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Effects of suprabasal progesterone levels and negative energy balance on estrous activity and fertility in dairy cows

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Contents

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The objective of the present study was to investigate if suprabasal P4 levels during estrus are related to negative energy balance and have negative effects on estrous activity and fertility in dairy cows. Therefore, 98 cows were examined. Examination period lasted from 2 wk before calving to first AI between 40 and 80 d p.p. Starting on d 35 p.p., ovulation was controlled sonographically every 48 h. Energy balance was monitored by estimation of BCS (1-5) and backfat-thickness every 2 wk, as well as by determination of NEFA. Blood samples for NEFA were collected weekly during the first 35 d p.p., then every 48 h until AI or until d 80 p.p. P4 was only determined for the day of ovulation (±24 h). Activity was measured with Heatime® estrus detection system. Cows with increased P4 concentrations tended to have a 1.2-fold higher decrease in BCS during the first 42 d p.p. Estrous activity did not depend on P4 levels, but cows with higher activity had lower NEFA and a more moderate BCS-loss. There was no correlation between NEFA and P4 levels. The non-return-rate after first AI was 44%. Neither NEFA nor P4 levels influenced the insemination success. Cows with a higher pregnancy rate tended to have a more moderate loss of BCS preceding insemination. In conclusion, cows with higher BCS loss and NEFA concentrations show a significant lower estrous activity and by tendency a reduced fertility. Both, activity and fertility are not affected by suprabasal P4 levels around the time of ovulation.

Keywords: Progesterone, estrous activity, negative energy balance, dairy cows

Effects of suprabasal progesterone levels and negative energy balance on estrous activity and fertility in dairy cows

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Influence of Progesterone and Energy Balance on Estrous Activity in Dairy Cattle. Purschke. Unrecognized or missed heats cause a tremendous financial loss in dairy farms. In research regarding the cause of silent heat, higher blood progesterone concentrations could be observed. Since high progesterone levels were also detected in cows that melted down adipose tissue, the present study was designed to investigate if progesterone concentrations during estrus are related to energy balance in postpartum dairy cows. Furthermore, effects of suprabasal progesterone levels or negative energy balance on estrous activity and fertility were evaluated.

ABSTRACT

The objective of the present study was to investigate if suprabasal progesterone (P4) levels during estrus are related to negative energy balance and have negative effects on estrous activity and fertility in dairy cows. Therefore, 98 Holstein Friesian cows were examined on a commercial dairy farm with a milk yield of 9,000 kg per cow and lactation. Examination period lasted from 2 wk before the expected calving date to time of first insemination between 40 and 80 d *post partum* (p.p.) or until 80 d p.p. in cows that did not show estrous behavior. Starting on d 35 p.p. (d 0 = parturition), time of ovulation was controlled by transrectal ultrasonography every 48 h. Two ultrasonographic pregnancy controls were performed 28 and 42 d after insemination. Energy balance was monitored by estimation of body condition score (BCS; 1-5 scale) and ultrasonographic measurement of backfat-thickness (BFT) every 2 wk, as well as by determination of non-esterified fatty acids (NEFA). Blood samples for NEFA analysis were collected weekly during the first 35 d p.p. (averaged as NEFA35), then every 48 h until an ovulation was observed, on d 7, 14 and 18 following ovulation, and again every 48 h until insemination was done or until d 80 p.p. (if cows were not inseminated before). Progesterone was determined in blood samples that were collected weekly between d 0 and 35 p.p. and on the day of ovulation (±24 h). Estrous activity was measured continuously after calving with Heatime® estrus detection system. Cows with increased (≥0.4 ng/ml) P4 concentrations at time of ovulation tended to have a 1.2-fold higher decrease in BCS during the first 42 d p.p. (BCS-L42; $P = 0.07$). Estrous activity did not ($r = 0.02$; $P > 0.10$) depend on P4 levels around the time of ovulation, but cows with higher activity had lower NEFA35 ($P < 0.05$) and a more moderate BCS-L42 ($P < 0.05$). There was no correlation between NEFA35 and P4 levels. Eighty-four cows were inseminated and 37 (44%) got pregnant after first insemination. Neither NEFA nor P4 levels at time of ovulation influenced the insemination success ($P > 0.10$). Cows with a higher pregnancy rate tended ($P = 0.07$) to have a more

moderate loss of BCS preceding insemination (during the first 28 d p.p.). In conclusion, cows with higher BCS loss and NEFA concentrations show a significant lower estrous activity and by tendency a reduced fertility. Both, activity and fertility are not affected by suprabasal P4 levels around the time of ovulation.

Keywords: Progesterone, estrous activity, negative energy balance, dairy cows

INTRODUCTION

While milk production in dairy cows increased, fertility decreased over the last decades (Walsh et al., 2011). Today, economic efficiency in dairy farms is mainly determined by fertility management. Besides aspects like housing, nutrition, milk yield, age and genetics, factors like intensity of shown heat and its detection have a growing influence on the reproductive outcome. Unrecognized or missed heats cause the second greatest loss in dairy farms, following mastitis (Maatje et al., 1997). Each undetected heat produces an economical loss of $ 42 by prolonging the time a cow is open by 21 d, no matter if in developing countries like Bangladesh (Shamsuddin et al., 2006) or industrial countries like the United States (Graves, 2009). Daily time effort and qualification of the farmer or employee for correct visual heat detection differ massively and therefore take a great influence on the reproductive efficiency (Reimers et al., 1985; Nebel et al., 1987; Nebel, 1988; Elmore, 1989). Some factors can hardly be influenced by management, e.g. number of cows in or near onset of heat at the same time, which distinctly increases the amount of shown estrous activity (Hurnik et al., 1975; Diskin and Sreenan, 2000). Pedometers using heat detection can be helpful to identify cows with increased walking activity or mounting and standing events. Sensitivities of these systems ranged from 61 to 96% (Liu and Spahr, 1991; Schofield et al., 1991; Roelofs et al., 2005; McGowan et al., 2007). Since ovulation may occur without shown signs of heat or even

a significant increase of walking activity (defined as silent heat in the current study), some heats just cannot be detected by common methods.

The incidence of silent heat is reported to be 34 or even up to 50% (Yániz et al., 2008; López-Gatius et al., 2008) and was 1.7-fold higher in high yielding compared to low yielding cows during the time between calving and first shown heat (Schopper et al., 1993). In the last mentioned study, an increase of milk yield by 1000 kg per year resulted in 0.27 more silent heats until the first shown heat after calving; these cows were 5.4 d longer in anestrus and had to be inseminated 0.51 times more often to establish pregnancy. In a more recent study (Ranasinghe et al., 2009), the influence of milk yield on the incidence of silent heat became manifest in prolonged intervals from calving to first AI of 18 d and an extended time to achieve pregnancy of 53 d more.

Considering the increase in milk yield, recent studies supposed a negative energy balance during the first 40 to 80 d of lactation to reduce fertility in cattle (Senatore et al., 1996; Domecq et al., 1997; Jorritsma et al., 2003; Pryce et al., 2004). Furthermore, several previous studies indicated that cows with a decrease in body condition score >1 (score 1-5) during the first 80 d p.p. had a conception rate of 17-38% after first insemination, whereas in cows with a BCS loss <1 conception rate was 53% or even higher (Butler and Smith, 1989; Garnsworthy and Webb, 1999; Butler, 2001). Plasma concentrations of non-esterified fatty acids (**NEFA**) were positively related to the degree of negative energy balance or the amount of activated body reserves (Block et al., 2001; Butler, 2003). Negative effects of NEFA on in vitro oocyte maturation and fertilization were evidenced (Leroy et al., 2005).

In a recent study (Rodrigues et al., 2010), performed on ovariectomized, non-lactating cows that were treated with intrauterine progesterone-releasing devices for more than 66 d, the lipophilic hormone progesterone (**P4**) was stored in the adipose tissues. After removing the progesterone-releasing device serum P4 levels decreased massively from >1.5 ng/ml to

negligible concentrations. Cows that were fed energy below maintenance and therefore melted down adipose tissues showed higher serum P4 levels compared to cows fed within maintenance without a negative energy balance. In that study, P4 concentrations of the low energy group even tended to become higher than 1 ng/ml between d 13 to 21 after the removal of the progesterone-releasing device. Since higher P4 concentrations were also observed during silent heat (ovulation was retrospectively defined by the lowest milk P4 concentration) in the mentioned study by Schopper et al. (1993), a possible association between P4 that is delivered by adipose tissue during lipolysis in negative energy balance, and silent heat in cows should be investigated.

The aim of the present study was to investigate if serum P4 concentrations during estrus are related to the amount of mobilised body fat reserves and if suprabasal P4 levels and negative energy balance have an effect on estrous activity and on subsequent fertility. To the best of our knowledge, this study was the first one that objectively measured activity during estrus to attend to this common problem in dairy cows.

MATERIALS AND METHODS

Animals, Feeding and Housing

The study took place on a northern German dairy farm with 400 year-round calving Holstein Friesian cows, which had an average yearly milk yield of 9,000 kg. Examinations began on August 15th, 2011 and were completed on March 30th, 2012. During this time period, environmental temperature ranged from -10°C to +18°C with the exception of only 2 d (-14°C and +28°C). Cows were kept all year inside a free stall barn with concrete floor and straw padded beds. Lactating cows were divided into three feeding groups according to their milk yield (>30 kg milk/d, 10-30 kg milk/d, <10 kg milk/d). Each group was milked three times a

day. Since examination period started 2 weeks before the expected calving date and ended with insemination (or 80 d *post partum* (**p.p.**) in cows that were not inseminated until then), all animals included in this study (n=106) were part of the high performance group for most of the time (average of 38 kg milk/d). This group was fed a total mixed ration once daily (22.3 kg dry mater, 159.2 MJ NEL, 3489 g crude protein, 2845 g crude fibre (70% structured), 185.3 g Ca, 81.2 g P, 69.2 g Mg, 448.4 g K, 8.7 g Na) and had *ad libitum* access to water.

In the examined cows, the average age at calving was (mean ± SD) 52.7 ± 20.8 months (range, 24–154 mo), including primiparous cows (n=28), second to fourth calvers (n=62), as well as cows with five or more calvings (n=16). Pluriparous cows (n=78) had an average calving interval of 421 ± 76.4 d (range, 317-667 d).

Body Condition Scoring, Back Fat Measurement and Activity Record

To quantify the body condition of each individual and the loss of body reserve adipose tissue in particular during the investigation period, two methods were applied: body condition scoring and backfat-thickness (**BFT**) measurement.

Based on visual and tactile cues of several body locations in the lumbar, thurl and tailhead regions, body condition score (**BCS**) was described as 5-point scale with 0.25-unit increments (below 2.5 and over 4.0 with 0.5-unit increments), according to Ferguson et al. (1994). During the study, BCS of each cow was evaluated at least 4 times (range, 4 – 7) at intervals of 14 (13 - 15) d by the same person.

Each body condition scoring was followed by a BFT measurement according to Staufenbiel (1992). In several studies, total body fat of Holstein Friesian cows correlated with BFT (1 mm BFT = 5 kg body fat; Staufenbiel, 1992; Schröder, 2000; Schröder and Staufenbiel, 2003) as

well as BCS (Schröder and Staufenbiel, 2006). To perform BFT measurement, a portable B-mode scanner (Honda HS 101 V, HONDA ELECTRONICS, Japan), equipped with a 5 MHz linear-array transducer, was used. A small region one palm cranial of the ischiadic tuber was prepared with 80% alcohol (clipping was not required). The transducer was held orthogonal on the prepared skin area, as lightly as possible to achieve a good image quality, since too much pressure might falsify the measurement of compressible adipose tissue (Brethour, 1992). A frozen image was documented to read BFT, rounded to the nearest full millimeter. Skin thickness of 5-6 mm was included in the result of BFT measurement and therefore was the lowest possible outcome, which was found in very poor conditioned animals.

Heat detection was managed with the automatic monitoring device Heatime^{TM} (Milkline S.r.l., Podenzano, Italy). Therefore, postpartum cows were equipped with a collar-transmitter inherent to this system within one day after calving. In the Heatime^{TM} system, an acceleration sensor, microprocessor and memory are used to collect data on the speed of movement as well as restless behavior. A proprietary algorithm analyzes these data and relates them to an individual index based on activity behavior during the previous 7 to 10 d. Different movements are distinguished according to amplitude, direction, intensity and duration. An increase of the same movement in many cows at the same time is recognized and considered in the algorithm to avoid false-positive results due to events like relocation or claw trimming. Each cow with an activity of more than 5 Heatime units (**HU**, units inherent to the system) is detected and announced by the system (exclusive information provided by the manufacturer). Since these units have no declared dimension, scoring was performed in the present study with 0 to 5 HU (defined as low activity (**LA**)) for silent or no heat, and > 5 to 10 HU (medium activity (**MA**)) as well as > 10 HU (high activity (**HA**)) for increasing activity levels of shown heat.

Experimental Design

Only data of animals that last throughout the whole study time (98 of 132) without major physical impairments (temperature >39.5°C for more than one day or illness for 2 d that required drug treatment) were analyzed.

Fourteen days before the expected calving date (14 d *ante partum* (**a.p.**)), the first examination, including BFT measurement, BCS rating and blood sampling from the coccygeal blood vessels, was performed in cows of the transition group. Measurements of BFT and BCS were repeated every 14 d until the end of examination.

On d 0 (defined as day of calving), 7 and 14 p.p. transrectal palpation of the uterus was done to control normal uterine involution. On d 21, 28 and 35 p.p. transrectal ultrasonographic examinations (using the ultrasound device Honda HS 101 V) were performed and, starting on d 35 p.p., an examination schedule with 2 d intervals was established to detect the first relevant ovulation after calving. Examination times included general examination, transrectal palpation and ultrasonography of uterus and ovaries as well as blood sampling. Ovulation was assessed retrospectively by ultrasonographic determination of a dominant follicle (diameter >12mm; Sartori et al., 2001) followed by the appearance of a corpus luteum (**CL**) in place of the follicle. The day when the dominant follicle was no longer visible for the first time, was defined as d 0 *post ovulationem* (**p.o.**). Examinations were then pursued weekly (d 7 and 14 p.o.) and continued every 2 d from d 18 p.o. on.

Collecting data and blood samples from each cow was completed when cows were inseminated or reached d 80 p.p. (if there was no insemination before). Pregnancy control was performed ultrasonographically 28 d after insemination (**p.i.**) and was repeated on d 42 p.i. (pregnancy diagnoses did not differ between d 28 and 42). All examinations, except for that

on d 14 a.p., were performed at noon (immediately following milking of the cows) in an automatic separation parlor to minimize stress due to fixation.

Serum Concentration of Progesterone and Non-esterified fatty acids

Blood samples were collected at the beginning of each examination (except for pregnancy control) from the coccygeal blood vessels into consistently labeled serum tubes without additive. After a minimum of 30 min (but not exceeding 120 min) of coagulation, blood serum was separated by centrifugation (3,000 x g, 20 min, 4°C), split into three aliquots and stored frozen (-20°C) until analysis. Detection of serum P4 (samples during the first 35 d p.p. and at time of ovulation) and NEFA (samples during the whole examination time) were performed by the laboratory of Veterinary Physiology in Posieux (Vetsuisse Faculty, University of Bern, Switzerland). A progesterone radioimmunoassay (**RIA**) kit IM1188 by Beckman Coulter GmbH (Krefeld, Germany) was used for determination of P4. Concentration of NEFA was assessed with an enzymatic Kit no. 994-75409 (Wako Chemicals, Neuss, Germany).

Statistical analysis

Data from 98 cows were used. Progesterone concentrations of four cows were excluded because of a deviation from the mean level at time of ovulation (0.48 ng/ml) of approximately 2 SDs (> 0.9 ng/ml); these values were treated as missing values. Length of the examined period ranged from 98 to 147 d (14 d a.p. to 83 d p.p. and 132 d p.p., respectively). The distribution of the quantitative data was tested visually (PROC CHART) and by means of the Kolmogorov-Smirnov-test (PROC UNIVARIATE). If data were not normally distributed, independent pairwise comparisons (between groups) were done with a Wilcoxon's two-

sample test (PROC NPAR1WAY), and for dependent pairwise comparisons (within groups), the paired Wilcoxon's signed rank test (PROC UNIVARIATE) was used. If data were normally distributed, Student's t-test was chosen for independent (PROC TTEST) and dependent (PROC MEANS) comparisons (two groups), and for independent comparisons between three groups variance analysis (PROC GLM) was performed. Comparisons of qualitative data were done with chi-square test and Fisher's exact test, respectively (PROC FREQ in each case), depending on sample sizes. Spearman's correlation coefficients (PROC CORR Spearman) were calculated to evaluate all pairwise relationships between P4, NEFA and Heatime on the day of ovulation, BCS and BFT 14 d a.p. (**BCS-14** and **BFT-14**), BCS and BFT 28 d p.p. (**BCS28** and **BFT28**), BCS and BFT 42 d p.p. (**BCS42** and **BFT42**), BCS- and BFT-loss during 28 d (**BCS-L28** and **BFT-L28**) and 42 d p.p. (**BCS-L42** and **BFT-L42**), respectively, average NEFA between 0 and 21 d (**NEFA21**) as well as between 0 and 35 d p.p. (**NEFA35**), appearance of a CL until 35 d p.p. (**CL35**), pregnancy rate 42 d p.i., and parity of cows. All statistical analyses were done with the Statistical Analysis System V9.3 (SAS Institute Inc., Cary, NC, USA); $P \leq 0.05$ was considered significant and $0.05 < P \leq 0.10$ as a tendency.

Grouping of cows was performed according to P4 concentrations (0.4 ng/ml was chosen as the cutoff point for fully regressed CL, according to Brusveen et al., 2009) and activity (being low (<5), medium (≥5 to 10) or high (>10), considering Heatime^{TM} recommendation to inseminate cows with an activity >5 HU) on the day of ovulation. Furthermore, groups of cows with low (≤0.7 mmol/ml) or high (>0.7 mmol/ml; as used by Ospina et al., 2010) average serum NEFA concentrations during the first 21 d and 42 d p.p., respectively, were compared in the present study.

RESULTS

In the present study, 98 of 132 cows were clinically healthy without any pathological signs of the uterus and ovaries. Approximately three-fourths (71%) of these cows developed a CL until 35 d p.p. (Tab. 1). First relevant ovulation occurred between 40 and 90 d p.p. (mean ± SD, 60.64 ± 14.73). Cows with HU > 5 or with distinct visual detected heat signs (n=84) were inseminated and 44% of inseminated cows became pregnant.

In this study, 26% of the animals were primiparous and 74% pluriparous (Tab. 1). Pluriparous cows had lower BCS-L28 ($P = 0.05$) and BCS-L42 ($P < 0.05$), lower P4 concentrations at time of ovulation ($P < 0.05$), and tended to have a lower chance of getting pregnant after first insemination than primiparous cows (38% vs. 62%, $P = 0.06$). Validating these results, cows with low periovulatory P4 levels (< 0.4 ng/ml) tended to be more often pluriparous ($P = 0.08$) and to have less BCS-L42 ($P = 0.07$).

Cows with low (LA), medium (MA) and high (HA) activity on the day of ovulation were analysed (Tab. 1). Serum P4 concentrations on the day of ovulation did not correlate ($r = 0.02$, $P > 0.10$) with estrous activity. Compared to HA cows, average NEFA21 concentrations were higher in LA and MA cows (both $P < 0.05$), respectively. Average NEFA35 was also higher in LA ($P < 0.05$) and tended to be higher in MA ($P = 0.06$) compared to HA cows. On d 28 p.p. BCS was higher ($P < 0.05$) and BFT tended to be higher ($P = 0.08$) in HA compared to LA cows, and on d 42 p.p. a higher ($P = 0.05$) BFT in HA compared to LA cows was observed. BCS-L42 was higher ($P < 0.05$) and BCS-L28 tended to be higher ($P = 0.07$) in MA than in HA cows. There was the tendency of a higher BFT-L42 in MA compared to HA ($P = 0.07$) and LA cows ($P = 0.10$). A CL before d 35 p.p. was more often found in HA cows than in LA ($P < 0.05$) or MA cows ($P = 0.05$). Success rate to first insemination was reduced by tendency ($P = 0.09$) in cows of group MA compared to those of group HA.

Body condition score and BFT of cows 14 d before the expected calving date positively correlated with BCS-L42 ($r = 0.71$ and $r = 0.42$, $P < 0.05$) and BFT-L42 ($r = 0.51$ and $r = 0.73$, $P < 0.05$). Cows with high NEFA35 had higher BCS-14 and BFT-14 (both $P < 0.05$; Tab. 1), as well as a more excessive BCS-L28, BCS-L42, BFT-L28 and BFT-L42 (all $P < 0.05$) compared to cows with low NEFA35. Furthermore, cows with high NEFA21 showed higher ($P < 0.05$) BFT28 than cows with low NEFA21 (mean ± SD, 18.94 ± 6.95 mm vs 15.12 ± 5.40 mm). A CL before d 35 p.p. was 1.3-fold more likely to be seen in cows with low than with high NEFA21 ($P = 0.05$), and low NEFA35 in cows resulted in a higher ($P < 0.05$) activity level at estrus compared to high NEFA35.

In cows with low serum P4 concentrations (<0.4 ng/ml) on the day of first ovulation, the tendency ($P = 0.07$) of a more moderate BCS-L42 was observed compared to cows with high P4 concentrations (≥ 0.4 ng/ml; Tab. 2). There was no correlation between periovulatory NEFA and P4 levels ($r = 0.006$, $P = 0.96$) as well as between concentrations of NEFA35 and P4 ($r = 0.05$, $P > 0.10$).

Cows with a CL on d 35 p.p. had a higher BCS28 ($P = 0.03$) and BCS42 ($P = 0.01$), as well as a higher BFT42 ($P = 0.03$) than cows with their first CL appearing later than 35 d p.p. (Tab. 2). Estrous activity was also higher ($P = 0.009$) in cows with than without a CL35. Furthermore, cows with a CL35 tended to have a higher chance ($P = 0.08$) of getting pregnant after first insemination, compared to those without a CL35. The highest P4 concentration during the first 35 d p.p. (weekly examination schedule) was 13.83 ± 8.26 ng/ml (mean ± SD; minimum, 1.20 ng/ml) in cows with a CL35 and 0.61 ± 0.18 ng/ml (maximum, 0.98 ng/ml) in cows without a CL35.

Cows that were not inseminated (because they were not noticed to be in estrus by adspectation and Heatime^{TM} system) had higher NEFA concentrations at time of ovulation than cows that were inseminated and got pregnant or non-pregnant (both $P < 0.05$) after insemination (Tab.

2). Furthermore, NEFA21 and NEFA35 levels were also higher in cows that were not inseminated than in pregnant or non-pregnant cows (all $P < 0.05$). Cows with a successful first insemination tended to have a lower BCS-L28 ($P = 0.07$) and were by tendency more likely to develop a CL before d 35 p.p. ($P = 0.09$) than cows with unsuccessful insemination. Primiparous cows tended to be more often found ($P = 0.06$) in the group that got pregnant after insemination than in the unsuccessfully inseminated group.

DISCUSSION

The present study did not find a correlation between estrous activity and serum P4 concentrations. In contrast, Schopper et al. (1993) observed a lower estrous activity and a higher incidence of silent heat in cows with higher serum P4 concentrations on the day of ovulation. The experimental design in their study compared to the field examinations in the current study could have led to the different outcome. Similar to these contrasting findings, Vailes et al. (1992) found a significant negative influence of administered, exogenous P4 (alone or in combination with estradiol compared to estradiol alone) on the number of mounting events in ovariectomized, non-lactating cows in a first experimental part of their study, but could not verify these findings in the second practical part with lactating cows and physiological hormone levels. Another experimental study (Duchens et al., 1995) also found detrimental influences of induced suprabasal P4 concentrations on estrous behavior, contrasting with an infield study (Bage, 2003) that could not verify this observation in cows with natural high P4 concentrations. Most of these studies indicate that progesterone is suitable to reduce estrous behavior experimentally, but has only an inferior standing within regulatory complexity in cows under field conditions. None of the mentioned authors found evidence for a reproducible explanation of this phenomenon.

In the present study, animals with higher P4 concentrations on the day of ovulation tended to have a more excessive loss of BCS before (during the first 42 d p.p.). Tendencies of a higher BCS-loss during 28 and 42 d p.p. and higher P4 concentrations on the day of ovulation were more often found in primiparous than pluriparous cows. Increased blood P4 concentrations during a negative energy balance period were also found in a recent study (Rodriguez et al., 2010) and were explained by the release of lipophilic P4 during melting down of adipose tissue. In contrast, studies by Villa-Godoy et al. (1988) and Spicer et al. (1990) indicated that cows with the highest amount of negative energy balance had lower P4 concentrations during the first ovulatory cycles after parturition. Other studies proclaimed a higher liver blood flow in lactating compared to non-lactating cows, even more during feed intake (Wiltbank et al., 2001), that resulted in a higher metabolic clearance of P4 as well as estradiol (Parr et al., 1993; Sangsritavong et al., 2002; Vasconcelos et al., 2003). In contrast to the mentioned studies, which were referring to the P4 levels during a whole cycle or metestrus, the current study applied to P4 at the time of ovulation, i.e. in the absence of a functional CL.

Cows with the highest activity during estrus (between d 35 and 80 p.p.) had lower losses in BCS and BFT before (during the first 28 d and 42 d p.p., respectively), as well as lower levels of NEFA (average serum concentration during the first 21 d and 35 d p.p., respectively) than cows with medium or low activity. A previous study (Edwards and Tozer, 2004) presented higher walking activity as a sign of health in cows, which results in a better feed intake and therefore could explain the findings of the recent study. Interestingly, BFT loss during the first 42 d p.p. was even higher in cows with medium than low activity. Since cows with the lowest activity also had lower condition already 14 d before calving, potential condition loss in these cows might be limited (Frood and Croxton, 1978) compared to cows starting lactation with a modest BCS (Garnsworthy and Topps, 1982; Ducker et al., 1985; Butler and Smith, 1989).

In the present study, no correlation between fertility and P4 concentrations at time of ovulation was found. In contrast, increased concentration of P4 on the day of artificial insemination (**AI**) was related to a decreased non-return-rate (Waldmann et al., 2000; Bage, 2003; Souza et al., 2007), possibly due to negative effects of elevated P4 on oocyte maturation and ovulation (Fair and Lonergan, 2012). Compared to the present study, the authors did not additionally investigate the influence of a negative energy balance or a loss of BCS on fertility or P4 concentrations. In our study, cows with a successful insemination tended to have a lower preceding loss in BCS (during the first 28 d p.p.) and were more likely in their second or further cycle. Cows with higher P4 concentrations tended to lose more of their BCS during the first 42 d p.p. in the present study. As shown by Gillund et al. (2001), cows with greater BCS loss (> 1.25) until AI, conceived only half as likely as cows with a more moderate loss. In other studies (Domecq et al., 1997; Buckley et al., 2003), a massive loss in BCS during early lactation and a poor BCS at the start of breeding were predictive of poor fertility performance. Furthermore, there were high correlations between body condition loss and serum concentrations of NEFA in the present as well as previous studies (Bauman, 2000; Meikle et al., 2004). Since maturation and fertilization of in vitro derived oocytes were reduced by high NEFA concentrations (Leroy et al., 2005), increasing serum levels of NEFA might be the reason for low fertility observed in cows with high body condition loss.

In the present study, cows with lower NEFA concentrations during the first 35 d p.p. were more likely to commit their first estrous cycle before d 35 after parturition. This observation was consistent with recent studies that found an association between negative energy balance and a prolonged anovulatory phase p.p. (Staples et al., 1990; Rhodes et al., 1998) as well as between positive energy balance and early resumption of cyclicity (Patton et al., 2007). In the current study, cows with a CL before d 35 p.p. had a higher activity during the following estrus (used for AI) and a greater success rate of insemination (50% compared to 29% in cows

without a CL before d 35 p.p.). This relationship between early commencement of estrous cycles p.p. and improved fertility after AI was well documented in several studies (Butler and Smith, 1989; Butler, 2001; Darwash et al., 1997, 2001).

In conclusion, estrous activity and fertility of high producing dairy cows are not affected by suprabasal progesterone concentrations during the periovulatory period under field conditions. However, negative energy balance during early lactation with increased serum concentrations of non-esterified fatty acids attenuates estrous activity, but reduces fertility only by tendency.

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Table 1. Differences (means ± SD or percentage as decimal number) in serum concentrations of progesterone (P4; ng/ml) and non-esterified fatty acids (NEFA; mmol/ml) as well as activity (Heatime; HU) on the day of ovulation, in body condition score (scale, 1-5) and backfat-thickness (mm) 14 d before (BCS-14 and BFT-14), and 28 d (BCS28 and BFT28) as well as 42 d (BCS42 and BFT42) after parturition (p.p.), in BCS- and BFT-loss during the first 28 d (BCS-L28 and BFT-L28) and 42 d (BCS-L42 and BFT-L42) p.p., in average NEFA concentrations between 0 and 21 d (NEFA21) as well as 0 and 35 d (NEFA35) p.p., in the appearance of a corpus luteum until 35 d p.p. (CL35), and in pregnancy rate 42 d after insemination between primiparous and pluriparous cows, cows with low (LA), medium (MA) and high (HA) estrous activity, and cows with NEFA35 concentrations of more or less than 0.7 mmol/ml. Furthermore, values for all cows are listed in the last column.

	Parity		Estrous activity			NEFA35		All
	primiparous	pluriparous	LA	MA	HA	≤0.7 mmol/ml	>0.7 mmol/ml	
n	*25*	*73*	*19*	*16*	*63*	*74*	*24*	*98*
P4	0.55 ± 0.16^{A}	0.45 ± 0.19^{B}	0.46 ± 0.20	0.52 ± 0.40	0.47 ± 0.21	0.47 ± 0.18	0.48 ± 0.19	0.48 ± 0.18
NEFA	0.87 ± 0.39	0.71 ± 0.50	0.27 ± 0.23	0.26 ± 0.25	0.23 ± 0.16	0.22 ± 0.16^{a}	0.32 ± 0.25^{b}	0.24 ± 0.19
Heatime	13.00 ± 8.04	13.01 ± 7.89	0 ± 0^{A}	7.19 ± 2.56^{B}	18.41 ± 2.35^{C}	14.19 ± 7.45^{A}	9.38 ± 8.25^{B}	13.00 ± 7.93
BCS-14	3.72 ± 0.55	3.53 ± 0.51	3.49 ± 0.61	3.72 ±0.47	3.58 ± 0.51	3.51 ± 0.52^{A}	3.81 ± 0.48^{B}	3.58 ± 0.52
BCS28	2.85 ± 0.43	2.86 ± 0.45	2.68 ± 0.39^{A}	2.78 ± 0.46^{A,B}	2.93 ± 0.44^{B}	2.87 ± 0.44	2.82 ± 0.44	2.86 ± 0.44
BCS42	2.62 ± 0.32	2.65 ± 0.39	2.54 ± 0.40	2.58 ± 0.37	2.69 ± 0.36	2.66 ± 0.39	2.59 ± 0.34	2.64 ± 0.37
BCS-L28	0.87 ± 0.39^{A}	0.71 ± 0.50^{B}	0.80 ± 0.49^{a,b}	0.94 ± 0.37^{a}	0.69 ± 0.50^{b}	0.68 ± 0.51^{A}	0.99 ± 0.29^{B}	0.75 ± 0.47
BCS-L42	1.10 ± 0.41^{A}	0.88 ± 0.44^{B}	0.95 ± 0.54^{A,B}	1.14 ± 0.40^{A}	0.88 ± 0.41^{B}	0.84 ± 0.43^{A}	1.22 ± 0.37^{B}	0.94 ± 0.43
BFT-14	22.48 ± 8.71	23.99 ± 8.14	20.84 ± 6.97	25.38 ± 8.45	23.98 ± 8.50	22.57 ± 7.71^{A}	26.79 ± 9.25^{B}	17.72 ± 8.29
BFT28	14.72 ± 5.56	16.90 ± 6.30	14.16 ± 5.56^{a}	16.50 ± 7.23^{a,b}	16.97 ± 6.00^{b}	15.85 ± 5.68	17.83 ± 7.40	16.34 ± 6.05
BFT42	12.90 ± 4.97	14.23 ± 5.83	11.68 ± 5.45^{A}	13.19 ± 6.78^{A,B}	14.54 ± 5.23^{B}	13.66 ± 5.44	14.08 ± 6.40	13.89 ± 5.61
BFT-L28	7.76 ± 4.49	7.32 ± 4.25	6.68 ± 3.59	8.88 ± 3.12	7.29 ± 4.69	6.93 ± 4.45^{A}	8.96 ± 3.39^{B}	7.43 ± 4.31
BFT-L42	10.08 ± 5.69	9.75 ± 5.26	9.16 ± 4.66^{a}	12.19 ± 4.46^{b}	9.44 ± 5.64^{a}	8.91 ± 5.26^{A}	12.71 ± 4.62^{B}	9.83 ± 5.37
NEFA21	0.61 ± 0.41	0.69 ± 0.38	0.80 ± 0.47^{A}	0.83 ± 0.52^{A}	0.59 ± 0.30^{B}	0.49 ± 0.17^{A}	1.21 ± 0.37^{B}	0.67 ± 0.39
NEFA35	0.54 ± 0.37	0.60 ± 0.31	0.70 ± 0.43^{A}	0.69 ± 0.38^{a}	0.52 ± 0.26^{B,b}	0.43 ± 0.13^{A}	1.06 ± 0.28^{B}	0.58 ± 0.33
CL35	0.68 (*17 of 25*)	0.73 (*53 of 73*)	0.53 (*10 of 19*)^{A}	0.56 (*9 of 16*)^{A}	0.81 (*51 of 63*)^{B}	0.73 (*54 of 74*)	0.67 (*16 of 24*)	0.71 (*70 of 98*)
Pregnancy rate	0.62 (*13 of 21*)^{a}	0.38 (*24 of 63*)^{b}	0.40 (*4 of 10*)^{a,b}	0.23 (*3 of 13*)^{a}	0.49 (*30 of 61*)^{b}	0.42 (*29 of 69*)	0.53 (*8 of 15*)	0.44 (*37 of 84*)

^{A,B,C} Within rows of each group assignment, values with different superscripts differ ($P \leq 0.05$) between groups.

^{a,b} Within rows of each group assignment, values with different superscripts differ by tendency ($0.05 < P \leq 0.10$) between groups.

Table 2. Differences (means ± SD or percentage as decimal number) in serum concentrations of progesterone (P4; ng/ml) and non-esterified fatty acids (NEFA; mmol/ml) as well as activity (Heatime; HU) on the day of ovulation, in body condition score (scale, 1-5) and backfat-thickness (mm) 14 d before (BCS-14 and BFT-14), and 28 d (BCS28 and BFT28) as well as 42 d (BCS42 and BFT42) after parturition (p.p.), in BCS- and BFT-loss during the first 28 d (BCS-L28 and BFT-L28) and 42 d (BCS-L42 and BFT-L42) p.p., in average NEFA concentrations between 0 and 21 d (NEFA21) as well as 0 and 35 d (NEFA35) p.p., in the appearance of a corpus luteum until 35 d p.p. (CL35), and in pregnancy rate 42 d after insemination between cows with P4 concentrations of more or less than 0.4 ng/ml, cows with or without a CL35, and between not inseminated cows and cows that got pregnant or not pregnant after insemination.

	Progesterone		CL activity		Insemination and pregnancy rate		
	<0.4 ng/ml	≥0.4 ng/ml	CL35	no CL35	not inseminated	inseminated, not pregnant	inseminated, pregnant
n	*35*	*59*	*70*	*28*	*14*	*47*	*37*
P4	0.28 ± 0.09^{A}	0.59 ± 0.12^{B}	0.46 ± 0.18	0.51 ± 0.19	0.46 ± 0.12	0.46 ± 0.20	0.50 ± 0.19
NEFA	0.25 ± 0.19	0.23 ± 0.20	0.26 ± 0.21	0.20 ± 0.14	0.39 ± 0.29^{A}	0.21 ± 0.14^{B}	0.23 ± 0.18^{B}
Heatime	13.43 ± 8.11	12.88 ± 7.72	14.43 ± 7.25^{A}	9.46 ± 8.43^{B}	3.57 ± 6.33	13.72 ± 7.26	15.68 ± 6.58
BCS-14	3.49 ± 0.57	3.64 ± 0.49	3.61 ± 0.53	3.51 ± 0.51	3.55 ± 0.69	3.65 ± 0.43	3.50 ± 0.55
BCS28	2.87 ± 0.52	2.86 ± 0.40	2.92 ± 0.45^{A}	2.71 ± 0.37^{B}	2.71 ± 0.48	2.90 ± 0.46	2.86 ± 0.40
BCS42	2.66 ± 0.46	2.64 ± 0.33	2.70 ± 0.39^{A}	2.50 ± 0.28^{B}	2.55 ± 0.36	2.68 ± 0.41	2.64 ± 0.34
BCS-L28	0.70 ± 0.62	0.78 ± 0.38	0.73 ± 0.51	0.80 ± 0.39	0.84 ± 0.50^{a,b}	0.82 ± 0.54^{a}	0.64 ± 0.38^{b}
BCS-L42	0.82 ± 0.44^{a}	0.99 ± 0.43^{b}	0.91 ± 0.45	1.01 ± 0.41	1.00 ± 0.53	0.98 ± 0.40	0.86 ± 0.45
BFT-14	24.46 ± 9.06	23.31 ± 7.90	24.03 ± 8.20	22.54 ± 8.49	24.07 ± 9.35	23.96 ± 6.95	22.97 ± 9.50
BFT28	16.56 ± 7.56	16.34 ± 5.48	16.87 ± 6.15	15.04 ± 6.13	15.71 ± 7.54	16.85 ± 5.62	15.95 ± 6.39
BFT42	14.23 ± 6.99	13.64 ± 4.92	14.53 ± 5.87^{A}	11.86 ± 4.64^{B}	12.93 ± 6.67	14.26 ± 5.51	13.46 ± 5.53
BFT-L28	8.37 ± 3.95	6.97 ± 4.28	7.40 ± 4.55	7.50 ± 3.65	8.36 ± 3.30	7.47 ± 3.75	7.03 ± 5.22
BFT-L42	10.23 ± 4.91	9.66 ± 5.52	9.50 ± 5.44	10.68 ± 5.08	11.14 ± 4.26	9.70 ± 4.65	9.51 ± 6.48
NEFA21	0.65 ± 0.34	0.68 ± 0.43	0.63 ± 0.36	0.76 ± 0.44	1.01 ± 0.55^{A}	0.59 ± 0.27^{B}	0.64 ± 0.39^{B}
NEFA35	0.56 ± 0.30	0.60 ± 0.35	0.55 ± 0.32	0.66 ± 0.35	0.85 ± 0.46^{A}	0.52 ± 0.26^{B}	0.56 ± 0.31^{B}
CL35	0.77 (*27 of 35*)	0.71 (*42 of 59*)	-	-	0.71(*10 of 14*)^{a,b}	0.64 (*30 of 47*)^{a}	0.81(*30 of 37*)^{b}
Pregnancy rate	0.37 (*11 of 30*)	0.48 (*24 of 50*)	0.50 (*30 of 60*)^{a}	0.29 (*7 of 24*)^{b}	-	-	-

^{A,B} Within rows of each group assignment, values with different superscripts differ ($P \leq 0.05$) between groups.

^{a,b} Within rows of each group assignment, values with different superscripts differ by tendency ($0.05 < P \leq 0.10$) between groups.

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