such as housing, nutrition, milk yield, age, and genetics, factors such as the intensity of shown heat and its detection have a growing influence on the reproductive outcome in dairy operations [3]. Unrecognized estrus or incorrect diagnosis of estrus can cause an estimated annual loss of over $300 million to the US dairy industry [4]. According to a previous study [5], each undetected estrus produces an economical loss of $42 by prolonging the open days of a cow. Success of heat detection with visual observation varies substantially between farms and therefore has a great impact on the reproductive efficiency of dairy operations [3,6]. Some factors that enhance estrous activity can only partly be influenced by management, such as the number of cows being simultaneously at heat [7,8]. Pedometers can be helpful to identify cows with increased walking activity or mounting and standing events. These systems reach sensitivities of up to 95% [9]. Because ovulation may occur without apparent signs of heat or any significant increase of walking activity, some heats cannot be detected by common methods.

Accounting for 42% [10] to 50% [11] of ovarian disorders during the postpartum (pp) period, silent ovulation was 1.7-fold higher in high-yielding cows compared to low-yielding cows during the time between calving and the first shown heat [12]. In the last mentioned study, an increase of milk yield by 1000 kg per year resulted in 0.27 more silent ovulations until the first visible estrus after calving and a prolongation of the calving-to-conception interval by 5.7 days. A recent study [13] showed that cows with at least one silent ovulation, excluding the first one pp, had prolonged intervals from calving to the first artificial insemination (AI) and from calving to pregnancy by 18 and 53 days, respectively.

Increased levels of milk yield were associated with a negative energy balance during early lactation and with reduced fertility in cattle [14–16]. Cows experiencing a severe body condition loss during the first 5 weeks pp had a 40% decline regarding the first-service conception rate compared to cows with milder body condition loss [17]. Plasma concentrations of nonesterified fatty acids (NEFAs) were positively related to the degree of negative energy balance and to the amount of activated body reserves [18,19]. Negative effects of NEFAs on *in vitro* oocyte maturation and fertilization were evidenced [20].

In a study that was performed in ovariectomized nonlactating cows treated with intravaginal progesterone (P4)-releasing devices for 70 days, it was found that the lipophilic hormone P4 was stored in the adipose tissues [21]. After the device was removed, serum P4 levels decreased to negligible concentrations. However, in cows that experienced fat mobilization because of restricted feeding, higher serum P4 levels were evidenced compared to cows fed within maintenance levels. Moreover, higher minimum P4 concentrations were observed during silent ovulation in the study by Schopper et al. [12]. Thus, the association between P4 concentrations delivered by the adipose tissue and silent ovulation should be investigated.

The objectives of the present study were to investigate (1) whether serum P4 concentrations during estrus are related to the amount of mobilized body fat reserves and (2) whether body condition loss or elevated P4 levels during estrus reduce estrous activity and fertility. To the best of our knowledge, this is the first study that monitors estrous activity in cows with different P4 concentrations in an objective way and relates it to the development of the body condition and NEFA levels pp.

2. Materials and methods

2.1. Animals, housing, and feeding

The study was performed at a northern German dairy farm with an average milk yield of 9000 kg per year between August 2011 and March 2012. The cows were kept in a freestall barn with full concrete floor and straw-padded beds.

Initially, 132 year-round calving Holstein Friesian cows were enrolled in the study. The cows were divided into three feeding groups according to their milk yield (>30 kg, 10–30 kg, and <10 kg milk/day) and were milked thrice daily. Because the examination period started 2 weeks before the expected calving date and ended with insemination (or 90 days after calving in cows that were not inseminated until then), all animals included in this study were allocated mostly to the high-performance group (average, 38 kg milk/day). This group was fed a total mixed ration once daily (22.3-kg dry matter, 159.2-MJ net energy for lactation, 3489-g crude protein, 2845-g crude fiber [70% structured], 448.4-g K, 185.3-g Ca, 81.2-g P, 69.2-g Mg, and 8.7-g Na) and had *ad libitum* access to water.

2.2. Experimental design

The first examination that included blood sampling, determination of body condition score (BCS), and backfat thickness (BFT) was performed in transition cows 14 days before the expected calving date. Because of deviations from the expected calving date, measurements were actually performed 13 $\pm$ 4 days before calving. The assessments of BCS and BFT were always carried out by the same person on the day of calving (defined as Day 0) and every 14 (13–15) days until the end of the examination period. On Days 0, 7, and 14 pp, general examination, blood sampling, and transrectal palpation of the uterus were made to control normal uterine involution. On Days 21, 28, and 35 pp, an additional ultrasonographic examination of the uterus and ovaries was performed. For transrectal ultrasonography, a portable B-mode scanner (Honda HS 101V; HONDA ELECTRONICS, Japan), equipped with a 5-MHz linear-array transducer, was used. Starting on Day 35 pp, an examination schedule with 2-day intervals was established to detect the first ovulation at that early period. Ovulation was assessed retrospectively by ultrasonographic determination of a dominant follicle (diameter, $\geq$12mm) [22] followed by the appearance of a CL in place of the follicle. The first day that the dominant follicle was no longer visible