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A randomized study on the effect of an extended voluntary waiting period in primiparous dairy cows on fertility, health, and culling during first and second lactation

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ABSTRACT

When the voluntary waiting period (VWP), defined as the days between calving and when the cow is eligible to receive the first insemination, is extended, high-yielding dairy cows may have better opportunities to regain energy balance before first insemination. This study investigated the effect of an extended (145–215 days in milk [DIM], n = 280) or conventional (25–95 DIM, n = 251) VWP treatment on fertility, disease incidence, and culling rate in cows during their first lactation. The cows were also followed through a second lactation without intervention regarding VWP, during which the farmers could decide when they wished to start the inseminations. This was done in a randomized-controlled study on 16 high-yielding commercial herds in southern Sweden, containing a total of 531 primiparous cows of the Holstein and Red Dairy Cattle breeds. Data from the Swedish national dairy herd recording scheme collected between August 2018 and September 2021 were used in the analysis, including records on breed, calvings, estrus intensity, inseminations, disease, somatic cell count, culling date, and culling reason. During first lactation, more cows receiving the extended VWP treatment showed strong estrus intensity (score 4–5, 55% vs. 48%) and fewer showed moderate estrus intensity (score 3, 35% vs. 43%) at first insemination, compared with cows receiving the conventional VWP treatment. First service conception rate (FSCR) was higher (67% vs. 51%) and number of inseminations per conception (NINS) was lower (1.6 vs. 2.0) during the first lactation for cows receiving the extended compared with the conventional VWP treatment. For disease incidence rate or culling rate expressed as number of events per cow-time in the study, we found no differences between the cows receiving the 2 VWP treatments in any lactation. Calving to first service interval during second lactation was longer (86 vs. 74 d) for cows with extended compared with conventional VWP. In conclusion, primiparous cows with extended VWP showed improved reproductive functions, in the form of higher estrus intensity, greater FSCR, and lower NINS, during the first lactation. However, we observed no apparent effect on these fertility measures during the following lactation (without VWP intervention) and no differences in disease prevalence or culling between cows receiving the 2 different VWP treatments in either lactation. Compliance with the planned VWP treatment was lower for cows with planned extended compared with planned conventional VWP treatment. We studied the "intention-to-treat" effect (i.e., the results for all cows randomized to each treatment regardless of whether the planned VWP was achieved or not) to identify any bias arising due to degree of compliance. However, we found no difference in culling rate between cows randomized to an extended VWP compared with those randomized to a conventional VWP. These findings can be used to support management decisions on VWP length in high-yielding dairy herds.

Key words: extended calving interval, extended lactation, reproduction, culling rate

INTRODUCTION

Delaying the first insemination by extending the days between calving and the time at which the cow is eligible to receive the first insemination, the voluntary waiting period (**VWP**), gives cows better possibilities to regain their energy balance, which may improve fertility (Butler, 2005). Estrus intensity, first service conception

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rate (**FSCR**), number of inseminations per conception (**NINS**), and insemination period length (**IPL**) are commonly reported in fertility studies, to reflect the cow's inherent ability to express estrus, conceive, and maintain early pregnancy. However, these variables are also affected by management factors such as accuracy of heat detection and insemination technique. Extending the VWP may reduce the frequency of transition periods per unit time for the individual cow and the herd. As reviewed by van Knegsel et al. (2022), this could lead to less negative impact from events associated with transition, such as diet change, dry-off, regrouping, start of lactation, and calving itself, which, in turn, is associated with decreased immunity, negative energy balance, and a need for physiological adaption, as reviewed by Pascottini et al. (2022). Theoretically, less frequent transition periods may thereby improve the long-term health of cows, as disease incidence is highest in early lactation (Ingvartsen et al., 2003; Bradley and Green, 2004).

Previous randomized studies of fertility measures in cows with extended VWP show conflicting results for NINS (Schneider et al., 1981; Niozas et al., 2019b; Burgers et al., 2022), FSCR (Arbel et al., 2001; Niozas et al., 2019b), and IPL (Schneider et al., 1981; Ratnayake et al., 1998; Niozas et al., 2019b); these results have mostly been reported for primi- and multiparous cows combined. The effect of extended VWP on health indicators show few conclusive results as well; however, Ma et al. (2022) found that cows with an extended VWP of 200 d had higher SCC in the beginning of the subsequent lactation than cows with a 50-d VWP. Previous findings on the effects of VWP length on culling are also inconsistent, as Niozas et al. (2019a) reported higher overall culling and culling due to low productivity in cows with 180-d compared with 40-d VWP, whereas Burgers et al. (2022) and Arbel et al. (2001) did not detect any increased risk of culling in cows with extended VWP. In a study by Larsson and Berglund (2000), a smaller proportion of cows with extended VWP were culled due to low fertility. Most previous studies have not examined effects on a subsequent lactation without VWP intervention, or whether dairy breed affects the outcome of the VWP intervention. The most common breeds in Sweden are the Holstein (**HOL**, 57%) and Red Dairy Cattle (**RDC**, 37%), and in most Swedish herds both breeds are represented (Växa Sverige, 2021).

Randomized-controlled studies may have inherent biases relating to degree of compliance with the planned treatments, choosing to report effects either as "intention-to-treat" outcomes divided by the study participants randomized to, but not necessarily receiving, each treatment regardless of compliance, or as "per-protocol" reporting outcomes for study participants actually receiving (complying to) the planned treatment (Mansournia et al., 2017). The per-protocol method addresses the "pure" effect of the applied treatment, whereas the intention-to-treat method may give more answers regarding application of the treatment in practice.

The main aim in this study was to investigate the per-protocol effect of an extended VWP on dairy cow fertility, measured as estrus intensity, FSCR, NINS, and IPL during a first lactation with extended VWP and a second lactation without VWP intervention. Additional aims were to compare disease incidence and culling rate and to investigate potential interactions between VWP treatment and breed. A further aim was to identify potential bias due to possible differences in compliance between cows randomized to the 2 VWP treatments; compliance and the intention-to-treat effect of extended VWP on culling rate were analyzed.

MATERIALS AND METHODS

Study Design and Selection of Herds and Cows

The study design and herd and cow selection for this study are described in detail in a previous publication (Edvardsson Rasmussen et al., 2023) and briefly summarized herein.

A randomized-controlled study of extended VWP was performed in commercial dairy herds in southern Sweden between August 2018 and September 2021, with ethical approval from Uppsala Ethics Committee for Animal Research, Uppsala, Sweden (protocol number 5.8.18-10126/2018). Initially, 19 herds volunteered to participate and met the inclusion criteria of yearly average milk production of more than 9,000 kg ECM, herd size of at least 100 cows, a system for daily milk recording, and mean calving interval (**CInt**) of less than 14 mo, based on data acquired from the Swedish national dairy herd recording scheme (**SNDRS**) 2016/2017. Failure to comply with the overall research protocol led to exclusion of 3 herds. The mean and range of main characteristics of the remaining 16 participating herds are presented in Table 1. The cow inclusion period in each herd started within 1 mo of September 1, 2018, and continued for 6 mo from the starting date. All heifers of the breeds HOL or RDC (defined as Swedish Red, Danish Red, and Swedish Ayrshire) having their first calf during this period were recruited.

The cows were randomly allocated by odd or even ear number to a conventional (35–85 d) or extended (155–205 d) VWP treatment, aiming at calving intervals of 12 and 16 mo. A range in VWP was applied to allow for variation between farms and to define the expected range of first insemination for each treatment, with the

Table 1. Main characteristics of participating herds (n = 16), presented as mean and range

Herd characteristic	Mean	Range
Average yearly milk production (kg of ECM)	10,623	(9,000–12,623)
Herd size (number of cows)	165	(102–305)
Mean calving interval (mo)	12.7	(11.8–13.8)
Holstein (%)	50	(5–97)
Red Dairy Cattle (%)	41	(2–90)
Crossbreeds or other breeds (%)	9	(0–34)

same range (50 d) for both treatments. In total, 7 cows were excluded from all results, 4 of which lacked information about inseminations during the first lactation and 3 of which did not have a complete first lactation before the data collection period ended in September 2021. The remaining 531 cows were monitored during their first lactation with VWP intervention. A total of 419 cows had a second calf, 62 of which were excluded from the second-lactation results. Of these, 42 cows in 1 herd were part of another VWP intervention study during their second lactation, 19 cows were still lactating at the end of data collection in September 2021, and 1 cow was lacking information about inseminations during the second lactation. A final total of 357 cows were monitored during a second lactation, during which the farmers could decide when they wished to start the inseminations (i.e., without VWP intervention), ending in either a third calving or culling before the end of data collection (Table 2).

Data Collection and Description of Variables Considered in the Analysis

Data from SNDRS collected between August 2018 and September 2021 were used in the analysis, including records on parentage, breed, calvings, estrus intensity, inseminations, diseases, SCC from monthly test milkings, culling date, and culling reason. Additionally, if the planned VWP treatment was not followed, the farmers were asked to report the reason why. Data on calvings, estrus intensity, inseminations, culling date, and culling reasons were reported to SNDRS by the farmers.

The CInt was calculated as the interval in days between 2 consecutive calving dates, and the calving to first service interval (**CFI**) was calculated in days. The IPL was defined as the interval in days between the dates of the first insemination and the insemination resulting in calving. The latter date was in turn defined as DIM at the last insemination in the interval 280 ± 14 d before the consecutive calving, to ensure accurate IPL calculation. The binary variable "conception at first insemination" was defined as cows that had a complete lactation and were estimated to have conceived at first insemination, or cows that had a positive pregnancy diagnosis after the first recorded insemination. To be able to account for the effect of farm, and because NINS makes less sense on individual basis, NINS was calculated as total number of inseminations divided by total number of conceptions, resulting in 1 value per herd and treatment subgroup for each lactation (number of subgroups, lactation 1: n = 32, and lactation 2: n = 30). All recorded inseminations were counted, including those given at an interval of just a few days during the same estrus. A conception was determined by either a positive pregnancy diagnosis or by a recorded calving. Repeated conceptions for the same cow during the same lactation were ignored and counted as 1 conception. Pregnancy loss was defined as either a cow with a recorded positive pregnancy diagnosis followed by a new insemination, a cow with a positive pregnancy diagnosis that did not have a calf, or a cow with a positive pregnancy diagnosis followed

Table 2. Number of cows randomized to conventional (CONV) or extended (EXT) voluntary waiting period (VWP) during their first lactation, following the different inclusion criteria and combinations of inclusion criteria applied in the data analysis, and of the 2 breeds Holstein or Red Dairy Cattle, during lactation 1 (lact. 1) with VWP intervention and lactation 2 (lact. 2) without VWP intervention

	Lactation 1			Lactation 2		
Inclusion criteria	CONV (n = 251)	EXT (n = 280)	Total lact. 1 (n = 531)	CONV (n = 186)	EXT (n = 171)	Total lact. 2 (n = 357)
VWP according to plan (VWP OK)[1]	204	178	382	166	128	294
Complete lactation	209	210	419	140	123	263
VWP OK + complete lactation	186	161	347	123	94	217
Number of inseminated cows (Ins.)	236	234	470	161	145	306
VWP OK + Ins.	204	178	382	143	110	253
Cow-years in the study with VWP OK	208	227	434	161	130	291
Holstein	145	181	326	106	108	214
Red Dairy Cattle	106	99	205	80	63	143

[1]Referring to the number of cows, in each lactation, with a VWP "per protocol" during lactation 1; during lactation 2 there was no VWP intervention, and the farmers were free to inseminate the cows whenever they choose.

by a negative pregnancy diagnosis. The estrus intensity score used was that recorded for the first insemination in each lactation. Estrus intensity was rated on an ordinal scale from 0 to 5 (with 0 representing no signs of estrus and 5 representing strong estrus signs). Scores 0 to 2 (representing no or weak estrus signs) were merged due to low frequency of observations, and scores of 4 and 5 were merged because they both represent cows with strong estrus expression and hence have the same biological and practical relevance (Nyman et al., 2016).

Disease events were recorded in SNDRS mainly by the treating veterinarian, although a few common diagnoses, such as mastitis, subclinical mastitis, inappetence, retained placenta, and leg or hoof disorder, were recorded by the farmers. The SCC values were retrieved from the monthly test milking records reported to SNDRS. During lactation 1, records of disease before the start of VWP intervention at 25 DIM were excluded. Recorded diseases were divided into 8 categories: mastitis, subclinical mastitis, reproductive disorders, leg or hoof lesion, puerperal paresis, accident or trauma, metabolic, and other. Disease incidence rate was calculated as total number of disease events divided by total number of days in each lactation, as well as in both lactations combined, per herd and VWP treatment subgroup, in the same manner as the NINS (number of subgroups, lactation 1: n = 32, and lactation 2: n = 30). The disease incidence rate was expressed as number of disease events per 100 cow-years in the study (time at risk). Time in the study per cow was calculated for each lactation as the CInt, or as time between calving and culling if the cow was culled before the next calving. The proportion of cows with SCC <100,000 cells/mL at first and last test milking in each lactation was calculated and presumed to represent cows with healthy udders (Jashari et al., 2016). Mean milk yield on d 4 to 33 for cows that were not inseminated according to the planned treatment, and on d 4 to 145 after calving for cows with a planned extended VWP treatment, was calculated from daily milk data recorded by the milking systems in each herd. Calculation of daily yield is described in detail in our previous publication (Edvardsson Rasmussen et al., 2023). Culling rate was calculated per herd and VWP treatment, with the number of culled cows divided by 100 cow-years in the trial per subgroup (number of subgroups, lactation 1: n = 32, and lactation 2: n = 30). Reasons for culling were divided into 7 categories: accident, (impaired) fertility, leg or hoof disorder, low milk yield, mastitis, sold (reported as a culling reason in the SNDRS), and other. Results are presented descriptively as number of culled cows per category per 100 cow-years in the study. Documentation, metadata, and supplemental files are published in the Swedish National Data Service catalog (Edvardsson Rasmussen et al., 2022). Due to restrictions in the agreement with the principal owner of the data, Växa Sverige, research data cannot be openly published.

Inclusion Criteria

For each variable studied 3 inclusion criteria were applied in different combinations; the numbers of cows fulfilling each criterion are presented in Table 2. The first criterion was that CFI deviating by a maximum of ±10 d from the planned VWP treatment was considered "per protocol" (i.e., 25–95 DIM for the conventional VWP treatment and 145–215 DIM for the extended VWP treatment). This inclusion criterion was applied to account for the per-protocol effect (i.e., the "pure" treatment effect, for all variables) and also to assess compliance with the planned VWP treatment. However, for culling rate, not only the per-protocol effect but also the results for all participating cows randomized to, and intended to receive, the 2 different treatments were used, to account for the intention-to-treat effect. This was done to investigate potential effects of lack of compliance to the VWP treatments that might stay unrevealed if cows failing to comply with the intended treatment were excluded.

The second criterion applied was a complete first or second lactation, defined as cows that had their second or third calf before September 2021, which was the endpoint of data collection. This criterion was applied for calculating all SCC variables and the time of the insemination that led to calving, which in turn was used for calculating DIM at insemination leading to calving, IPL, and NINS. The third criterion was daily milk yield collected from the milking systems in the herds, where "reliable daily yield records" was defined as no more than 10 d of missing milk yield data in the first 40 d of lactation. This criterion was applied to the calculation of mean daily yield on 4 to 33 d after calving for cows with extended (n = 74) and conventional (n = 33) VWP where the VWP plan was not followed. For daily yield on d 4 to 145 for cows receiving (n = 159) and not receiving (n = 63) the planned extended VWP treatment, an additional criterion of no more than 50% of daily yield observations missing during the whole lactation was applied.

Statistical Analyses

Microsoft Excel 2016 was used for initial data organization and GraphPad Prism version 9.5.0 (GraphPad Software, 2022) was used to visualize estrus intensity, culling reason, and disease incidence rate. R software (R Core Team, 2022) and RStudio version R-4.1.2

(RStudio Team, 2022) were used for statistical analysis. Planned VWP treatment (2 levels), breed (2 levels), and the interaction between planned VWP treatment and breed were included as fixed factors, and herd (first lactation: 16 levels; second lactation: 15 levels) was included as a random factor in all models, unless otherwise stated. If the interaction was not significant, it was removed from the model. The confidence level was set to 0.95.

For the variables' compliance with the planned VWP treatment, estrus intensity, first service conception, pregnancy loss, and SCC <100,000 cells/mL, generalized binomial linear mixed models were used. These were fitted by Laplace approximation, using the glmer function in R (Bates et al., 2022). Post hoc tests were conducted using the emmeans function in R (Lenth et al., 2022). The ordinal data for estrus intensity score were analyzed with 1 binomial model for each score group described previously. Results are presented as percentage and proportion of cows (n/N, where n is the number of cows with each specific estrus intensity score that conceived at first insemination, with pregnancy loss, or with SCC <100,000 cells/mL, and N is the total number of animals included with each VWP treatment). For hypothesis testing, the binary models were analyzed with an analysis of deviance table. Type II Wald chi-squared tests were used to determine which of the fixed factors were significant.

Linear mixed models were applied with the lmer function from the package lme4 in R (Bates et al., 2022) for the continuous data (CFI, IPL, and milk yield variables). The emmeans function (Lenth et al., 2022) was used for post hoc tests. The results are presented as least squares means ± standard error of the mean. Differences in disease incidence rate, NINS, and culling rate between cows receiving the 2 different VWP treatments were analyzed with a negative binomial generalized linear mixed model using the glmmTMB function in R (Brooks et al., 2017), due to the presence of underdispersion in the count data. The emmeans function in R (Lenth et al., 2022) was used for post hoc tests. In the NINS, disease incidence rate, and culling rate models, breed was not included as a factor because all breed–VWP treatment combinations were not represented among the included cows on all farms.

RESULTS

The numbers of cows receiving the planned VWP treatment and following specific inclusion criteria and combinations of these, as well as numbers of cows of the 2 breeds, are shown in Table 2. Of the 531 cows included, 280 were randomized to the extended VWP treatment and 251 to the conventional VWP treatment. However, we found that compliance with the planned VWP treatment was significantly lower ($P < 0.001$) in the extended than in the conventional VWP treatment (65% and 83%, respectively).

Fertility

As intended, CFI and CInt were longer for the cows randomized to and receiving the extended than the conventional VWP treatment (Figure 1). During first lactation, more cows receiving the extended VWP treatment (55%) than the conventional VWP treatment (48%) had strong estrus intensity (score 4–5) at first insemination ($P < 0.001$), and fewer cows with extended VWP treatment (35%) than with conventional VWP treatment (43%) had moderate estrus intensity (score 3, $P < 0.001$; Figure 2). We detected no differences in estrus intensity scores between cows receiving the 2 different VWP treatments during lactation 2 (Figure 2).

In the first lactation, FSCR was greater (67% vs. 51%, $P = 0.001$; Table 3), NINS was lower (1.6 vs. 2.0, $P = 0.005$; Table 4), and IPL was shorter (15 ± 4 vs. 26 ± 4 d, $P < 0.001$) for cows receiving the extended compared with the conventional VWP treatment. However, we found no difference in FSCR, NINS, or IPL between the VWP treatments during the second lactation and no difference in the extent of pregnancy loss between the VWP treatments in either lactation (Table 3). The planned VWP treatment resulted in mean CFI of 156 and 71 d for cows receiving the extended and conventional VWP treatments, respectively, during the first lactation, and a 12-d-longer ($P < 0.001$) CFI during the second lactation without VWP intervention, for cows with extended compared with conventional VWP treatment (Table 4). We found no interaction between VWP treatment and breed for any of the fertility traits considered (data not shown).

Health Records

We found no difference between the VWP treatments or breeds regarding proportion of cows with SCC <100,000 cells/mL of milk, and thereby a presumably healthy udder, during the first test milking in lactation 2 (n = 201, 60 vs. 61%, $P = 0.90$, for cows with extended and conventional VWP, respectively). However, at the last test milking, an interaction was detected between VWP treatment and breed in both lactations. For HOL cows, we found no difference between cows receiving different VWP lengths, but at the end of the first lactation a higher proportion of RDC cows with extended VWP than RDC cows with conventional VWP had SCC <100,000 cells/mL (70% vs. 51%, $P < 0.05$; Table 5). At the end of the second lactation, however,

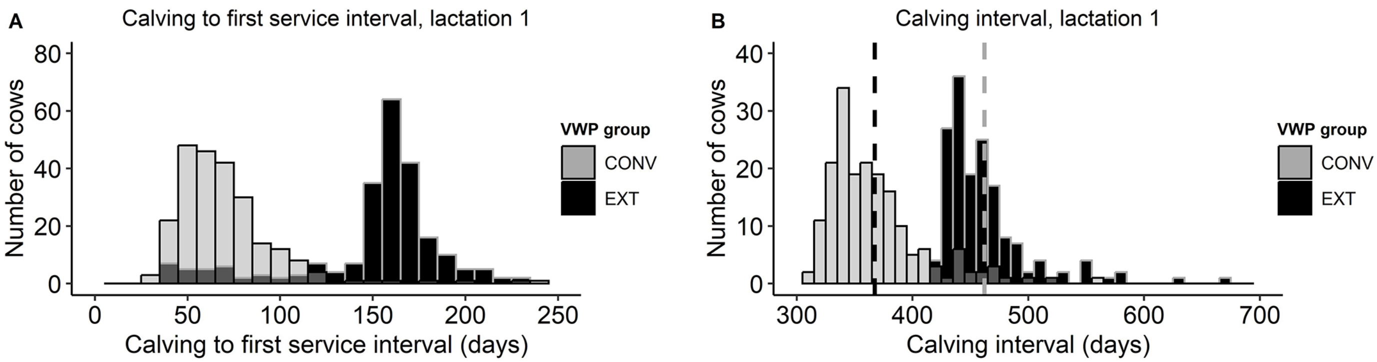

Figure 1. Distribution of (A) interval between calving and first insemination, for cows randomized to each treatment ("intention to treat") and receiving a first insemination, with conventional (CONV, gray, n = 236) and extended (EXT, black, n = 234) voluntary waiting period (VWP) treatment. (B) Distribution of calving interval and mean calving interval for each VWP treatment are represented with dashed lines (CONV, black, mean = 367; EXT, gray, mean = 462) for cows following the planned VWP treatment and having a complete lactation 1, with CONV (gray, n = 186) and EXT (black, n = 161) VWP. The dark gray bars represent overlap between the 2 VWP treatments.

the proportion of cows with SCC <100,000 cells/mL was lower for RDC cows with extended compared with conventional VWP (34% vs. 61%, $P < 0.05$; Table 5).

We detected no difference in disease incidence rate, expressed as number of disease events per 100 cow-years, between cows receiving extended and conventional VWP in lactation 1 (8.24 ± 2.96 vs. 6.72 ± 2.57, $P = 0.57$), lactation 2 (32.9 ± 8.81 vs. 30.8 ± 8.12, $P = 0.76$), or both lactations combined (17.7 ± 5 vs. 18.3 ± 5, $P = 0.87$). We also found no difference in culling rate, expressed as the number of culled cows per 100 cow-years in the trial, between cows receiving the planned extended or conventional VWP treatment per protocol during either the first (7.5 ± 2.0 vs. 8.7 ± 1.8, $P = 0.67$) or the second lactation (26.2 ± 4.1 vs. 26.7 ± 4.5, $P = 0.93$; Tables 6 and 7). Disease incidence rates per disease category are illustrated in Figure 3, and culling rates per culling reason category for cows receiving extended or conventional VWP are shown in Figure 4.

Intention-to-Treat Analysis and Culling

We found no difference in milk yield per day during the first 4 to 33 d of lactation for cows randomized to but not following their planned extended or conventional VWP treatment (25.9 vs. 26.3 kg, $P = 0.72$). In contrast, for cows allocated to an extended VWP but not following their planned VWP treatment (i.e., that failed to comply), the mean yield during 4 to 145 DIM was 8% lower than for cows that were following the planned extended VWP treatment (i.e., that succeeded in complying; 23.5 vs. 28.4 kg/d, $P < 0.001$). Culling rate did not differ between cows randomized "intention to treat" to the extended or conventional VWP treatment in either lactation 1 (20.6 ± 3.1 vs. 15.8 ± 2.8, P

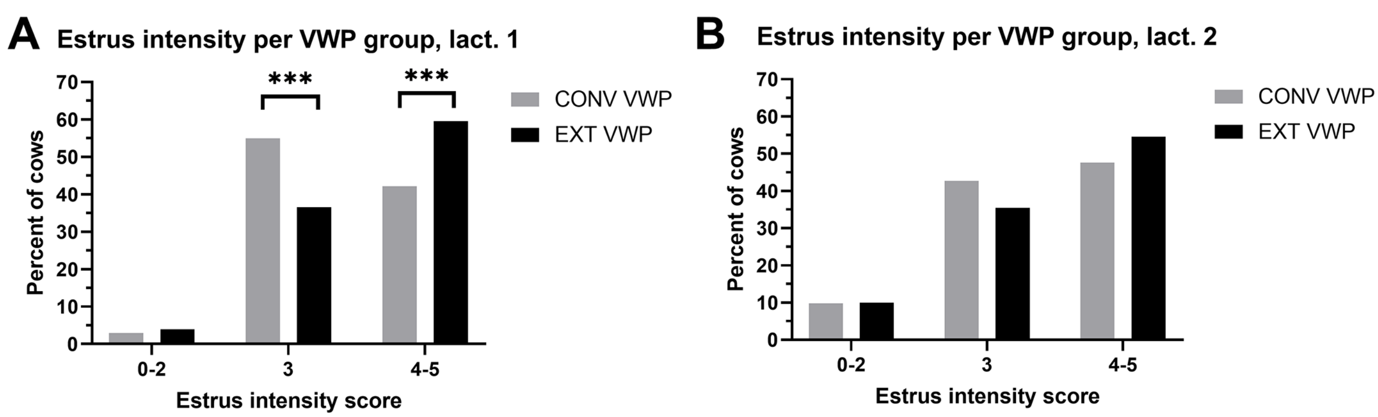

Figure 2. Estrus intensity scores at first insemination on a scale from 0 to 5, where 5 represents the strongest estrus intensity, and with scores 0–2 and 4–5 merged (***$P < 0.001$). Displayed as proportion of cows receiving the intended conventional (CONV) or extended (EXT) voluntary waiting period (VWP) treatment with each estrus intensity score in (A) lactation 1 (lact. 1, n = 382) and (B) lactation 2 (lact. 2, n = 253).

Table 3. First service conception rate (FSCR) and pregnancy loss for all inseminated animals with voluntary waiting period (VWP) according to plan in lactation 1[1]

		CONV		EXT		
Variable and lactation	n_{tot}	%	n/N	%	n/N	*P*-value
Lactation 1						
FSCR	382	51[b]	104/204	67[a]	119/178	0.001
Pregnancy loss	382	3	6/204	7	13/178	0.05[2]
Lactation 2						
FSCR	253	45	65/143	45	49/110	0.93
Pregnancy loss	253	6	8/143	7	8/110	0.51

[a,b]Mean values within a row with different superscripts differ significantly ($P < 0.05$).

[1]Data presented as proportion of cows, as percentage and n/N, where n is the number of cows with first service conception or pregnancy loss, N is the total number of cows in the conventional (CONV) and extended (EXT) VWP treatments in each lactation, and n_{tot} is total number of cows in each analysis.

[2]$P = 0.0503$.

= 0.18) or lactation 2 (27.8 vs. 25.2, $P = 0.67$; Tables 6 and 7). Culling reasons for cows randomized to each VWP treatment per lactation are shown in Figure 4.

DISCUSSION

This randomized-controlled study investigated the effects of extended VWP in primiparous cows on fertility, health, and culling during their first lactation and also during their second lactation (without VWP intervention). One strength of random-controlled studies of VWP is that this limits the risk of a confounding effect of poor fertility compared with retrospective observational field studies. In the latter case, it is often unknown weather a long CFI is due to a voluntary decision of extended VWP (and in that case, why) or due to poor fertility, which may influence the results. Another strength of random-controlled studies is that having cows with both treatments simultaneously in several herds makes it possible to compensate for all the different factors that might influence fertility in the herds. However, observational studies have the advantage of allowing inclusion of more cows.

Fertility

The fertility results clearly revealed that estrus intensity, FSCR, NINS, and IPL were all improved in cows receiving extended VWP during the first lactation, although the effect on IPL seemed largely to be explained by the FSCR (results not shown). As we found no interaction between breed and VWP treatment, the results regarding fertility were valid for both RDC and HOL cows. The CFI in lactation 2 was longer for cows receiving an extended VWP during the first lactation, for reasons unknown. An explanation may be that the farmers knew these cows could manage an extended lactation and were more liberal regarding the VWP, although no difference in CInt occurred during the second lactation (Edvardsson Rasmussen et al., 2023). During the second lactation, no VWP intervention occurred, and the farmers were free to choose their own VWP for each cow or farm; however, these VWP were not registered but might to some extent be reflected by the CFI during lactation 2.

Several possible reasons could explain the diverging results in previous randomized studies of fertility in

Table 4. Number of inseminations per conception (NINS) in lactations 1 and 2, and calving to first insemination interval (CFI) in lactation 2[1]

Variable and lactation	n	CONV	EXT	*P*-value
NINS lactation 1	382	$2.0^{a} \pm 0.1$	$1.6^{b} \pm 0.1$	0.009
NINS lactation 2	294	2.2 ± 0.1	2.1 ± 0.1	0.57
CFI lactation 1[2] (d)	382	71 ± 4	156 ± 4	
CFI lactation 2 (d)	288	$74^{b} \pm 4$	$86^{a} \pm 4$	<0.001

[a,b]Mean values within a row with different superscripts differ significantly ($P < 0.05$).

[1]NINS was calculated as number of inseminations per number of pregnant cows, and the results were calculated per herd and voluntary waiting period (VWP) subgroup (number of subgroups lactation 1, n = 32, and lactation 2, n = 30). The values presented are LSM ± SEM for cows with conventional (CONV) or extended (EXT) VWP.

[2]Result of the intervention and therefore not tested.

cows with extended VWP (Schneider et al., 1981; Ratnayake et al., 1998; Niozas et al., 2019a). The study by Schneider et al. (1981) was conducted about 40 years ago, and the average 305-d yield was reported to be below 8,000 kg for the cows included in the analysis. The cows in the study by Ratnayake et al. (1998) milked approximately 9,000 kg per lactation. For modern, high-yielding cows such as those included in the present study, longer VWP has been associated with shorter IPL and fewer NINS, whereas the opposite has been found for cows with yearly milk production of less than 9,000 kg (Römer et al., 2020). However, our results support findings by Ratnayake et al. (1998) regarding estrus intensity and by Niozas et al. (2019b) regarding NINS, FSCR, and IPL.

During early lactation at the end of the conventional VWP, many dairy cows are in a state of negative energy balance, as milk production increases at a higher rate than can be supported by dry matter intake. This negative energy balance has been linked to lower fertility (de Vries and Veerkamp, 2000; Butler, 2003; Walsh et al., 2011). To meet the energy deficit, the cow must mobilize fat from body reserves, leading to increased nonesterified fatty acid levels in the blood. The increased nonesterified fatty acid level may be reflected in follicular fluid (Leroy et al., 2004) and may negatively affect oocyte development (Ruebel et al., 2022) and early embryo physiology (Van Hoeck et al., 2011). With an extended VWP, cows have better opportunities for regaining their energy balance before the first insemination. Moreover, Stangaferro et al. (2018) noted that cows with extended VWP had better uterine health (i.e., fewer polymorphonuclear cells) at the end of their VWP.

The BCS of the cows in the present study were not recorded systematically in the herds. According to the idea of the "high fertility cycle" (Middleton et al., 2019; Fricke et al., 2022), cows should become pregnant by 130 DIM to avoid getting a high BCS at the end of lactation, with following elevated risk for health and reproductive disorders. Burgers et al. (2021) reported a higher BCS during the last 12 wk before dry-off for multiparous cows receiving an extended VWP of 200 d, compared with cows with a 50- or 125-d VWP. However, in primiparous cows, these authors did not observe any effect of VWP on BCS either in late lactation or during the first 6 wk of the next lactation. Further research is needed to shed more light on the effect of VWP on BCS.

Health Records and Culling

Taken together, our results agree with previous findings that mastitis incidence (Niozas et al., 2019a; Ma

Table 5. Total number of cows (n_{tot}) in each analysis, and proportion of cows (% and n/N), where n is the number of cows with SCC less than 100,000 cells/mL and presumably good udder health at the last test milking (TM) of the first or second lactation, and N is the total number of cows included with conventional (CONV) or extended (EXT) voluntary waiting period (VWP) treatment within the breeds Holstein (HOL) and Red Dairy Cattle (RDC)

		HOL					RDC					
		CONV		EXT			CONV		EXT			
SCC at last TM	n_{tot}	%	n/N	%	n/N	HOL *P*-value	%	n/N	%	n/N	RDC *P*-value	Interaction *P*-value
Lactation 1	347	65	72/110	58	58/100	0.28	51[b]	39/76	70[a]	43/61	0.03	0.02
Lactation 2	217	47	27/58	53	39/73	0.43	61[a]	22/36	34[b]	17/50	0.01	0.02

[a,b]Mean values within a row with different superscripts differ significantly ($P < 0.05$).

Table 6. Culling rate per 100 cow-years in the study (time at risk) for all cows randomized to (intention-to-treat) and receiving the planned (per-protocol) conventional (CONV) and extended (EXT) voluntary waiting period (VWP) treatment[1]

Culling rate	Per-protocol				Intention-to-treat			
	n	CONV	EXT	*P*-value	n	CONV	EXT	*P*-value
Lactation 1	382	8.7 ± 2.0	7.5 ± 1.8	0.67	531	15.8 ± 2.8	20.6 ± 3.1	0.18
Lactation 2	294	26.7 ± 4.1	26.2 ± 4.5	0.93	357	25.5 ± 3.8	27.8 ± 4.0	0.67

[1]Results were calculated per herd and VWP treatment subgroup (number of subgroups lactation 1, n = 32, and lactation 2, n = 30). Values presented are LSM ± SEM.

et al., 2022) and SCC in the beginning of first lactation (Österman et al., 2005; Ma et al., 2022) or second lactation (Ma et al., 2022) are not affected by altering the VWP of primiparous cows. However, those studies also found no differences between the VWP treatments regarding SCC in any part of the lactation.

In the present study, the proportion of cows with good udder health (indicated by low SCC), in the late stage of the first lactation, was higher among RDC cows receiving an extended compared with a conventional VWP. However, in late second lactation the pattern was the opposite, with a lower proportion of RDC cows with low SCC in the extended compared with the conventional VWP treatment. We did not find any previous data supporting these results and do not have a theory on their cause. In this study the HOL cows had higher milk yield at dry-off than the RDC cows (Edvardsson Rasmussen et al., 2023), which has been connected with increased risk of mastitis (Rajala-Schultz et al., 2005). However, the higher proportion of RDC cows with good udder health in late first lactation did not transfer to the beginning of the subsequent lactation, when no difference was found between the VWP treatments regarding proportions of cows with low SCC. Lower milk yield has also been linked to higher SCC (Hagnestam-Nielsen et al., 2009). However, the RDC cows in our study had lower milk yield at the last test milking before dry-off in both lactations (Edvardsson Rasmussen et al., 2023), so this does not explain the contrasting results between the 2 lactations.

In previous studies of VWP, Ratnayake et al. (1998) reported a lower need for treatments of ovarian disorders in cows with extended VWP, and Burgers et al. (2022) found that VWP length did not affect the number of veterinary treatments per cow. We hypothesized that the number of disease cases described per unit time in the study might be lower for cows with extended VWP due to lower frequency of transition periods (when disease incidence is highest) per unit of time (Ingvartsen et al., 2003). Moreover, a lower dry-off yield, as was found in lactation 1 for cows with extended VWP (Edvardsson Rasmussen et al., 2023), has been connected to a reduced risk for mastitis at dry-off (Rajala-Schultz et al., 2005). However, we found no difference in disease incidence rate between cows receiving the 2 different VWP treatments in either lactation or in both lactations combined.

Compliance and Intention-to-Treat Analysis

Because this study was conducted in the field, on commercial herds with different management routines, we had limited scope to influence compliance with the planned VWP treatments, which varied between the

Table 7. Recorded diseases and number of culled cows per lactation and 100 cow-years in the study (time at risk) for all cows randomized to (intention-to-treat) and receiving (per-protocol) the planned conventional (CONV) and extended (EXT) voluntary waiting period treatment[1]

Variable and lactation	Per-protocol		Intention-to-treat	
	CONV	EXT	CONV	EXT
Lactation 1				
Diseases recorded from 25 DIM	19	23	23	33
Culled cows	18	17	42	70
Cow-years in study from 25 DIM	194	215	235	304
Cow-years in study	208	227	252	323
Lactation 2				
Diseases recorded	66	59	81	73
Culled cows	43	34	46	48
Cow-years in study	161	130	181	173

[1]Diseases and cow-time in study recorded for d 0 to 25 in lactation 1, before the start of the intervention, were excluded.

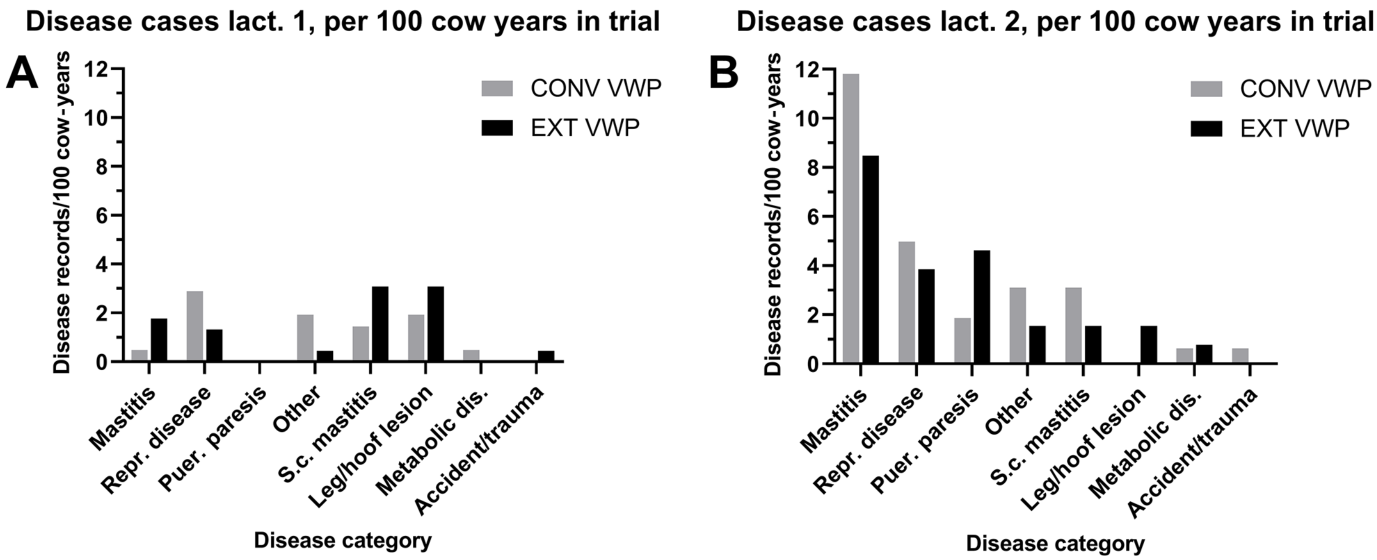

Figure 3. Disease incidence rate for each disease category per 100 cow-years in the study for cows receiving an extended (EXT; lactation 1 [lact. 1], n = 178; lactation 2 [lact. 2], n = 128) or conventional (CONV; lact. 1, n = 204; lact. 2, n = 166) voluntary waiting period (VWP), respectively, during (A) lact. 1 and (B) lact. 2, for cows receiving the intended VWP. Diseases recorded on d 0 to 25 in lact. 1, before the start of the intervention, were excluded. Results are presented descriptively. Repr. disease = reproductive disease; Puer. paresis = puerperal paresis; S.c. mastitis = subclinical mastitis; Metabolic dis. = metabolic disorders.

herds (Edvardsson Rasmussen et al., 2023). Compliance was lower for the extended than for the conventional VWP treatment. One explanation is that it might be easier to comply with a conventional management routine, in this case regarding which cows to inseminate at what time, as a new routine increases the requirement for precision among staff performing inseminations at herd level. When asked why the planned VWP treatment was not followed, several farmers also reported that it was difficult to resist the temptation to inseminate a cow with strong estrus signs, leading to cows randomized to the extended VWP treatment being inseminated earlier than planned (Figure 1). However, the most common answer in both groups was due to "unknown reason," and the second most common answer for the cows randomized to the extended VWP treatment was due to "mistakes" and, for the conventional VWP treatment, due to "fertility issues." The most commonly reported "fertility issue" was that the cows had not shown estrus during the intended conventional VWP treatment range (results not shown). These cows with late onset of estrus were excluded from the analysis, which might have affected the results to some extent. However, this reason for lack of compliance was reported for the group with higher overall compliance; thus we do not find it likely to have a large effect on the results.

As the risk of culling a nonpregnant cow is greater than for a pregnant cow (Gröhn et al., 1998), the cows with longer VWP had a longer period of higher "risk" of culling before insemination. However, this gives the herd manager more time to gather information about the cows and their yield, potentially leading to better-informed decisions about culling. This theory, in combination with the possibility that the farmers may have had preconceptions that high-yielding cows may be better suited to extended VWP, based on results from previous studies (Arbel et al., 2001; Römer et al., 2020), was supported by our findings on milk yield per day during early lactation in cows not receiving their planned VWP treatment. From 4 to 33 DIM, we found no difference in average daily yield between the VWP treatments, indicating that at the start of intervention for the conventional VWP treatment, compliance did not depend on milk yield. However, on looking at milk yield per day over a longer period, up to 145 DIM (thus allowing comparison only between cows with planned extended VWP treatment), we found higher yield for cows receiving, in contrast to not receiving, an extended VWP. Additionally, we found an apparent difference in proportion of cows culled due to low milk yield during lactation 1 (Figure 4), between cows with extended and conventional VWP treatment.

To reveal potential structural bias introduced by difference in compliance, we performed an intention-to-treat analysis for the culling rate. We found no difference in culling rate between the 2 VWP treatments, either for cows randomized to "intention to treat" or

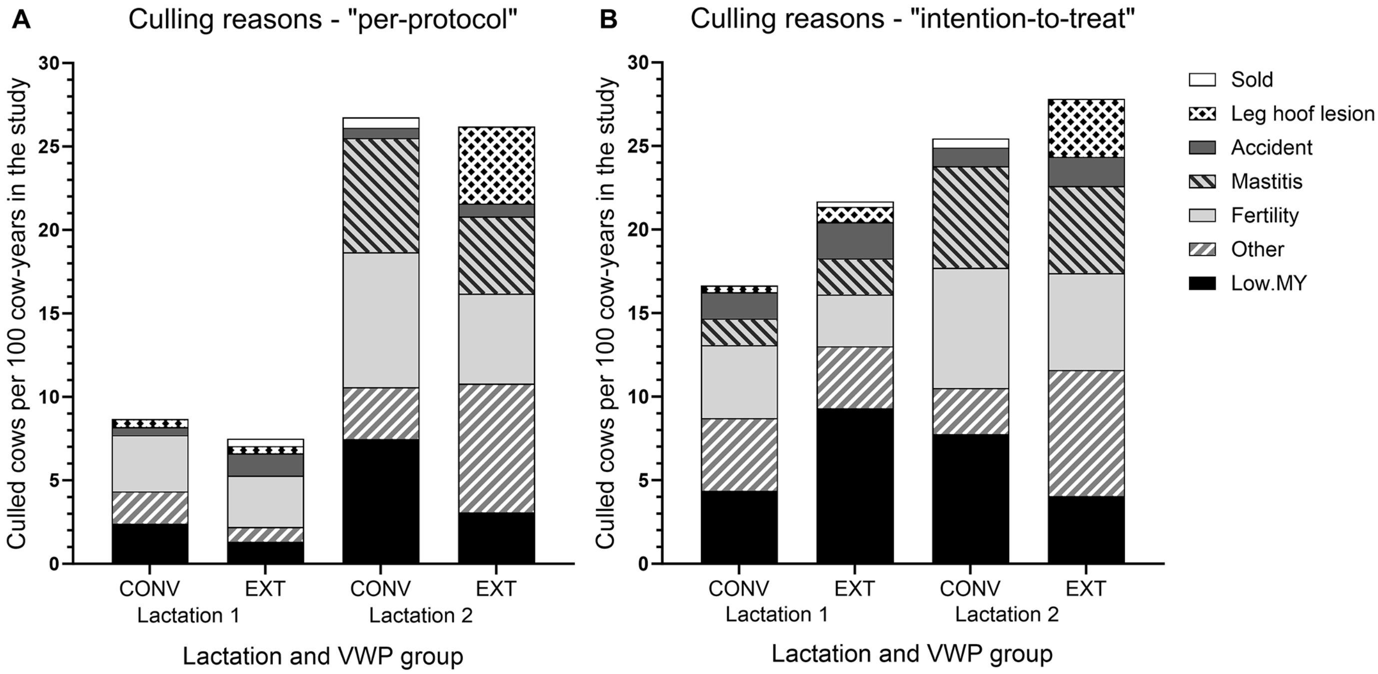

Figure 4. Reported culling reasons, presented as the number of culled cows per culling reason category and 100 cow-years in the study in each lactation, for (A) cows receiving their planned conventional (CONV) or extended (EXT) voluntary waiting period (VWP) treatment, "per protocol" (lactation 1, n = 382; lactation 2, n = 294), and (B) all cows randomized to each VWP treatment, "intention to treat" (lactation 1, n = 531; lactation 2, n = 357). MY = milk yield.

receiving "per protocol" the extended or conventional VWP treatment, which is in line with the results from Burgers et al. (2022) and Arbel et al. (2001).

General Considerations

Because farmers themselves reported most of the data to SNDRS, the reliability and completeness of data may differ between herds. To account for potential differences between herds, herd was included as a random factor in all models. We assumed that the management routines regarding estrus detection, insemination technique, and so on, were comparable for cows receiving the 2 different VWP treatments, as both treatments were represented in each herd. However, some routines might have been affected by the intervention, as by differences in management regimen between the VWP treatments or by the possibility to observe the cows with extended VWP for a longer period before the inseminations. These effects would be interesting to evaluate in future research, as they may be relevant to the implementation of extended VWP in herd management.

Considering the results from our previous paper on milk yield (Edvardsson Rasmussen et al., 2023) in combination with the current results, it appears that for primiparous cows with an extension of VWP to 145 up to 205 d, milk yield may be sustained, and reproductive performance, in the form of estrus intensity, NINS, and FSCR, may be improved without apparent detrimental effects on health or culling.

CONCLUSIONS

In this prospective randomized-controlled study, we found that primiparous cows receiving an extended VWP had improved reproductive functions during the first lactation, as reflected by stronger estrus intensity, higher FSCR, and lower NINS. During the following lactation without VWP intervention, we found no effect on these fertility parameters and no difference in disease prevalence or culling between cows receiving the 2 different VWP treatments in either lactation. Compliance with the planned VWP treatment was lower for cows with extended compared with conventional VWP. However, when we investigated the "intention-to-treat" effect of extended VWP on culling rate, to identify any bias due to varying compliance with the planned VWP treatments, we found no difference between cows randomized to an extended compared with a conventional VWP. These findings can be used to support management decisions on VWP length in high-yielding dairy herds. However, lack of compliance for cows randomized to an extended VWP indicates that further research, for example on the customization of VWP for individual cows, might give room for further improvements.

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