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Consequences of extending the voluntary waiting period for insemination on reproductive performance in dairy cows

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ABSTRACT

The aim of the study was to evaluate the effect of extended voluntary waiting period (**VWP**) on ovarian cyclicity and reproductive performance of dairy cows. Holstein-Friesian dairy cows (N = 154) were blocked and randomly assigned to one of 3 groups with different VWP (50, 125 or 200 d: **VWP-50**, **VWP-125** or **VWP-200**). Milk samples were collected 3 times a week and analysed for progesterone concentration. Ovarian cycles were classified as: normal (18–24 days), short (<18 days) or prolonged (>24 days). For cows that became pregnant within 100 days after VWP, a VWP-200 d was related with fewer days until pregnancy after end of the VWP (19.4 d) compared with VWP-50 or VWP-125 (35.5, 37.3 d respectively). During 100 days (−50 until 50 d) around the end of VWP, cows in VWP-200 had a greater percentage of normal cycles (91.9 vs 58.0 %, $P < 0.01$) and a lower percentage of prolonged cycles (6.0 vs 32.7 %, $P = 0.01$) compared with cows in VW*P*-50. In the 4 weeks around the end of the VWP, cows in VWP-125 and VWP-200 had a lower milk yield compared with cows in VWP-50 (32.0, 27.5 vs 37.4 kg/d, $P < 0.01$). Inseminations continued until 300 days in milk, resulting in fewer pregnant cows for longer VWPs. In conclusion, extending the VWP from 50 to 125 or 200 days resulted in a greater percentage of cows with normal ovarian cycles and a lower milk yield around the end of VWP. Moreover, VWP-200 reduced days open after the end of the VWP, compared with VWP-50.

1. Introduction

The voluntary waiting period until first insemination (**VWP**) is the postpartum period during which cows are deliberately not inseminated, to give cows time to recover from negative energy balance (**NEB**) and resume normal ovarian cyclicity during this period (Chen et al., 2015). Traditionally, most dairy farms apply a VWP of 40–60 d, aiming at a 12-month calving interval for high milk production and economic reasons (Österman and Bertilsson, 2003). Using this traditional VWP, however, artificial insemination (**AI**) starts during a period of high milk yield (Ancker et al., 2006) when most of the cows are in NEB and mobilizing body reserves. After the traditional VWP, not all cows are immediately in estrous (Kawashima et al., 2012; Cheong et al., 2016) and first-service conception rates can vary from 26.7 % to 50.7 % (Tillard et al., 2008; Siddiqui et al., 2013). Starting AI during peak milk yield is associated with high reproductive failure (Santos et al., 2009), indicated by low conception rates after first insemination, more inseminations per

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pregnancy, and more days open (Lucy, 2001; Pryce et al., 2004; Inskeep and Dailey, 2005).

Extending the VWP by deliberately postponing the first insemination postpartum allows cows more time to recover from calving and the NEB. It can be hypothesized that extending the VWP will result in more regular ovarian cyclicity at the end of the VWP, which can be expected to be beneficial for reproductive performance. In an earlier study, extending the VWP from 60 to 150 d resulted in increased calving interval from 12 to 15 months, improved conception rate (50 % vs 41.5 %), reduced veterinary treatments for anestrus (5.3 % vs. 28.6 %) (Ratnayake et al., 1998), and decreased the number of inseminations per conception (1.9 vs 1.6) (Larsson and Berglund, 2000). In a more recent study, increasing the VWP from 40 to 120 or 180 days reduced the number of days open after the end of the VWP, and increased pregnancy rates for both extended VWP groups (Niozas et al., 2019). In another study, first service conception rate was similar (52.2 % vs 52.4 %) when VWP was extended from 50 d to 230 d (Bertillson et al., 1997). Furthermore, it has been discussed that extension of the VWP might increase the risk of development of ovarian cysts as well as weaker heat symptoms, making heat detection more difficult (Larsson and Berglund, 2000). Ambiguous consequences of a deliberately extended VWP on reproduction, as reported in earlier studies, might be related to limited contrast between study groups in VWP, in milk yield or in body condition after extending the VWP. It can be hypothesized that if extending the VWP results in a lower milk yield, better EB, and better body condition at insemination, this may be beneficial for ovarian cyclicity and further reproductive measures including pregnancy rate/AI and days open after the end of VWP. Therefore, the objective of this study was to investigate the consequences of a VWP of 50, 125 or 200 d on ovarian cyclicity, pregnancy rate/AI, and days open after the end of VWP, and to relate the days open after the end of VWP to ovarian cyclicity, milk yield, body condition score and body weight around the end of the VWP.

2. Materials and methods

2.1. Animals and housing

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Wageningen University & Research (the Netherlands) and complies with the Dutch law on Animal Experimentation (protocol number 2016. D-0038.005). This experiment was described earlier (Burgers et al., 2021b). In short, the experiment was conducted at Dairy Campus research farm (WUR Livestock Research, Leeuwarden, the Netherlands) and included 154 Holstein-Friesian dairy cows which were followed from December 2017 until January 2020. Of the cows in the experiment, 32 % were primiparous and 68 % were multiparous. These cows were selected based on: being pregnant with a Holstein-Friesian calf (no twin gestation), no clinical mastitis or high somatic cell count (**SCC**>250.000 cells) during the last 2 test days before dry off, and expected to be able to accomplish a full lactation. Cows were milked twice daily around 6 am and 6 pm in a 40-cow rotary milking parlor (GEA, Dusseldorf, Germany).

2.2. Experimental design

The experiment started with drying-off at 45 days before the expected calving date and cows were monitored for a complete subsequent lactation. Animals that were culled were followed until they were culled.

Cows were blocked for parity, expected calving date, calving interval, breeding value for persistency (**CRV**, Arnhem, the Netherlands) and expected fat and protein corrected milk (**FPCM**) based on their previous lactation in multiparous cows and breeding value for milk production in primiparous cows. The experiment included 154 cows in total, each block consisted of 3 cows. First, 50 blocks of 3 cows were formed. After removal of 2 cows before the end of VWP because of culling, 2 more blocks of 3 cows were added. Cows in each block were randomly assigned to one of the 3 treatments: a VWP of 50 d (**VWP-50**), 125 d (**VWP-125**), or 200 d (**VWP-200**).

Cows were artificially inseminated as soon as heat was detected after the end of VWP of 50, 125 or 200 days. Heat was detected by either the Nedap Smarttag (Nedap, Groenlo, the Netherlands) or visually by the animal caretaker. Heat attentions were noted by the animal caretakers and reported to AI service (CRV, Arnhem, the Netherlands) the same evening. The day after (around 7.00 am), all cows were rechecked to see whether new cows in estrus could also be inseminated that morning. All cows in heat were inseminated between 7.30 and 8.30 am. At 35–49 days after insemination, a veterinarian checked for pregnancy using ultrasound scanning, according to the standard protocol of Dairy Campus. Cows in all 3 groups were inseminated until 300 days in milk, thus cows in VWP-50, VWP-125, and VWP-200 were insemination during a period of 250, 175, and 100 days after the end of the VWP, respectively.

2.3. Rations

Ration composition was described earlier in detail (Burgers et al., 2021b). In short, dry cows received one ration over the entire dry period (45 d) with 5.66 MJ/kg dry matter and 13 % protein. The roughage in the dry period ration consisted of grass silage and corn silage (ratio 70:30) and was supplemented with wheat straw (30–40 % of the total ration). Cows received 1 kg concentrate per day from 10 days before the expected calving date onwards. Lactating cows received a partial mixed ration for their expected milk production (at 60 DIM: 36 kg/d; and at 305 DIM: 22 kg/d). The partial mixed ration consisted of grass silage, corn silage, and soybean meal supplemented with wheat straw for 22 kg milk, including 1 kg concentrate in the milking parlor. In addition, concentrate was supplied separate from the partial mixed ration in concentrate feeders (Manus VC5, DeLaval, Steenwijk, the Netherlands). Individual concentrate allowance was built up in 21 days to 9 kg for primiparous cows or 10 kg for multiparous cows. from 100 DIM onwards, concentrate allowance depended on milk production of the last 5 days. The ration was switched to the dry period ration a week before next dry-off.

2.4. Measurements

2.4.1. Milk sampling and progesterone assay

Each Monday, Wednesday and Friday from calving until confirmed pregnancy (as checked by the veterinarian), milk samples were collected in 10 mL tubes with bronopol as a preservative and stored at − 20 °C until analysis. Milk samples were used to determine ovarian cyclicity from calving until pregnancy using progesterone (**P4**) levels in milk analysed with a commercial ELISA kit (Ridgeway Science, Gloucester, UK). Analysis of milk progesterone was carried out according to the protocol of the manufacturer (Ridgeway Science as described earlier (Roelofs et al., 2006)). The intra-assay and inter-assay coefficients of variance were 9.2 % and 11.6 %, respectively.

Onset of luteal activity (**OLA**) was defined as two or more successive milk samples with P4 concentration of 2 ng/mL or higher. Ovarian cycle length was defined as the interval between OLA in one ovarian cycle and OLA in the next ovarian cycle. Based on the P4 profile, the luteal activity intervals for each cow were classified into 1 of 3 categories (Chen et al., 2015): short (<18 days), normal (18–24 days) or prolonged (>24 days). For statistical analyses, the number of cycles of each category were counted per cow over two different periods: in the 100 days around the end of the VWP (i.e., from −50 until 50 days relative to the end of the VWP), and from calving until pregnancy.

2.4.2. Milk yield, body condition and body weight

Milk yield was recorded at every milking, from day of calving until dry-off. Milk yield and FPCM yield per day of CI were calculated per week and averaged over the 4 weeks around the end of VWP, i.e., week − 2 until 2 relative to the end of VWP. Milk was converted to FPCM using the following formula (CVB, 2012):

$$\text{FPCM (kg)} = \text{milk (kg)} * (0{\cdot}337 + 0.116 * \text{fat (\%)} + 0.06 * \text{protein (\%)})\cdot$$

Body condition was scored (**BCS**) by the same technician every month on a 1–5 scale (Ferguson et al., 1994). Body weight was recorded twice daily after each milking and averaged over the 4 weeks around the end of VWP, i.e., week − 2 until 2 relative to the end of VWP. Body weight development was computed as the change in weight from week − 2 to 2 relative to the end of the VWP.

2.5. Statistical analyses

Six cows were not inseminated due to early culling (4 cows due to lameness, 2 cows due to accidents), and were excluded, resulting in 148 cows in total for the statistical analysis. Analyses were performed for all 148 cows and for the subgroups of cows that became pregnant within 100 days after the end of the VWP or within 300 days in milk. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC). A Pearson chi-square was used to assess whether the percentage of cows that became pregnant within 100 days after the end of the VWP depended on VWP treatment (PROC FREQ).

Effects of the fixed factors VWP (50 d, 125 d, or 200 d), parity class (1, or ≥2) and their interaction on fertility variables were assessed using different models, depending on the variable distribution. Regarding variables for ovarian cyclicity, OLA was analysed using a linear model (PROC MIXED); the number of cycles (in the 100 days around the end of the VWP or from calving to pregnancy) using a generalized linear model with a negative binomial regression (PROC GLIMMIX); and the percentage of normal, prolonged, or short cycles each with a logistic regression (PROC LOGISTIC). Regarding reproductive performance, days open, days until pregnancy after the end of the VWP, and number of inseminations per conception were analyzed using a linear model. Pregnancy rate/ AI was analyzed using a generalized linear model with a binary distribution and the default logit link function (PROC GLIMMIX). To evaluate time to pregnancy or time to the first AI from calving or from end of VWP, a survival analysis (PROC LIFETEST) was used to obtain Kaplan-Meier curves. To evaluate statistical differences of Kaplan-Meier curves among the VWP treatments, a Cox proportional hazards model (PROC PHREG) was used. Last, milk yield, body weight, body weight development and body condition score in the 4 weeks around the end of the VWP were analysed using linear models. Values were regarded significant if $P < 0.05$ and as a tendency if $0.05 \leq P < 0.10$. LSMEANS were presented and a Bonferroni correction was used for post-hoc pairwise comparisons between VWP treatments.

3. Results

3.1. Milk production per day of calving interval

Results about lactation yield were already published by (Burgers et al., 2021b). In short, VWP did not affect the milk yield per day of calving interval. For cows in parity = 1, milk production per day of calving interval was 23.3, 22.7 and 23.5 kg/d for cows with VWP-50, VWP-125 and VWP-200, respectively. For cows in parity≥ 2, milk production per day of calving interval was 29.5, 28.0 and 25.6 kg/d for cows with VWP-50, VWP-125 and VWP-200, respectively. Effect of VWP on FPCM depended on parity, the VWP did not affect FPCM yield per day of calving interval for cows in parity = 1, whereas FPCM yield per day of calving interval was higher in VWP-50 compared with VWP-200 for cows in parity≥ 2 (30.4 vs 27.4 kg/d for VWP-50 vs VWP-200, respectively, $P < 0.01$).

3.2. Ovarian cyclicity of all cows within 100 days around the end of the VWP

In the 100 days around the end of the VWP, cows in VWP-125 had more ovarian cycles than cows in VWP-50 (Table 1). Cows in VWP-125 and VWP-200 had a greater percentage of normal ovarian cycles than cows in VWP-50 ($P < 0.01$). Cows in VWP-200 had a lower percentage of prolonged cycles than cows in VWP-50 and VWP-125 ($P = 0.01$).

3.3. Fertility characteristics of cows that became pregnant within 100 days after the VWP

Of cows that became pregnant within 100 days after the end of the VWP, cows in VWP-125 and VWP-200 had more ovarian cycles from calving until pregnancy than cows in VWP-50 ($P < 0.01$). Cows in VWP-200 tended to have a greater percentage of normal cycles from calving until pregnancy than cows in VWP-50 or VWP-125 ($P = 0.09$) (Table 1).

Due to experimental treatment, cows in VWP-200 had the longest days open, followed by cows in VWP-125 and VWP-50 ($P < 0.01$; Table 2). In contrast, cows in VWP-200 had fewer days open after end of the VWP than cows in VWP-50 or VWP-125 ($P = 0.03$). Cows in VWP-200 had a longer time after calving to first AI than cows in VWP-50 and VWP-125 due to the deliberately extended VWP length (Fig. 1a). There was, however, no difference in time to first AI after the end of the VWP among three VWP lengths (P > 0.1) (Fig. 1b). Cows in VWP-200 had a shorter time to pregnancy after the end of the VWP compared with cows in VWP-50 (Hazard Ratio 0.43, confidence interval 0.27–0.68, $P < 0.01$) and cows in VWP-125 (Hazard Ratio 0.46, confidence interval 0.29–0.73, $P < 0.01$) (Fig. 1d).

3.4. Milk production, body condition and body weight around the end of the VWP

Extending the VWP from 50 d to 125 d or 200 d reduced milk yield in the 4 weeks (−2 to 2 weeks) around the end of the VWP by 5.4 kg/d or 9.9 kg/d, respectively, and FPCM yield by 4.5 kg/d or 7.6 kg/d, respectively (Table 3). Moreover, cows in VWP-50 were still losing body weight in the 4 weeks around the end of the VWP, while cows in VWP-125 were gaining body weight in this period. In the 4 weeks around the end of VWP, multiparous cows had a higher milk yield and FPCM yield than primiparous cows in VWP-50 or VWP-125, but not in VWP-200. For multiparous cows, milk yield in the 4 weeks around the end of the VWP decreased when VWP was extended from 50 to 125 or 200 d, but not for primiparous cows (Fig. 2). Days open after the end of the VWP was weakly correlated with milk yield ($r = 0.31$, $P < 0.01$) and FPCM yield ($r = 0.23$, $P = 0.01$), but not with body weight and body weight development around end of the VWP (scatter plots shown in Fig. 3). When correlations were performed separately for the three VWP treatments or the six VWP × parity classes, none were significant.

3.5. Fertility characteristics of cows that were pregnant within 300 DIM after different VWP

Cows in this experiment were inseminated until 300 DIM, irrespective of VWP length. In this period, 48 of 52 inseminated cows were pregnant in VWP-50, 42 of 49 in VWP-125, and 38 of 47 in VWP-200 (Table 4). Including all cows that were pregnant within 300 DIM, extending the VWP from 50 d to 200 d, but not from 50 to 125 d, increased conception rate after first AI ($P = 0.03$). Cows in VWP-125 and VWP-200 had 4.8 d and 35 d fewer days until pregnancy after the end of the VWP than cows in VWP-50 ($P < 0.01$). Within all cycles until pregnancy for pregnant cows or until 100 days after the end of VWP for cows that were not pregnant, cows in VWP-125 or VWP-200 had a higher percentage of normal cycles and lower percentage of prolonged cycles than cows in VWP-50.

Table 1
Ovarian cyclicity of all cows around the end of the voluntary waiting period (VWP), and ovarian cyclicity from calving until pregnancy for cows that became pregnant within 100 days after the end of the VWP. Cows had a VWP of 50, 125 or 200 days. Values represent LSMEANS and maximal SEM.

	Voluntary waiting period			SEM	*P*-value		
50 d	125 d	200 d			VWP	Parity (P)	VWP×P
All Cows, *n*	52	49	47				
OLA[1] (d)	24.3	24.8	28.3	2.9	0.32	0.73	0.28
Cycles per cow (100 days[2] around the end of VWP)	1.6^{a}	2.3^{b}	2.1^{ab}	0.2	0.02	0.51	0.41
Normal cycle (100 days[2] around the end of VWP in %)	58.0^{a}	77.3^{b}	91.9^{b}	0.1	< 0.01	< 0.01	0.82
Prolonged cycle (100 days[2] around the end of VWP in %)	32.7^{a}	19.0^{a}	6.0^{b}	0.1	0.01	0.04	0.43
Short cycle (100 days[2] around the end of VWP in %)	9.3	3.7	2.1	0.0	0.33	0.06	0.16
Cows pregnant within 100 days after VWP, *n*	39	36	38				
OLA[2] (d)	23.2	26.6	27.2	2.8	0.38	0.99	0.49
Cycles per cow (calving until pregnancy in %)	1.9^{a}	4.8^{b}	7.0^{c}	0.4	< 0.01	0.87	0.15
Normal cycle (calving until pregnancy in %)	53.8	64.6	71.4	0.2	0.09	0.05	0.26
Prolonged cycle (calving until pregnancy in %)	37.5	28.2	22.9	0.1	0.19	0.30	0.01
Short cycle (calving until pregnancy in %)	8.6	7.2	5.7	0.1	0.85	0.14	0.14

[a,b]Values within VWP within a row with different superscript letters differ ($P < 0.05$)
[1]OLA: Days to onset of luteal activity after calving;
[2]100 days: − 50 until 50 days around the end of VWP;

Table 2
Fertility characteristics of all cows and cows that became pregnant within 100 days after the end of the voluntary waiting period of 50, 125 or 200 days. Values represent LSMEANS and maximal SEM.

	Voluntary waiting period			SEM	*P*-value		
	50 d	125 d	200 d		VWP	Parity (P)	VWP×P
Cows (all), *n*	52	49	47				
Pregnancy rate/AI	43.8	42.0	63.3		0.17	0.59	0.52
Inseminated cows that were pregnant within 100 days after VWP	39	36	38				
Days open	85.5[a]	162[b]	219[c]	7.2	< 0.01	0.55	0.15
Days until pregnancy after end VWP	35.5[a]	37.3[a]	19.4[b]	7.2	0.03	0.55	0.15

[a,b]Values within VWP within a row with different superscript letters differ ($P < 0.05$)

4. Discussion

Extending the VWP from 50 to 200 days reduced the days until pregnancy after the end of the VWP. This is in accordance with Larsson and Berglund (2000), who reported fewer days from insemination to pregnancy after extending the VWP from 50 to 140 days. Ratnayake et al. (1998) also reported fewer days from first insemination to pregnancy by extending the VWP from 150 days to 240 days. Other studies, however, found no difference in days until pregnancy after the end of the VWP between a conventional versus extended VWP (Schindler et al., 1991; Arbel et al., 2001).

Fewer days until pregnancy after the end of an extended VWP likely resulted from an increase in conception rate and fewer inseminations per pregnancy. In the current study, number of inseminations per pregnancy was 0.5 lower for cows in VWP-200, compared with cows in VWP-125. In addition, conception rate after first insemination was 63.3 % for cows in VWP-200, compared with 43.8 % and 42.0 % for cows in VWP-50 and VWP-125. In earlier studies, an increase in the VWP was also related with an increase in conception rate (Foote and Riek, 1999; Caraviello et al., 2006) and a decrease in the number of AIs per conception (Larsson and Berglund, 2000). This was explained by the delay of insemination until a cow is likely no longer in a state of NEB. The NEB in early lactation is typically accompanied by low glucose and increased free fatty acid and ketone body concentrations, which may impair oocyte quality and embryo development (Jorritsma et al., 2003; Leroy et al., 2006; Fouladi-Nashta et al., 2007).

In earlier studies, more cycles or ovulations prior to insemination were related with an increase in conception rate after first AI (Stevenson et al., 1983; Butler and Smith, 1989; Butler, 2003). Furthermore, ovarian cycles of normal length (18–24 days) were related with an increased pregnancy rate and fewer days open (Lamming and Darwash, 1998; Shrestha et al., 2004; Ma et al., 2020). In the current study, cows in VWP-125 and VWP-200 had a greater percentage of normal ovarian cycles around the end of the VWP, and cows in VWP-200 had a lower percentage of prolonged ovarian cycles than cows in VWP-50. Prolonged cycles are a common ovarian disturbance in dairy cows (Shrestha et al., 2004), and have been associated with a reduced pregnancy rate and extended days open (Ranasinghe et al., 2011). In our study, the increased percentage of normal cycles in VWP-125 and VWP-200 and reduced percentage of prolonged cycles for the cows in VWP-200 were not associated with improved conception rates, but may have contributed to the fewer days until pregnancy after the end of the VWP in VWP-200. The higher percentage of prolonged cycles for cows in VWP-50 could be related with the higher milk yield around end of the VWP.

We hypothesized that after an extended VWP, a reduction in milk yield during the breeding period might contribute to an improvement in reproductive performance. High milk production, as a consequence of genetic selection and improvements in nutrition and management, has coincided with a corresponding decline in fertility (Hansen et al., 1983; Roxström et al., 2001). Moreover, a high milk yield is one of the major risk factors for a prolonged luteal phase in dairy cows (Kafi et al., 2012), possibly due to its association with a catabolic state (Santos et al., 2009) and inflammatory status in the uterus (Ribeiro and Carvalho, 2018; Pascottini et al., 2020). Other previous reports were ambivalent about the relation between milk yield and fertility and discussed that the interaction between milk yield and fertility is complex (Faust et al., 1988; López-Gatius et al., 2006; Bello et al., 2013). In the current study, extending the VWP from 50 to 125 or 200 days decreased milk yield and FPCM around the end of the VWP. In our experiment, however, milk yield was only weakly correlated with days until pregnancy after the end of the VWP. This indicated that a lower milk yield at time of insemination can only explain part of the less days open after the end of VWP after an extended VWP in our study. Other aspects of an extended VWP, such as a longer period for uterine recovery and insemination at a time of more neutral energy balance, may contribute to this less days open after the end of VWP as well (Ma et al., 2020).

Next to milk yield, also an improvement in body condition or body weight might be related to more regular ovarian cyclicity, as indicated by a greater percentage of normal cycles around the end of the VWP, and improved reproductive performance for cows with an extended VWP. Several studies have shown a negative relationship between reproductive performance and body condition in early lactation. Veerkamp et al. (2001) reported that the correlation between BCS and calving interval and days to first service was between − 0.44 and − 0.59. In earlier work, greater BW loss during the early postpartum period did not affect fertilization of oocytes, but was hypothesized to be associated with an increase in the percentage of degenerated embryos by 7 days after insemination, thus providing important evidence for a carryover effect of NEB on embryo development 6–7 weeks later (Britt et al., 1986). Cows that lost more body condition in the first 65 days postpartum were more likely to be anovular, had decreased pregnancy per AI, and increased risk of pregnancy loss (Santos et al., 2009). In our study, we found no difference of BCS around end of the VWP in different groups, but cows in VWP-50 were still losing BW around end of the VWP, while cows in VWP-125 and VWP-200 were gaining BW around the end of the VWP. Additionally, cows in VWP-125 gained most weight (5.3 kg) in the 4 weeks around the end of VWP. This is probably because the

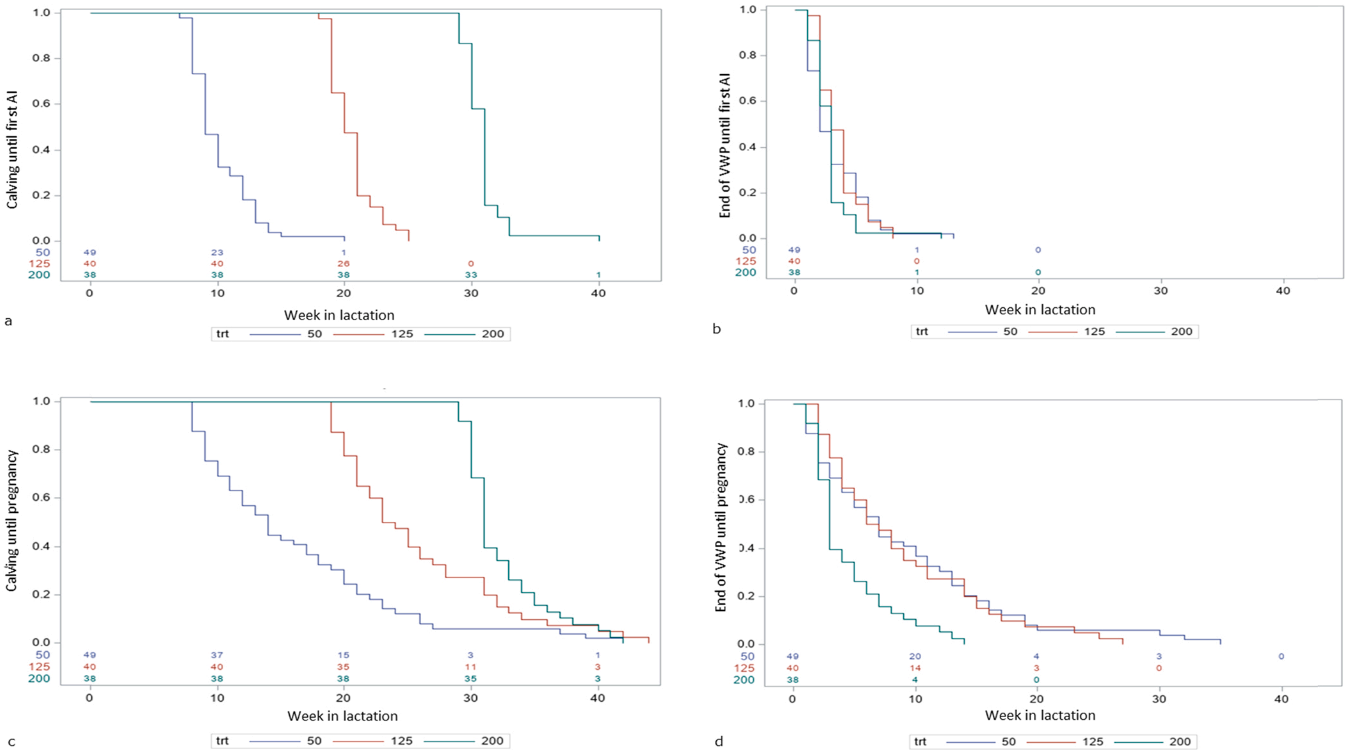

Fig. 1. a-d: Kaplan-Meier survival curves (with number of subjects at risk). The survival function is (a) the time of survival for no AI after calving, (b) the time of survival for no AI after the end of VWP, (c) the time of survival for no pregnancy after calving, (d) the time of survival for no pregnancy after the end of voluntary waiting period (VWP).

Table 3
Milk production, FPCM[1], body condition and body weight around the end of the voluntary waiting period (VWP) for all cows with a VWP of 50, 125 or 200 days. Values represent LSMEANS and maximal SEM.

	Voluntary waiting period			SEM	*P*-value		
	50 d	125 d	200 d		VWP	Parity (P)	VWP×P
Cows, *n*	52	49	47				
Milk production (−2 to 2 weeks around the end of VWP), kg/d	37.4^{a}	32.0^{b}	27.5^{c}	1.5	< 0.01	< 0.01	< 0.01
FPCM[1] (−2 to 2 weeks[2] around the end of VWP), kg/d	36.8^{a}	32.3^{b}	29.2^{c}	1.4	< 0.01	< 0.01	< 0.01
Body weight (−2 to 2 weeks[2] around the end of VWP), kg	621	628	645	14	0.14	< 0.01	0.83
Body weight change (−2 to 2 weeks[2] around the end of VWP), kg	-3.3^{b}	5.3^{a}	1.9^{ab}	3.6	0.04	0.06	0.32
Body condition score (−2–2 weeks[2] around the end of VWP)	2.5	2.5	2.4	0.2	0.61	0.05	0.32

^{a,b}Values within VWP within a row with different superscript letters differ (P < 0.05)
[1]FPCM: Fat and protein corrected milk production.
[2]Weeks around end of VWP (week −2, −1, 1, 2 relative to end of the VWP).

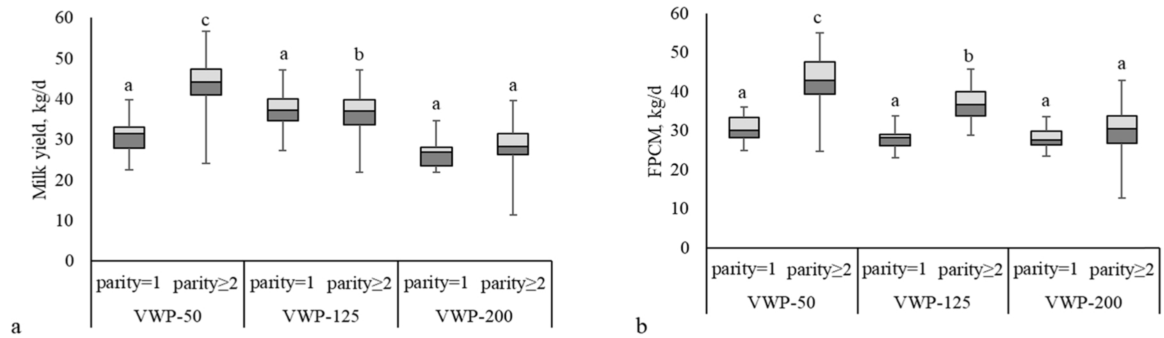

Fig. 2. a, b: Milk yield (a) and FPCM (fat- and protein-corrected milk production) (b) during − 2–2 weeks around the end of VWP for cows of parity 1 or ≥ 2 and a VWP of 50 d, 125 d, or 200 d. Letters a and b above box plots indicate differences between parities within VWP class.

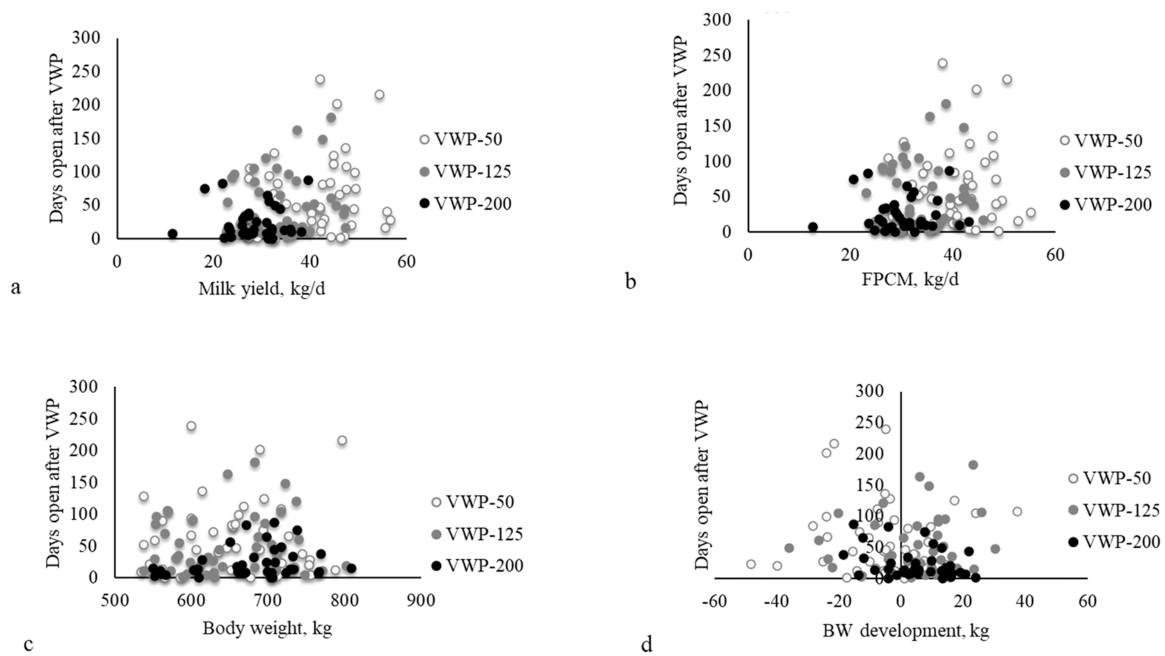

Fig. 3. Relationship between days until pregnancy after end of VWP with Milk yield (a), FPCM (fat- and protein-corrected milk production) (b), Body weight(c) and Bodyweight (BW) development(d) within − 2–2 weeks around the end of VWP for cows with a VWP of 50 d, 125 d, or 200.

Table 4
Fertility characteristics of all cows[1] until pregnancy or until 100 days after the voluntary waiting period of 50, 125 or 200 days[3], and of pregnant cows[4]. Values represent LSMEANS and maximal SEM.

	Voluntary Waiting Period			SEM	P-value		
	50 d	125 d	200 d		VWP	Parity (P)	VWP×P
Cows (all)[1], n	52	49	47				
OLA (d)[2,3]	23.8	25.5	27.2	2.9	0.51	0.89	0.56
Number of ovulations[3]	3.3[a]	6.4[b]	8.1[c]	0.5	< 0.01	0.94	0.18
Cycles per cow[3]	2.2[a]	5.4[b]	7.0[c]	0.5	< 0.01	0.80	0.11
Normal cycles[3]	49.8[a]	65.4[b]	71.4[b]	0.2	0.02	0.03	0.36
Short cycles[3]	6.8	6.9	5.7	0.0	0.95	0.21	0.15
Pregnant cows[4], n	48	42	38				
Days open	104.4[a]	174.6[b]	219.4[c]	15.0	< 0.01	0.17	0.74
Days open after VWP	54.4[a]	49.6[b]	19.4[c]	15.0	< 0.01	0.17	0.74
Inseminations per conception	2.1[ab]	2.2[a]	1.4[b]	0.3	0.05	0.76	0.36

[a,b] Values within VWP within a row with different superscript letters differ ($P < 0.05$)
[1]Number of cows in the complete experiment;
[2]Days to onset of luteal activity after calving;
[3]Variables related with ovarian cyclicity were determined until 100 days after the end of the voluntary waiting period or until pregnancy (whichever came first).
[4]Cows which became pregnant in the complete period where experimental treatments were applied (until 300 DIM).

peak dry matter intake occurs between 70 and 140 days post calving (Tesfaye and Hailu, 2019), and cows are restoring body condition after the period of NEB, whereas cows in VWP-200 have already recovered from the NEB. That the number of days until pregnancy after the end of the VWP were not reduced for cows in VWP-125 might be related with a too positive energy balance (Ma et al., 2020).

In our experiment, ovarian cyclicity and reproductive measures were intensively monitored for the first 100 days after the end of the VWP. This makes sense when comparing physiology of cows during the peri-insemination period, but for practical implications a more extended period, beyond 100 days after end of the VWP, might be relevant. In the current study, cows of all treatments were inseminated until 300 DIM. This implies that cows with a shorter VWP had more days to conceive after the end of the VWP. Within 300 DIM in our experiment, cows in VWP-200 had fewer days until pregnancy and less inseminations per pregnancy compared with cows in 50-d VWP. Although cows in VWP-200 had a better fertility, there was less time (100 d after end of the VWP) to inseminate the cows, which resulted in fewer cows being pregnant at 300 DIM. In this study, 92 %, 86 % and 81 % of cows in VWP-50, VWP-125, and VWP-200 were pregnant by 300 DIM. To avoid increased culling for fertility reasons in practice, farmers should either apply a short VWP, or continue to inseminate the cows that do not conceive within 100 days after the VWP until later in lactation. Additionally, cows with calving intervals of ≥ 15 months (Lehmann et al., 2017) or days open after calving ≥ 130 days (Middleton and Pursley, 2019) had higher BCS at next calving, which might compromise health and metabolism during the subsequent transition period and start of lactation (Walsh et al., 2011; Schuh et al., 2019). At commercial farms applying an extended VWP for part of the herd or the total herd, farmers were accepting towards an extended calving interval with extended VWP, and were more inclined to inseminate a cow with difficulties to conceive multiple times, rather than replacing that cow (Burgers et al., 2021a).

Deliberately extending the lactation of dairy cows is of interest because it reduces the frequency of challenging events in a cow's life like drying off, calving and start of a new lactation (Bertillson et al., 1997; Knight, 2005). This reduction in challenging events can be beneficial for cow health and fertility, but also can reduce the number of surplus calves, and labor associated with drying off, calving and disease treatments (as reviewed by Van Knegsel et al., 2022). Concerns associated with an extended VWP are that cows are not persistent enough to be milked for an extended lactation or fatten at the end of the extended lactation (Van Knegsel et al., 2022). Moreover, to our knowledge it is unknown how long the VWP of high-producing dairy cows can be extended. Reports are present which extended the VWP till 180 (Niozas et al., 2019) or 230 d (Bertillson et al., 1997), which is in line with the 200 d VWP in the current study, but these studies lack an economic evaluation of interest for farmers to decide on the practicality of an extended VWP. Most economic consequences of an extended lactation were estimated using a modeling approach including retrospective analysis of data (e.g., Dijkhuizen et al., 1985, Strandberg and Oltenacu, 1989, Kok et al., 2019). In addition, in these modeling studies the economic consequences were based on milk yield data and it was unknown what the reasons were that cows had an extended lactation, e.g., if it was a deliberate choice by the farmer or that it was due to impaired health or fertility of the cow. More recently, some studies reported the economic result of a deliberately extended VWP (Arbel et al., 2001; Stangaferro et al., 2018; Burgers et al., 2022), including besides milk yield data, also other variables including veterinary costs, culling rate, labor and benefits and costs for calf rearing. In the study of Arbel et al. (2001), yearly net return increased by $0.19 per day of calving interval for primiparous cows when the VWP was extended from 93 to 154 d, and increased with $0.12 per day per calving interval for multiparous cows with extended VWP from 71 to 124 d, only high-producing cows were included in this study. In another study, when extending VWP from 60 to 88 d, the yearly cashflow was numerically increased for primiparous cows and numerically decreased for multiparous cows (Stangaferro et al., 2018). In a stochastic simulation model of Kok et al. (2019), considering costs related to AI and calving management, extending lactations by 2 months reduced the net partial cash flow by k€7 for cows or k€2 for heifers per herd per year, or €70 for cows or €19 for heifers per cow per year. Based on data of the current experiment, the yearly net partial cashflow did not differ between cows with a VWP-50, VWP-125, and VWP-200 (Burgers et al., 2022). Cows with VWP-50 had greater yearly revenues, but also greater yearly costs, compared with cows with VWP-200, revenues or costs of cows with VWP-125 were not different from cows with VWP-50 or with VWP-200 (Burgers et al., 2022). Lastly, specifically for multiparous cows, there was a large variation in revenues and costs of an extended VWP among cows, which indicates that some cows are better suited for an extended VWP than other cows.

5. Conclusions

Extending the VWP from 50 d to 200 d improved ovarian cyclicity around the end of the VWP, as indicated by a greater percentage of normal ovarian cycles, a lower percentage of prolonged ovarian cycles, and resulted in fewer days open after the end of the VWP. Extending the VWP from 50 to 125 d also improved the percentage of normal cycles in the 100 days around the end of the VWP but did not affect the days open after the end of VWP. In addition, extending the VWP from 50 to 125 or 200 days decreased the milk yield and FPCM around the end of the VWP, which could partially explain the improvement of the ovarian cyclicity and the reduction in days until pregnancy after the end of the VWP.

CRediT authorship contribution statement

Junnan Ma: Methodology, Data curation, Investigation, Formal analysis, Vizualisation, Writing-original draft, Writing-review & editing. **Eline E.A. Burgers**: Methodology, Data curation, Investigation. **Akke Kok**: Methodology, Formal analysis, Writing-review & editing, Supervision. **Roselinde M.A. Goselink**: Conceptualization, Funding acquisition, Methodology, Investigation, Project administration. **Theo J.G.M. Lam**: Conceptualization, Writing-review & editing, Supervision. **Bas Kemp**: Conceptualization, Methodology, Writing-review & editing, Supervision. **Ariette T.M. van Knegsel**: Conceptualization, Methodology, Funding acquisition, Project administration, Writing-review & editing, Supervision.

Declaration of Competing Interest

The authors declare the absence of economic or other types of conflicts of interests.

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