Extended lactation in high-yielding dairy cows. I. Effects on reproductive measurements

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ABSTRACT

The objective of this prospective field study was to evaluate the effects of extending the lactation period on various reproductive measurements of high-yielding Holstein cows. On 40 d in milk (DIM), cows were gynecologically examined (transrectal palpation, sonography, vaginoscopy). Cows without signs of clinical endometritis were blocked by parity and were randomly allocated to 1 of 3 experimental groups with a voluntary waiting period (VWP) of 40, 120, and 180 d, respectively (G40, $n = 135$; G120, $n = 141$; G180, $n = 139$). Cows of G120 and G180 were reexamined at the end of the VWP. If natural estrus was detected within 46 d after the end of the VWP, an artificial insemination was performed. If no estrus was detected, the respective cows were synchronized by applying the classical Ovsynch protocol. We found no difference in the proportion of cows in which estrus was detected between 40 to 86 DIM or in the days to first estrus between the 3 groups. Estrus detection in this period was lower in cows with body condition score <3 on 90 DIM compared with body condition score ≥ 3 (61.5 vs. 76.0%) and in cows with high energy-corrected milk production (ECM) on 92 DIM [58.6 vs. 70.1%, for cows with higher and lower than the median (39.9 kg) ECM, respectively]. The proportion of cows that estrus was detected within 46 d after the VWP was greater in G120 (88.9%) and G180 (90.8%) compared with G40 (70.4%). These effects were more apparent in cows with high ECM. The rate of estrus detection and of becoming pregnant in this period was greater for G120 (hazard ratio = 2.2 and 1.6, respectively) and for G180 (hazard ratio = 2.4 and 1.8) compared with

G40. Cows in both groups with extended lactation had greater overall first service conception rates (G120 = 48.9%; G180 = 49.6%) and a lower number of services per pregnant cow (G120 = 1.56 ± 0.1 ; G180 = 1.51 ± 0.1) compared with G40 (36.6%; 1.77 ± 0.1). We observed no difference in pregnancy loss or in the proportion of cows culled up to 305 d of lactation between the 3 groups. The number of Ovsynch protocols per 1,000,000 kg of ECM was reduced by 75% in G180 and by 74% in G120 compared with G40 (5.9 vs. 7.1 vs. 25.1). In conclusion, extending the lactation of dairy cows can improve main reproductive measurements in high-yielding cows.

Key words: extended lactation, dairy cow, fertility

INTRODUCTION

Parity is the prerequisite for milk production. A 12-mo calving interval is usually proposed traditionally as the economically most cost-effective breeding pattern (Holmann et al., 1984); that is, cows should conceive before 85 DIM. However, the remarkable increase in milk yield in dairy cattle over the past decades has been accompanied by a decrease in reproductive efficiency (Lucy, 2001; Pryce et al., 2004; Inskeep and Dailey, 2005), which makes a 12-mo calving interval difficult to achieve.

The voluntary waiting period (**VWP**) as a component of reproduction management describes the postpartum interval during which the cows are deliberately not inseminated. On most commercial dairy farms, a VWP of 40 to 60 d is applied. In high-yielding dairy cows, a substantial increase of the VWP to prolong the lactation period may prove beneficial for various reasons. First, it is generally accepted that negative energy balance during early lactation has detrimental effects on fertility (Beam and Butler, 1999; Butler, 2005; Wathes et al., 2007) and may be avoided if the first service is

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performed in later lactation. Second, a longer VWP may prevent drying off during high milk production, which can negatively affect the health status of udder during the dry period and the subsequent lactation (Bates and Dohoo, 2016). Third, the transition period represents a time of increased disease and culling risk for the cow, as 50% of production diseases and 30% of involuntary cullings occur in the first 30 DIM (Roemer, 2011). Thus, the replacement of many short lactations with fewer longer ones may improve longevity of cows. Finally, with short VWP, too many calves are being born, as the broad use of sexed semen has facilitated the production of replacement heifers and the prices for male Holstein calves are very low.

To overcome the aforementioned problems, the extension of the lactation period has been proposed (Borman et al., 2004; Knight, 2005) and studied mostly in pasture-based systems (Butler et al., 2006; Kolver et al., 2007; Kay et al., 2009) but also, to a smaller extent, in intensive production systems (Arbel et al., 2001). Several retrospective studies have analyzed and evaluated data from cows with long calving intervals (Vargas et al., 2000; Grossman and Koops, 2003; Mellado et al., 2016). However, those studies included cows that failed to conceive during the desired period (i.e., subfertile animals). Only a few preplanned studies tested the effect of extending the VWP on fertility. Larsson and Berglund (2000) reported that extending the calving interval from 12 to 15 mo had weak, albeit positive, effects on the reproductive efficiency of high-yielding cows. Other studies revealed an improvement in conception rates at first AI (Schindler et al., 1991) or a reduction of the number of AI per conception (Harrison et al., 1974; Whitmore et al., 1974) when the animals were first inseminated later in lactation (Tenhagen et al., 2003; Chebel and Santos, 2010). In contrast, the results of Schneider et al. (1981) suggested negative effects of a longer VWP. For animals whose breeding period started at first observed estrous after either 50 or 80 DIM, the average number of AI per conception was 1.50 and 1.95, respectively. Unfortunately, further reproductive measurements were not evaluated in that study and the results are outdated.

The purpose of our study was to examine the effects of extending the lactation in high-yielding cows by extending the VWP up to 180 DIM on different aspects of productivity and profitability. The hypothesis we tested was that the extension of the VWP would result in enhanced fertility of high-yielding dairy cows.

MATERIALS AND METHODS

Animals and Experimental Design

The study took place at a commercial dairy farm in Saxony, Germany. Based on data from 2012, the farm had a total of 1,092 lactating Holstein-Friesian cows and a mean milk production of 11,488 kg/305 d (4.09% fat, 3.27% protein). Herd fertility was characterized by a 32% first-service conception rate (**FSCR**), 2.6 inseminations per pregnancy and a calving interval of 422 d on average. Animals were housed indoors in a freestall barn with a concrete slatted floor and cubicles with mattresses. All animals at the farm were housed, fed, and milked under the same conditions and they were dried off 6 wk before the expected calving date or when the average daily milk production was under 15 kg/d for 5 consecutive days. Lactating cows were milked with a total of 21 automatic milking systems (**AMS**; Lely Astronaut A4, Maassluis, the Netherlands). On average, each AMS was used for 47 cows (3.2 milkings per cow per day). A mixed ration (Table 1) was allocated by a stationary conveyor belt 4 times a day ad libitum, leading to refusals before the next feeding of roughly 2%. Additional amounts of the concentrate used in the mixed ration (Table 2) were fed at the AMS based on the individual milk production level of each cow. Cows producing 34 kg of ECM received 2.7 kg of concentrates per day and 0.173 kg/kg of additional milk yield. Thus, in total, up to 7.2 kg of concentrates were offered at the AMS. Feeding of dry cows was performed twice daily using a feed mixer (Tables 1 to 3). The dairy herd software HERDE (DSP Agrosoft GmbH, Paretz, Germany) was used to record production and health data. The herd was vaccinated and checked for bovine herpesvirus 1 twice a year; serologically positive animals were culled. The farm was free from bovine viral diarrhea virus infections.

For the purpose of the study, animals that calved between January 2013 to December 2013 underwent daily health evaluation between parturition and 10 DIM. Cows that did not suffer severe dystocia, puerperal diseases (septicemic mastitis or metritis accompanied with a rectal temperature of $\geq 39.5^{\circ}\text{C}$), or life-threatening illness were selected to undergo an examination of their reproductive system at 40 DIM by transrectal palpation, vaginoscopy, and sonography

Table 1. Constituents of the mixed ration fed during the study period

Item (% of DM)	Lactation	Far off ¹	Close up ²
Corn silage	43.2	9.1	46.9
Grass silage	28.4	49.0	21.7
Grass straw		28.2	13.0
Molasses	3.2		
Concentrates	25.2	13.8	18.4

¹Day of drying off until d 21 before expected calving.
²Day 21 before expected calving until calving.

Table 2. Composition of the concentrates (% of DM) fed during the study period

Item	Lactation	Far off ¹	Close up ²
Rapeseed meal	32.9		
Aldehyde-treated rapeseed meal ³	5.9		
Expeller soybean meal	18.3	13.0	61.4
Sunflower meal	7.2	34.0	2.1
Malt culms		42.4	26.0
Dried molasses	9.7		
Wheat middlings	9.5		
Wheat	1.3		
Corn	5.9		
Calcium salt of palm fatty acids ⁴	6.3		
Minerals	2.0	5.4	9.7
Calcium carbonate	0.7	0.9	
Sodium chloride	0.3	4.2	0.7
DCAD (mEq)	209	288	69

¹Day of drying off until d 21 before expected calving.²Day 21 before expected calving until calving.³Mervo Best, Nuscience, Utrecht, the Netherlands.⁴Nurisol, Heinrich Nagel KG, Hamburg, Germany.

(Tringa linear, Esaote/Pie Medical, Maastricht, the Netherlands) equipped with a 5-MHz linear transducer. Assessed findings during the examination included the presence and characteristics of vaginal discharge, size, contractility, and horn symmetry of the uterus and the ovaries with their corresponding structures. Reproductive disorders were diagnosed based on the following criteria: cows exhibiting abnormal vaginal discharge or hyperechogenic content in the uterus were considered to suffer from an endometritis and were excluded from the study. Additionally, all cows with incomplete involution of the uterus (e.g., no repositioning in the pelvic canal, no symmetry of the horns, or both) were excluded from the study. Cows with cystic ovarian follicles (**COF**) were diagnosed based on the presence of thin-walled

(<3 mm) cystic structure on the ovary with a diameter greater than 25 mm in the absence of a corpus luteum (Vanholder et al., 2006) and were treated with a single intramuscular injection of GnRH (gonadorelin; Oestracton, IDT Biologika, Dessau-Roßlau, Germany). No animals were detected with luteinized cystic structures. Animals with follicles smaller than 12 mm in diameter without a corpus luteum and no recorded estrus up to 40 DIM were characterized as animals with potential inactive ovaries (**PIO**). No treatment was undertaken for these cows.

Sample size estimation was performed and a difference of 10% in main binary variables (i.e., proportion of cows with estrus detection, FSCR) was considered significant in a univariate analysis. Animals were blocked by parity and were randomly (based on the last digit of the ear tag) allocated to 1 of the 3 experimental groups, VWP of 40 d (**G40**; n = 135), 120 d (**G120**; n = 141), and 180 d (**G180**; n = 139). A second ultrasonographic examination of the reproductive system was performed at the end of the VWP of groups G120 and G180 (i.e., at 120 and 180 DIM, respectively). Documentation and treatment of these animals was the same as described above.

Reproductive Management of Animals, Estrus Detection, and Pregnancy Diagnosis

Estrus detection was based on the Lely Qwes-HR System, which evaluates animal locomotion and rumination activity collected via the AMS and on supplementary observation from the personnel of the farm. Estrus signs (vulvar hyperemia and clear mucus discharge) were confirmed with vaginoscopy by means of a speculum.

Table 3. Rations fed during the study period

Item	Lactation 37 kg/d ECM	Lactation 50 kg/d ECM	Far off ¹	Close up ²
Assumed DMI (kg/d)	22.65	24.90	12.5	12.3
MJ of NE _L /kg of DM	7.30	7.33	5.5	6.6
MJ of NE _L /d	165.3	182.5	68.8	81.2
CP (g/d)	3,740	4,413	1,568	1,781
Undegradable protein (g/d)	1,067	1,389	319	448
Crude fat (g/d)	1,093	1,273	318	358
Crude fiber (g/d)	3,464	3,706	3,436	2,360
Starch (g/d)	4,600	4,906	562	2,597
Sugar (g/d)	1,150	1,425	538	407
Calcium (g/d)	162	198	60	36
Phosphorus (g/d)	100	120	42	46
Sodium (g/d)	42	54	36	24
Magnesium (g/d)	63	72	25	49
Potassium (g/d)	329	329	235	173
Sulfur (g/d)	56	56	30	52

¹Day of drying off until d 21 before expected calving.²Day 21 before expected calving until calving.

The animals of all groups received an AI at observed estrus during the first 40 d after the end of the VWP. A subsequent pregnancy check was conducted once a week by sonography between d 42 and 49 postinsemination. Findings used as criteria for a pregnancy diagnosis included the presence of an intact embryo with obvious heart pulse. In case of dubious findings, the sonographical examination was repeated a week later. A second pregnancy check by rectal palpation was performed within 90 to 100 d after the successful AI. For cows diagnosed as pregnant 40 d postinsemination and non-pregnant 100 d postinsemination, embryonic or early fetal mortality was stated.

Cows exhibiting estrus before pregnancy diagnosis were reinseminated with deposition of the semen in the cranial part of the cervix. In case of multiple inseminations, the date of the successful insemination was determined based on the sonographic findings and confirmed retrospectively from the calving date. Cows with a negative pregnancy diagnosis as well as those that did not exhibit estrus during the first 40 d after the end of VWP were synchronized using the classical Ovsynch protocol [100 µg of GnRH; 7 d later 0.5 mg of cloprostenol (PGF Veyx forte, Veyx-Pharma, Schwarzenborn, Germany); 48 h later 100 µg of GnRH; insemination 14–20 h thereafter using frozen-thawed semen from multiple sires of proven fertility]. Ovsynch was initiated once a week for practical reasons. Thus, the actual estrus-detection period was extended up to 46 d after the end of the VWP for the cows with VWP ending on a Tuesday. A cow found not pregnant but without having showed estrus signs was resynchronized following the same protocol. Cows that were not pregnant after a total of 3 inseminations were classified as subfertile.

All animals in G40 received at least 1 AI, whereas 6 animals of G120 and 8 animals of G180 had to be removed from the study because they were culled before the VWP ended. Thus, in our study, effective sample size was 401 cows. Moreover 4 animals in G40, 6 animals in G120, and 6 animals in G180 were culled before having received 3 AI (i.e., before completing the experimental protocol). These animals were eligible for analysis only for a part of the reproductive measurements.

Variables Under Investigation

The BCS of all cows was scored on the day of the parturition (BCS0) and on 60, 90 (BCS90), 120, and 180 DIM using a score between 1 and 5 in 0.25 increments (Edmonson et al., 1989). Based on the measurements at d 0 and 90, cows were divided into groups of low (BCS0 n = 49; BCS90 n = 322) or high BCS (BCS0

n = 352; BCS90 n = 79) if the score was <3.0 or ≥3.0, respectively. The effect of BCS change between d 0 and 90 was estimated, with animals categorized in those losing <1 (n = 267) and ≥1 (n = 134) BCS. Season was divided in warm (from May 1 to October 31, n = 215) and cool (from November 1 to April 30, n = 186) period. Additionally, the cows were classified into 2 parity groups: primi- (n = 152) and pluriparous cows (n = 249). Monthly visits were conducted in the farm by the State Control Association (Landeskontrollverband, Lichtenwalde, Germany) to perform production measurements at cow level (milk yield, fat, protein, SCC, and so on). For the purpose of the present study, milk yield was documented based on the third monthly test between 79 and 104 DIM (lower and upper quartile). It was calculated as ECM (4.0% fat and 3.4% protein; GfE, 2001):

$$\text{ECM (kg)} = \text{milk (kg)} \times (0.38 \times \text{fat\%} + 0.21 \times \text{protein\%} + 1.05) / 3.28.$$

Animals were categorized as low (n = 197) and high (n = 198) milk production cows (ECM3) based on the median ECM, which was 39.9 kg for all cows in the third monthly test. Data from 6 cows were not available due to technical reasons. Additionally, daily milk yield at the end of the VWP (d 40, 120, and 180 for G40, G120, and G180, respectively) and the ECM at the nearest time point to the end of the VWP (second monthly test between 46 and 64 DIM for G40, fourth monthly test between 115 and 142 DIM for G120, and sixth monthly test between 187 and 206 for G180, lower and upper quartiles) have been recorded.

Statistical Analysis

Statistical analyses were conducted using SAS version 9.3 (SAS Institute, Cary, NC). To compare the 3 groups, G40, G120, and G180, with respect to reproductive, productive, and body measurements, we first performed a univariate analysis. Differences in binary variables were evaluated by Chi-squared analysis (PROC FREQ). Differences in continuous variables were analyzed using the Kruskal-Wallis and Wilcoxon's 2 sample test (PROC NPAR1WAY) due to the underlying distribution of the data.

To determine the effect of a set of variables on binary outcomes, generalized linear mixed models using PROC GLIMMIX were used. In the statistical models, the following effects were included: group (G40 vs. G120 vs. G180), parity, season, BCS0 and BCS90, BCS change between parturition and d 90, and ECM3. All 2-way interactions with the group were included into the mod-

els. Backward stepwise selection with level of stay of 0.10 was used. When group had a significant effect on a variable, pairwise comparisons were performed using the LSmeans statement. The rate of estrus detection between 40 and 86 DIM and 46 d after the end of the VWP, the rate of becoming pregnant, and the median days to estrus and to pregnancy were evaluated using the Kaplan Meyer survival curves (PROC LIFETEST) and Cox's proportional hazards regression models (PROC PHREG). Endpoint for a cow to express estrus was set to 46 d and to become pregnant to 200 d after the end of the VWP. The aforementioned effects were tested in these models. Results are presented as mean $\pm$ standard error of the mean and median $\pm$ median absolute deviation, unless stated otherwise. Differences were considered to be statistically significant at $P < 0.05$ and as a tendency at $0.05 \leq P < 0.10$.

RESULTS

No significant differences were detected between the 3 groups with respect to mean lactation number, BCS0, BCS90, and BCS loss between d 0 and 90 ($P > 0.10$). The median BCS0 and BCS90 were 3.25 and 2.5 for all cows, respectively. The median BCS loss between d 0 and 90 was 0.75. We found a significant decrease of the BCS between d 0 and 60 in all groups (0.75 ± 0.25 , $P < 0.0001$, for all groups). No significant decrease of the BCS was noticed between d 60 and 120 for G120 and between d 60 and 180 for G180 (0 ± 0 and 0 ± 0.25 , $P = 0.62$ and $P = 0.41$, respectively). The mean lactation number averaged for all cows in the experiment was 2.34 ± 0.08 . Daily milk production at the end of the VWP differed between G40 and G180 (44.1 ± 0.7 vs. 39.1 ± 0.7 , $P < 0.0001$) and between G120 and G180 (42.8 ± 0.7 vs. 39.1 ± 0.7 , $P = 0.001$), but not between G40 and G120 ($P = 0.42$). Regarding the ECM at the nearest time to the end of the VWP, this differed between G40 and G180 (40.1 ± 0.6 vs. 36.7 ± 0.6 , $P = 0.0005$) and tended to differ between G120 and G180 (38.6 ± 0.6 vs. 36.7 ± 0.6 , $P = 0.08$), but not between G40 and G120 ($P = 0.21$).

Reproductive Measurements Between d 40 and 86 of Lactation

The gynecological examination that took place at 40 DIM revealed no difference between the 3 groups regarding the proportion of animals that showed PIO (15.6% for both G40 and G120 and 22.1% for G180, $P = 0.27$). Moreover, we found no significant differences between the 3 groups regarding the proportion of animals that exhibited COF ($P = 0.41$, Table 4). We observed no group effect on the proportion of animals in which estrus was detected between d 40 and 86 of the lactation (70.4 vs. 58.5 vs. 64.1% for G40, G120, and G180, respectively; $P = 0.13$; Table 4). We noted no group effect on days to first estrus after the 40-d period (22 vs. 25 vs. 20.5 median days for G40, G120, and G180, respectively; $P = 0.54$). We found a statistical tendency ($P = 0.08$) for an effect of parity on the proportion of PIO (21.7% in first parity vs. 15.3% in greater parities) and an interaction of parity with group ($P = 0.06$). Specifically, in G40 and G180, more first-parity cows showed PIO (19.2 vs. 13.3% and 34.8 vs. 15.3%, respectively), whereas in G120 this effect was reversed (13% in first parity compared with 17.3% in greater parities). Fewer animals with PIO ($P = 0.0004$), with low BCS90 ($P = 0.02$), and with high milk production ($P = 0.01$) were detected in estrus in this period compared with cows with active ovaries (46.5 vs. 68.2%), with higher BCS90 (61.5 vs. 76.0%), and with lower milk production (58.6 vs. 70.1%; Table 5). Only PIO had a significant effect on days to first estrus after the 40-d period (27 median days for cows with PIO compared with 22 d when ovaries were active; $P = 0.03$).

Reproductive Measurements Within 46 d After the End of the VWP

Group had an effect on the proportion of animals with PIO at the end of the VWP (15.6 vs. 8.9 vs. 2.3% for G40, G120, and G180, respectively; $P = 0.0002$). Moreover, an interaction with parity was evident (P

Table 4. Reproductive performance between DIM 40 and 86 for cows grouped according to a voluntary waiting period of 40 (G40), 120 (G120), and 180 d (G180)

Variable	Group (no./no.)			P-value
	G40	G120	G180	
PIO ¹ (%)	15.6 (21/135)	15.6 (21/135)	22.1 (29/131)	0.27
COF ¹ (%)	3 (4/135)	4.4 (6/135)	1.5 (2/131)	0.41
Estrus detection (%)	70.4 (95/135)	58.5 (79/135)	64.1 (84/131)	0.13
Days to first estrus ²	22 (95)	25 (79)	21 (84)	0.54

¹PIO = potential inactive ovaries; COF = cystic ovarian follicle.

²From 40 DIM onward (median).

Table 5. Effect of parity, ECM yield, BCS and its loss, and season on main reproductive parameters

Variable	Primiparous	Pluriparous	ECM ³ <39.9 kg/d	ECM ³ ≥39.9 kg/d	BCS ⁹⁰ ² <3	BCS ⁹⁰ ² ≥3	BCS loss ³ <1	BCS loss ³ ≥1	Warm season ⁴	Cold season ⁴
Number of cows	152	249	197	198	322	79	267	134	215	186
Estrus detection 40–86 DIM (%)	66.5	63.1	70.1*	58.6*	61.5*	76.0*	67.0	59.0	62.3	66.7
Days to 1st estrus 40–86 DIM (median)	20	24	20.5	24	23	20	22	24	22	24
PIO ⁵ 40 DIM (%)	21.7	15.3	20.3	15.2	17.4	19.0	17.6	17.9	19.5	15.6
COF ⁶ 40 DIM (%)	1.3	4.0	2.5	3.5	3.4	1.3	2.3	4.5	3.3	2.7
FSCR ⁵ (%)	51.3*	41.1*	50.0	40.4	45.9	41.0	46.6	41.8	45.3	44.6
AI per pregnancy (mean ± SEM)	1.58 ± 0.1	1.64 ± 0.1	1.57 ± 0.1	1.67 ± 0.1	1.60 ± 0.1	1.69 ± 0.1	1.62 ± 0.1	1.61 ± 0.1	1.61 ± 0.1	1.63 ± 0.1
DO – VWP ⁵ (median)	33	37	33*	40*	35	35	35	35	35	35
Subfertile ⁶ (%)	8.9*	19.7*	12.3	18.0	15.7	15.1	14.8	17.2	17.1	13.9

¹Milk yield calculated as ECM per day at the third monthly test.²Body condition score (scale 1–5) at 90 DIM.³Loss of BCS points (scale 1–5) between 1 and 90 DIM.⁴Warm season: May 1–October 31; cold season: November 1–April 30.⁵PIO = potential inactive ovaries; COF = cystic ovarian follicle; FSCR = first service conception rate; DO = days open, VWP = voluntary waiting period.⁶Defined as the proportion of cows that did not conceive after the third AI.*Values within a row with significant ($P < 0.05$) effect on the corresponding reproductive parameter.

= 0.09). Specifically, more primiparous cows (19.2%) compared with pluriparous (13.3%) had PIO in the G40, whereas this effect was reversed in the G120 and G180 (1.9 vs. 13.6% and 0 vs. 3.5% for primi- and pluriparous, respectively).

Group had a highly significant effect on the proportion of animals that exhibited estrus ($P < 0.0001$). Specifically, 70.4% of the G40 cows showed estrus within 46 d after the VWP compared with 88.9% and 90.8% of the G120 and G180 cows, respectively (Table 6); moreover, a significant interaction with milk production was apparent ($P = 0.001$). In the subgroup of cows with low ECM3, we found no statistical difference regarding the proportion of animals that exhibited estrus within 40 d between the 3 groups (81.7 vs. 87.5 vs. 90.7% for G40, G120, and G180, respectively; $P = 0.3$). However, in high-milk production cows, only 57.1% showed estrus within 46 d in G40 compared with 91.7 and 93.3% in G120 and G180, respectively ($P < 0.0001$). Additionally, the median days from the end of the VWP to estrus was shorter in G120 and the G180 compared with G40 (Table 6 and Figure 1). An interaction between group and ECM3 was evident in this variable as well ($P = 0.06$). Potentially inactive ovaries at the end of the VWP influenced the proportion of animals that exhibited estrus within 46 d (84.9% showed estrus with active ovaries vs. 66.7% with PIO; $P = 0.005$). Moreover, we found a significant interaction with group ($P = 0.01$), as this effect was noticed only in G40 (73.7% with active ovaries were detected in estrus vs. 52.4% with PIO), but not in the G120 (83.3 vs. 89.4%, respectively) or in the G180 (100 vs. 90.6%, respectively). No significant differences were detected between the 3 groups regarding the proportion of animals with COF at the end of the VWP due to the low number of animals with this condition in the present study ($P = 1.0$; Table 6).

Regarding the proportion of animals that became pregnant within 46 d after the end of the VWP, the same pattern as mentioned above was evident; group, ECM3, and their interaction proved significant. Cows assigned to G40 showed lower overall conception rate (34.1%) within 46 d compared with G120 and G180 (56.4 and 56.5%, respectively; $P = 0.0001$; Table 6). However, we found no difference in the number of services per conception between the 3 groups (1.18 ± 0.1 vs. 1.25 ± 0.0 vs. 1.18 ± 0.0 for G40 vs. G120 vs. G180, respectively; $P = 0.43$; Table 6). The lower conception rates were more apparent in high-milk production cows, in which a subgroup only 17.5% of G40 conceived within 46 d compared with 53.3 and 56% of the G120 and G180 cows, respectively ($P < 0.0001$). Generally, high-milk production cows had lower conception rates in this period than low-milk production cows (42.9 vs.

Table 6. Reproductive performance within 46 d after the end of the voluntary waiting period of 40 d (G40, DIM 40–86), 120 d (G120, DIM 120–166) and 180 d (G180, DIM 180–226), respectively

Variable	Group (no./no.)			P-value
	G40	G120	G180	
PIO ¹ (%)	15.6 (21/135) ^a	8.9 (12/135) ^b	2.3 (3/131) ^b	0.0002
COF ¹ (%)	3.0 (4/135)	3.0 (4/135)	3.1 (4/131)	1.0
Estrus detection (%)	70.4 (95/135) ^a	88.9 (120/135) ^b	90.8 (119/131) ^b	<0.0001
Days to first estrus ²	22 (95) ^a	12 (120) ^b	9 (119) ^b	<0.0001
Pregnant (%)	34.1 (46/135) ^a	56.4 (75/133) ^b	56.5 (74/131) ^b	0.0001
AI per pregnancy	1.18 ± 0.1 (46)	1.25 ± 0.0 (75)	1.18 ± 0.0 (74)	0.43

^{a,b}Values within a row with different superscripts differ ($P < 0.05$).

¹PIO = potential inactive ovaries; COF = cystic ovarian follicle.

²From the end of the voluntary waiting period (median).

³Two cows of the G120 were culled before first pregnancy examination.

55.4%, $P = 0.01$). However, number of services per conception did not differ between cows with low (1.19 ± 0.0) versus high (1.21 ± 0.0 , $P = 0.87$) milk production. Although in univariate analysis parity had a significant effect on conception rates as well (primiparous 56.7% vs. pluriparous 44.2%; $P = 0.02$), this effect did not remain in the final model, as it was evident only in the G40 group (primiparous 50.0% vs. pluriparous 24.1%; $P = 0.002$) and not in the extended lactation groups (primi- vs. pluriparous 65.4 vs. 50.6% for G120, and

54.4 vs. 57.5% for G180; $P = 0.10$ and $P = 0.71$, respectively).

Reproductive Measurements Overall

Group and parity had an effect on the overall first service conception rate and group had an effect on the number of services of pregnant cows. Specifically, 36.6% of the G40 conceived with 1 AI, whereas this proportion was 48.9% for G120 and 49.6% for G180 (P

Figure 1. Kaplan-Meier survival curves for the proportion of cows not seen in estrus between the end of the voluntary waiting period (VWP) and 46 d onward in the 3 lactation groups. Median days to the expression of estrus were 31 d for VWP of 40 d (G40; 95% CI = 26–37), 14 d for VWP of 120 d (G120; 95% CI = 11–16), and 12 d for VWP of 180 d (G180; 95% CI = 9–18). The rate of estrus detection was higher for G120 (hazard ratio = 2.2; 95% CI = 1.7–2.9) and for G180 (hazard ratio = 2.4; 95% CI = 1.8–3.2) compared with G40 (both $P < 0.0001$).

= 0.05, Table 7). Cows of the G40, G120, and G180 required 1.77 ± 0.1 , 1.56 ± 0.1 , and 1.51 ± 0.1 services to conceive, respectively ($P = 0.04$, Table 7). Primiparous cows showed FSCR of 51.3% compared with 41.1% for pluriparous cows ($P = 0.04$, Table 5). Although a greater proportion of the G120 conceived after an OvSynch protocol compared with G40 and G180 (53.3 vs. 27.5 vs. 33.3% for G120, G40, and G180, respectively), the number of animals in the extended lactation groups was too low (15 in G120 and 12 in G180) to detect statistical differences. The median days from the end of the VWP to pregnancy were shorter and the rate of pregnancy was greater in G120 and G180 compared the G40 (Table 7 and Figure 2). Milk yield influenced this variable as well, as low-milk production cows needed 33 d to become pregnant after the end of the VWP compared with 40 d for high-milk production cows ($P = 0.04$, Table 5). The proportion of subfertile cows (i.e., >3 AI) was influenced only by parity (primiparous 8.9% vs. pluriparous 19.7%; $P = 0.002$). Pregnancy loss and proportion of animals culled up to 320 d of the study did not differ between the 3 study groups (Table 7); however, we noted a tendency ($P = 0.09$) for a greater percent of animals to be culled from begin to end of study in the extended lactation groups (11.1 vs. 17 vs. 20.9% for G40, G120, and G180, respectively).

DISCUSSION

The prospective field study presented allowed us to characterize the effects of varying the VWP on herd fertility, as all cows experienced uniform feeding, housing, and milking management on a large-scale dairy

farm. The idea behind the extension of the VWP is that the onset of breeding takes place fairly after the high-risk period of puerperium and postpartal negative energy balance (NEB; Knight, 2005), which should result in improved herd fertility. This is crucial, as the genetic selection toward milk production has led to a high predisposition of dairy cows to NEB and NEB-mediated diseases (Veerkamp et al., 2003; Leroy et al., 2017) associated with a reduced fertility (Lucy, 2007; Lucy, 2016).

The underlying pathophysiologic mechanisms explaining the interaction between NEB and subfertility are complex and include (1) the suppression of pituitary luteinizing hormone release in face of a NEB (Jolly et al., 1995; Beam and Butler, 1999); (2) negative effects of peripheral metabolite concentrations being typical for cows experiencing NEB (e.g., high nonesterified fatty acids and low serum glucose, insulin, and IGF-I levels; Butler, 2001; Butler, 2005; Schlamberger et al., 2010) on oocyte quality, follicular growth, estrogen production from dominant follicles, estrus expression; and embryo survival (Leroy et al., 2004; Wathes et al., 2007; Lüttgenau et al., 2016); and (3) an increase of hepatic hormone clearance of progesterone and estrogens during peak lactation (Sangsritavong et al., 2002; Vasconcelos et al., 2003). Thus, the period of postpartal anovulation may be prolonged in high-yielding dairy cows (Rhinehart et al., 2009) and, concomitantly, the anestrus period is extended (Beam and Butler, 1999; Pryce et al., 2004; Butler, 2005).

Exhibition of estrus and its subsequent detection is pivotal for a good herd reproductive efficiency. It is largely influenced by numerous environmental, management, and health factors, such as barn surface type (Vailes

Table 7. Overall reproductive performance of cows grouped according to a voluntary waiting period of 40 d (G40), 120 d (G120) and 180 d (G180)

Variable	Group (no./no.)			P-value
	G40	G120	G180	
FSCR ¹ (%)	36.6 (49/134) ^{2b}	48.9 (65/133) ^{2a}	49.6 (65/131) ^a	0.05
AI per pregnancy	1.77 ± 0.1 (106) ^a	1.56 ± 0.1 (112) ^b	1.51 ± 0.1 (107) ^b	0.04
DO ¹	92 (106) ^c	148 (112) ^b	204 (107) ^a	<0.0001
DO-VWP ¹	52 (106) ^a	28 (112) ^b	24 (107) ^b	<0.0001
Subfertile ³ (%)	19.1 (25/131) ⁴	13.2 (17/129) ⁴	14.4 (18/125) ⁴	0.38
Pregnancy loss ⁵ (%)	3.9 (4/104) ⁶	3.6 (4/112)	1 (1/105) ⁶	0.37
Culled $\leq$ 320 DIM (%)	7.4 (10/135)	11.4 (16/141)	10.1 (14/139)	0.53

^{a-c}Values within a row with different superscripts differ ($P < 0.05$).

¹FSCR = first service conception rate; DO = days open (median); VWP = voluntary waiting period.

²Two cows of the G120 and 1 of the G40 were culled before first pregnancy examination.

³Defined as the proportion of cows that did not conceive after the third AI.

⁴Four cows of the G40 and 6 cows of the G120 and G180 were culled before receiving 3 AI.

⁵Defined as the proportion of cows being pregnant at d 40 postinsemination and nonpregnant at d 100 post-insemination.

⁶Two cows of the G40 and G180 were culled before 100-d confirmation of pregnancy.

Figure 2. Kaplan-Meier survival curves for the proportion of nonpregnant cows in the 3 lactation groups. Median days to the pregnancy was 65 d for voluntary waiting period (VWP) of 40 d (G40; 95% CI = 52–74), 41 d for VWP of 120 d (G120; 95% CI 26–50), and 26 d for VWP of 180 d (G180; 95% CI 22–41). The rate of becoming pregnant was higher for G120 (hazard ratio = 1.6; 95% CI = 1.2–2.0) and for G180 (hazard ratio = 1.8; 95% CI = 1.4–2.4) compared with G40 ($P = 0.001$ and $P < 0.0001$, respectively).

and Britt, 1990; Saint-Dizier and Chastant-Maillard, 2012), methods for estrus detection (Saint-Dizier and Chastant-Maillard, 2012), and lameness (Walker et al., 2010) or ketosis (Rutherford et al., 2016). Especially in high-milk production cows, the efficiency of estrus detection is low due to poor or total absence of visible estrus, known as silent ovulation (Yoshida and Nakao, 2005; Yániz et al., 2008). This phenomenon may affect one third of cows in the first 90 DIM (Ranasinghe et al., 2010), which is very close to the results of the present study (Table 4 and 5). In the study of Lüttgenau et al. (2016), higher nonesterified fatty acid levels were associated with a lower probability of inseminating a cow. Consistently, a high level of milk production and a low BCS90 had a significant negative effect on the proportion of animals without estrus detection between 40 and 86 DIM. Reasonably, estrus could be easier detected in cows of G120 and G180 after the end of the extended VWP. This observation indicates that high-milk production cows are those who profit mainly in terms of estrus expression by extending the VWP. This positive relationship between length of VWP and estrus detection was also evident in the study of Larsson and Berglund (2000). In accordance with their results, in our study only one third of the cows in G120 and G180

compared with the control cows in G40 needed to be synchronized hormonally due to the inability to detect estrus within 46 d after the VWP.

Although our definition of inactive ovaries (PIO) was not conclusive due to the structure of our protocol, associations between cows defined as inactive (with follicles smaller than 12 mm in diameter, without a corpus luteum, and no recorded estrus up to 40 DIM) and fertility traits from that point on were evident, especially in early lactation. Specifically, in accordance with the aforementioned studies, our results showed that, regardless of the groups, both the interval from parturition to first visible estrus and the proportion of cows not showing estrus within the first 86 DIM were greater for animals with PIO in early lactation (i.e., at 40 DIM) than in animals with definite functional ovaries. The extension of the VWP offered those cows the time needed to resume their ovarian activity at the beginning of the breeding period (i.e., at the end of the VWP), as indicated by the significant difference in the proportion of animals with PIO at the end of the VWP between control and experimental groups (Table 6). In addition, PIO early in lactation tended to occur more frequently in primiparous than in pluriparous animals. These findings agree with the study of Tanaka et al.

(2008), who showed that primiparous heifers need more time to resume their ovarian activity postpartum than pluriparous cows.

The results of our study showed that cows with a longer VWP had a better first AI conception rate and a lower number of AI per conception. The overall enhanced fertility (both estrus detection and successful insemination) in the extended lactation groups resulted in the reduction of the expected difference of open days between the control and the experimental groups. Thus, a difference of 80 d in VWP (between G40 and G120) resulted in a median difference of 56 open days. These results are in accordance with those from other studies that examined the fertility of cows deliberately inseminated at various DIM intervals. Larsson and Berglund (2000) found a decrease in the number of AI per conception (1.6 vs 1.9) and a tendency for improved first AI conception rate (56 vs. 49%) between cows bred for a 15-mo and for a traditional 12-mo calving interval (Larsson and Berglund, 2000). Gordon et al. (2010) reported that the efficiency of Ovsynch increases if the protocol is initiated between 76 to 105 DIM compared with 60 to 75 DIM. The positive effect of an extension of the VWP on the reproductive efficiency of cows timed inseminated after synchronization is further supported from the results of Stangaferro et al. (2018). Although in our study the low number of synchronized cows did not allow safe statistical conclusions to be drawn, a similar trend could be implied from the available results. Recent studies using algorithms for the construction of alternating decision trees also found a positive relationship between the length of the VWP and the reproductive efficiency, as indicated by conception rates (Scheifers et al., 2010) and the proportion of pregnant animals after the first AI (Caraviello et al., 2006).

Another aspect to consider in the context of extended lactation protocols are the cows with puerperal disorders. We deliberately chose to exclude endometritis cases to exclude a presumably major confounding factor that needed precise definition and diagnosis. However, uterine disorders interfere with ovarian cyclicity but improve with time, even without treatment (Tsousis et al., 2009). Moreover, animals with NEB have a delay in uterus clearance and involution (Wathes et al., 2007). Hence, we believe that the positive effect of extended lactation protocols on the reproductive efficiency of dairy cows under commercial farm conditions would have been even greater if endometritis cases would have been included. However, based on the results of the present study, we could not determine precisely the extent to which the enhanced fertility in the extended lactation groups was attributable to a better energy balance of the cows, to the increased DIM up to estrus

detection and insemination, or to their interaction, which additionally are factors confounded by nature. Cows in the extended lactation groups did not show any further decline of their BCS from d 60 onwards, which could have a positive effect on the reproductive measurements noticed in the present study.

According to the literature, our study provides clear evidence that the expenditures for fertility can be substantially reduced by extending the VWP and, thereby, the lactation period. Interestingly, no distinct differences in respect to the analyzed reproductive measurements were obvious comparing G120 and G180. Thus, in contrast to other studies, even a VWP of 180 d appears to be feasible as long as an appropriate persistency assures the economic viability of a lactation period of 500 d (results not shown). This approach, however, seems to be most appropriate for high-yielding herds with superior management; an involuntary extended lactation may be detrimental for substandard farms.

Dairy farmers' revenue is overwhelmingly based on the mean daily milk production of the cow, whereas disposal of the calf constitutes only minimal income. Nonetheless, the key figures and expenditures for fertility in a herd are still calculated based on the individual cow (e.g., insemination index, expenditures per successful insemination). Based on the economical framework, it may be more useful to relate the expenditures to the amount of milk produced as we did using the means of the 3 groups investigated. Accordingly, 30 to 35% less inseminations were needed in G180 and G120 per 10,000 kg of ECM compared with G40 ($G40 = 1.50$; $G120 = 1.02$; $G180 = 0.97$ inseminations/10,000 kg of ECM). Even more impressive was the number of Ovsynch protocols needed to produce 1,000,000 kg of ECM ($G40 = 25.1$, $G120 = 7.1$, $G180 = 5.9$). Hormone programs used to overcome the subfertility of high-yielding dairy cows have been highly criticized by the consumer in recent years (Pieper et al., 2016). Thus, a management tool to reduce their application in practice by 75% without affecting milk production may be attractive in the future. These considerations are the basis to suggest that new key indicators which are related to the amount of milk produced should be implemented to characterize herd fertility. The positive effects of extended lactations on fertility are only relevant to dairy farmers if economic viability is guaranteed. Further aspects of extended lactations are studied in a companion study (Niozas et al., 2019).

CONCLUSIONS

The results of the present study indicate that the extension of the VWP up to 180 DIM can significantly improve main reproductive measurements of dairy cows.

Moreover, these positive effects are more prominent in the higher yielders (in our study 50% of cows with the higher milk production). This criterion can be used to adjust breeding strategies in a farm. The productive and economic impact of such reproductive protocols is the subject of additional research.

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