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Extended voluntary waiting period before first insemination in primiparous dairy cows

Effects on milk production, fertility, and health

Anna Edvardsson Rasmussen

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Anna Edvardsson Rasmussen
Faculty of Veterinary Medicine and Animal Science
Department of Animal Nutrition and Management
Uppsala

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Abstract

Extension of the voluntary waiting period (VWP) before first insemination after calving, could decrease the frequency of demanding transition periods both at cow and herd level. In the initial study of this thesis, the cows were randomized to an extended (155-205 d) or conventional (35-85 d) VWP and assessed during their first and second lactation. In a later study on customized extended VWP, cows expected to be suited for extended VWP were selected based on high genomic persistency index, calving difficulties or disease in early lactation, and high early lactation yield, and then randomly allocated to extended (≥185) or conventional (≤90) VWP.

For cows with randomized extended compared with conventional VWP, milk yield per day in the calving interval was maintained during the first lactation and higher during the second lactation, milk yield before dry-off was lower and reproductive performance was improved. Cows with customized extended VWP showed similar improvements compared with cows expected to be suited for an extended but receiving conventional VWP, but in contrast to the randomized study, the dry period was not longer for cows randomized to customized extended VWP. Extending the VWP had no effect on disease incidence and culling rate. Extended VWP for primiparous cows in high-yielding herds can thus make use of modern dairy cows' great potential for milk production and fertility, thereby potentially increasing flexibility and resilience in dairy herds.

Keywords: Extended calving interval, extended lactation, customized calving interval, customized lactation, milk yield, reproduction, dairy cattle

Author's address: Anna Edvardsson Rasmussen, Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, P.O. Box 7024, 750 07, Uppsala, Sweden

Förlängd frivillig väntetid innan första insemination för förstakalvare

Effekter på mjölkproduktion, fertilitet och hälsa

Sammanfattning

Genom att förlänga den frivilliga väntetiden (FVT) innan första insemination efter kalvning kan frekvensen av övergångsperioder minska på både ko- och besättningsnivå. Inom ramen för denna avhandling undersöktes effekten av förlängd FVT på mjölkproduktion, fertilitet och hälsa för förstakalvare. I en första randomiserad studie lottades förstakalvare till förlängd eller konventionell FVT under första laktationen och följdes under två laktationer. I en påföljande studie individanpassades kalvningsintervallet med hjälp av tre selektionskriterier; genomiskt index för uthållig laktationskurva, kalvningssvårigheter eller sjukdom i tidig laktation samt hög mjölkavkastning första månaden efter kalvning.

Kor som randomiserats till förlängd FVT behöll mjölkmängd per dag i första kalvningsintervallet och hade högre mjölkmängd per dag i det andra kalvningsintervallet, dessutom var mjölkmängden innan sinläggning lägre och fertiliteten förbättrad. Kor med individanpassad förlängd FVT hade till skillnad från kor som randomiserats till förlängd FVT inte längre sinperiod, jämfört med konventionell FVT. Förlängd FVT hade ingen effekt på sjukdomsincidens eller utslagningsfrekvens i någon av studierna. Resultaten visar att förlängd frivillig väntetid, genom att ta till vara på moderna mjölkkors stora potential, kan minska frekvensen övergångsperioder med bibehållen mjölkproduktion och hälsa, samt förbättrad fruktsamhet i högproducerande besättningar. Att ha förlängd FVT som ett alternativ kan öka flexibiliteten och därmed resiliensen i mjölkbesättningar.

Nyckelord: Förlängt kalvningsintervall, förlängd laktation, individanpassat kalvningsintervall, mjölkavkastning, reproduktion, mjölkkor

Författarens adress: Anna Edvardsson Rasmussen, Sveriges lantbruksuniversitet, Institutionen för husdjurens utfodring och vård, Box 7024, 750 07, Uppsala, Sverige

Preface

“Determining the optimal VWP from field data is difficult and unlikely to happen.” Inchaisri et al. 2011

Dedication

♡ To my beloved family, I will always love you ♡

&

☆ To all my excellent supervisors and teachers through the years ☆

༄ This thesis would not exist without you ༄

❀

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Edvardsson Rasmussen, A., Holtenius, K., Båge, R., Strandberg, E., Åkerlind, M., & Kronqvist, C. (2023). A randomized study on the effect of extended voluntary waiting period in primiparous dairy cows on milk yield during first and second lactation. *Journal of Dairy Science* vol. 106 (No. 4), pp. 2510-2518.

II. Edvardsson Rasmussen, A., Båge, R., Holtenius, K., Strandberg, E., von Brömssen, C., Åkerlind, M., & Kronqvist, C. (2023). 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. *Journal of Dairy Science*, (accepted).

III. Edvardsson Rasmussen, A., Holtenius, K., Båge, R., Strandberg, E., Åkerlind, M., & Kronqvist, C. Customized voluntary waiting period in primiparous dairy cows. I. Effect on milk production (manuscript).

IV. Edvardsson Rasmussen, A., Båge, R., Holtenius, K., Strandberg, E., Åkerlind, M., & Kronqvist, C. Customized voluntary waiting period in primiparous dairy cows: II. Effect on fertility, health and culling (manuscript).

The contribution of Anna Edvardsson Rasmussen to the papers included in this thesis was as follows:

I. Participated in planning the study, in collaboration with the supervisors. Had main responsibility for coordinating and running the study, with help from the supervisors. Analyzed the results with help from the statistical unit and supervisors. Drafted and revised the manuscript in collaboration with the co-authors.

II. Participated in planning the study, in collaboration with the supervisors. Had main responsibility for coordinating and running the study, with help from the supervisors. Analyzed the results with help from the statistical unit and supervisors. Drafted and revised the manuscript in collaboration with the co-authors.

III. Had main responsibility for planning, coordinating, and running the study, with help from the supervisors. Analyzed the results, and drafted and revised the manuscript in collaboration with the co-authors.

IV. Had main responsibility for planning, coordinating, and running the study, with help from the supervisors. Analyzed the results, and drafted and revised the manuscript in collaboration with the co-authors.

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Abbreviations

BCS	Body condition score
BHB	Beta-hydroxybutyrate
CFI	Calving to first insemination interval
CInt	Calving interval
DIM	Days in milk
DPL	Dry period length
ECM	Energy-corrected milk
FSCR	First service conception rate
GnRH	Gonadotropin-releasing hormone
HOL	Holstein
IGF-1	Insulin-like growth factor 1
LH	Luteinizing hormone
NEB	Negative energy balance
NEFA	Non-esterified fatty acids
NINS	Number of inseminations per conception
PI	Persistency index
RDC	Red dairy cattle
SNDRS	Swedish national dairy herd recording scheme
VWP	Voluntary waiting period

1. Introduction

Milk production is the largest category (13%) of animal-source agricultural output in the EU (European Commission 2022). In Sweden, around 60 L of milk, 20 kg of cheese, and 30 kg of other dairy products were consumed per person in 2022 (Swedish Board of Agriculture 2023). Despite this high demand, many dairy farmers are under severe economic pressure and the number of dairy farms in Sweden has decreased dramatically in the past 30 years (Figure 1). Many small farmers lacking the financial resources to invest and grow have resigned. At the same time, there are increasing demands from society regarding productivity, sustainability, and animal welfare (Barkema et al. 2015; Widmar et al. 2017; Britt et al. 2018; Ritter et al. 2022).

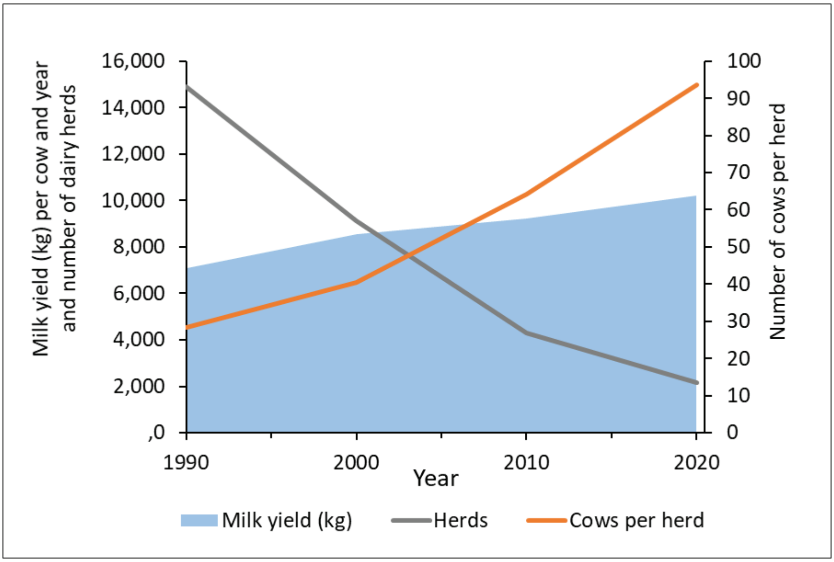

Figure 1. Trend in number of dairy herds, cows per herd and milk production per cow and year in Sweden, during the past 30 years (Växa Sverige 2023b).

Furthermore, we live in an ever-changing world and herd managers may have to adapt quickly to altered conditions. Also, the modern dairy cow have evolved dramatically during the past decades, and old management routines might need to be updated to optimize the potential for production and increase cow welfare. For this reason, it is of critical importance to investigate new possible management routines to allow for flexibility and thereby resilience, and to question old 'truths'. One of the practices that has been challenged in several studies over many years (Arbel et al. 2001; Gaillard et al. 2016; Römer et al. 2020; Burgers 2022) is that a cow should have one calf per year to be most profitable (Strandberg & Oltenacu, 1989; Inchaisri et al. 2011; Steeneveld & Hogeveen, 2012).

A dairy cow is pregnant for nine months (or about 280 days), so to have a traditional calving interval (CInt) of about 12 months she needs to become pregnant within three months after calving. However, it takes time for the cow to recover and regain sexual cyclicity after calving (Crowe et al. 2014). Therefore, a voluntary waiting period (VWP) before the first insemination is generally applied to give the cow time to recover after calving, start cycling and showing estrus again. Even so, many cows do not conceive at the first insemination, an average of about two inseminations is needed for a cow to become pregnant (Växa Sverige 2023b), and normally each estrus cycle is about 21 days. Moreover, there is a large individual variation in the number of inseminations needed per conception (NINS) (Burgers et al. 2021b).

On many farms, the VWP is around 50 days, but the management strategy differs between herds, as well as within herds, for different groups of cows. The aim is commonly that heifers should be around 23-25 months old at their first calving (Steele 2020; Atashi et al. 2021), but as these animals are still growing and not fully mature, they do not have as high peak milk yield in their first lactation as older, multiparous cows (Gaillard et al. 2016). This proportionally lower yield during the first lactation has been used to justify keeping the first CInt, and therefore the VWP, as short as possible. On the other hand, the lactation curve of primiparous cows generally shows higher persistency than that of multiparous cows (Österman & Bertilsson, 2003) (Figure 2), and lactation persistency has been proposed as one of the most important traits for cows suitable for extended VWP (De Vries 2006; Lehmann et al. 2019; Römer et al. 2020).

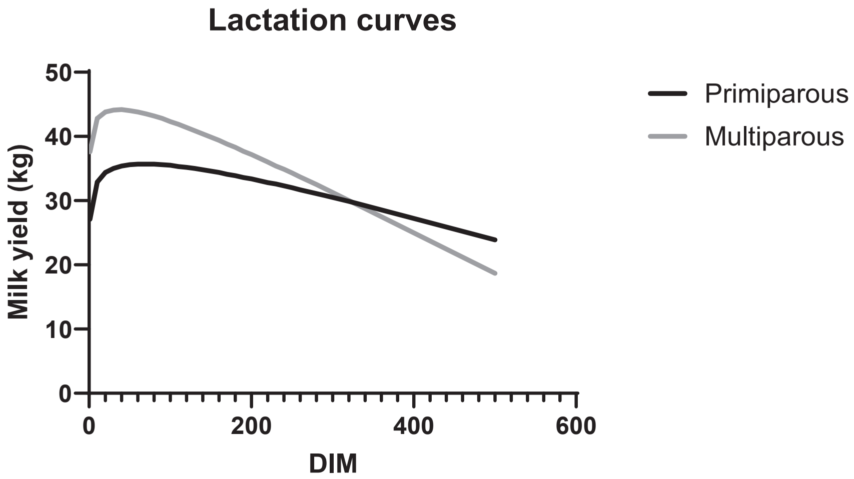

Figure 2. Schematic lactation curves for primiparous and multiparous Red dairy cattle, calculated using the NorFor model (Volden 2011). DIM = days in milk.

2. Background

2.1 Extended calving interval

By extending the VWP while maintaining the conventional dry period length (DPL) (usually about 50 days), the time to the next dry-off and calving is extended. Provided that the cow can maintain sufficient milk production, this will lead to a longer lactation and thereby a higher proportion of lactating days compared with dry days. This may result in a higher proportion of productive days during the cow's life. It will also result in the cow spending proportionally more time in the later stages of lactation, when she needs less energy-rich feed, which in turn may lower feed costs. For most cows, there is a decline in yield over time, which means that cows with extended VWP may have lower yield before dry-off (Niozas et al. 2019a). This is beneficial from an udder health and cow welfare perspective, as abrupt dry-off at high yield may be painful (Bertulat et al. 2013) and may increase the risk of mastitis (Rajala-Schultz et al. 2005; Odensten et al. 2007).

Furthermore, an extended CInt will reduce the relative time the cow spends in the sensitive transition period (Burgers et al. 2021a). As the first insemination is delayed, the cow has more time to recover after calving and to improve her energy balance (Figure 3), which in turn may improve fertility (Butler 2005).

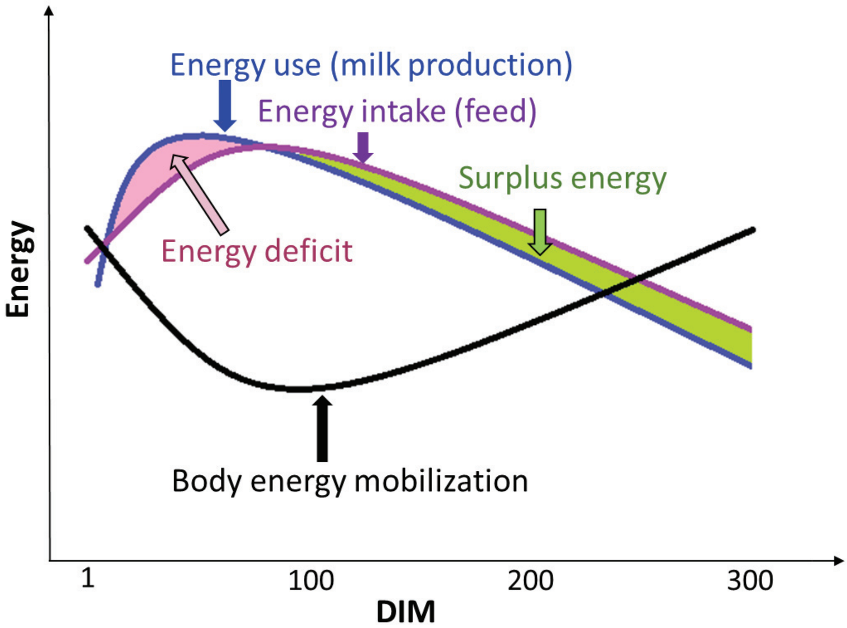

Figure 3. Schematic diagram of energy balance during the lactation. Modified after Strucken et al. (2015). DIM = days in milk.

When the interval between calving and first insemination increases, the farmer gets more time to gather information about cow performance and hence to decide which cows should be kept in the herd and inseminated, allowing for more qualitative culling decisions. As the time around calving is the most disease-affected period in the cow's life (Bradley & Green 2004; Ingvartsen 2006) (Figure 4), prolonging the CInt may lead to fewer disease cases per unit time (van Knegsel et al. 2022). Moreover, extended CInt has been linked to better longevity (Owusu-Sekyere et al. 2023) and longer productive life (Remmik et al. 2020; Römer et al. 2020).

At herd level, changing to an extended VWP for primiparous cows (which usually make up about 30% of the herd) may have consequences for the flow of replacement animals. First, the number of calves born per unit time would decrease. This might be one way of reducing the number of surplus dairy calves, which is a problem in some countries (Bolton & von Keyserlingk, 2021). On the other hand, having fewer calves could also result in fewer replacement heifers, which might lead to slower genetic progress. A possible solution, as proposed in a recent opinion paper by Veissier et al. (2023) and shown in a simulation study by Clasen et al. (2019), could be to use sexed semen for genetically promising cows to produce replacement heifers, and beef semen or embryo transfer for cows with less promising genetic merit.

Second, the proportion of lactating cows to dry cows would increase with an extended VWP. Assuming a fixed culling rate per lactation, this would lower the yearly requirement for replacement heifers in the herd, matching the lowered supply (Lehmann et al. 2019). Further, the proportion of cows in their first lactation would increase. Applying an extended VWP would also reduce the number of transitions on herd level (Burgers et al. 2021a), which might decrease the workload for farm staff regarding dry-offs, calvings, calf care, and regrouping of animals.

However, for cows that are unable to maintain milk production for an extended lactation, extended VWP has been linked to a longer dry period (Rehn et al. 2000), and higher body weight (Schneider et al. 1981). Excessively high body condition score (BCS) at the end of the CInt has also been reported, especially with greater extension of the VWP (Niozas et al. 2019a) and in multiparous cows (Burgers et al. 2021a). High BCS at dry-off and at calving has been linked to detrimental effects on fertility and health (Chebel et al. 2018; Fricke et al. 2023). High body condition at the end of lactation increases the risk of body condition loss during the dry period, which may increase the incidence of uterine disease and the need for drug treatments, reduce the chances for pregnancy post-partum, lower milk yield (Chebel et al. 2018) and increase the risk of culling (Melendez et al. 2020). Further, high BCS at calving has been linked to body condition loss in early lactation, which in turn has been associated with decreased health (Barletta et al. 2017) and reproductive performance (Carvalho et al. 2014). On the other hand, too low body condition is also detrimental for reproductive performance, health, and milk yield (Roche et al. 2009).

Hence, customizing VWP for cows that may have a higher chance of maintaining milk production through an extended CInt could reduce the risk of high body condition at the end of lactation and also give cows with too low body condition a chance to regain body condition before dry-off. This might improve health, reproduction, and production in the subsequent lactation.

2.2 The transition period

The weeks around calving, when the cow moves from a non-lactating state to a lactating state, is usually called the transition period. This period involves multiple physiological, metabolic, immunological, and endocrinal changes that may be demanding for the dairy cow. Starting about three weeks before calving, there are social changes, which may involve agonistic interactions during the establishment of a social hierarchy, as animals are usually regrouped to prepare for calving and lactation. All these events may be stressful for the cow.

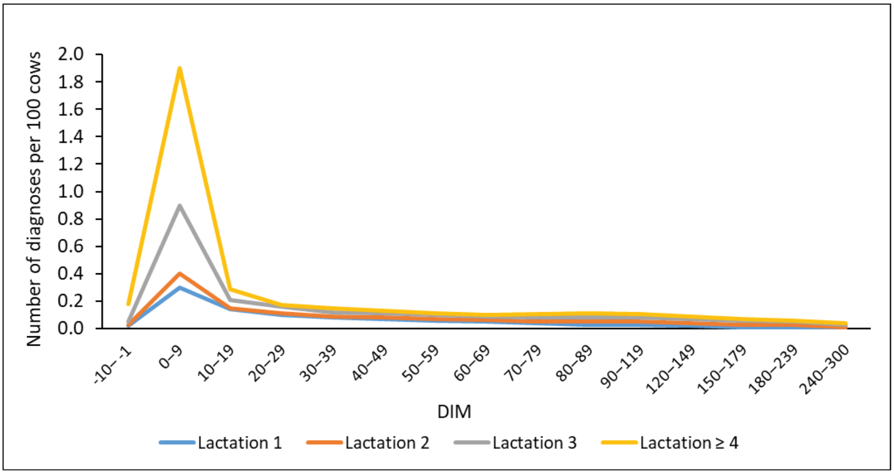

Figure 4. Disease incidence in Swedish dairy herds at different time periods during lactation 1, 2, 3 and ≥ 4. Modified after Växa Sverige (2023a).

Furthermore, the considerable physiological changes and immense acceleration of metabolism and milk synthesis during the transition period add extra pressure on the cows. Local inflammation of the reproductive tract, induced by calving in combination with increased metabolism and mobilization of body tissues, has been associated with systemic inflammation (Medzhitov 2008; Pascottini et al. 2022). At the same time, the function of the immune system is reduced during the weeks around calving (Goff & Horst, 1997; Abuelo et al. 2023). For the most part, these changes are physiological and necessary, and most cows adapt. However, some cows fail to adapt, making them susceptible to a number of disorders of metabolic (*e.g.*, ketosis, displaced abomasum, retained placenta, and milk fever), inflammatory (*e.g.*, endometritis), and infectious (*e.g.*, mastitis and metritis) nature, as reviewed by Esposito et al. (2014) and Pascottini et al. (2022). This is manifested as a rise in disease incidence in the time period around calving (Figure 4). Several health disorders have also been found to be connected to decreased fertility, with carry-over effects of several months (Ribeiro et al. 2016). It is debated whether common transition diseases are in part caused by, or in fact themselves partly cause, the energy deficit in dairy cows, by reducing feed intake and increasing energy consumption of the immune system (Roche et al. 2009; Horst et al. 2021; Pascottini et al. 2022). Either way, extending the VWP makes transition periods less frequent, both for the individual cow and on herd level, which may be beneficial for both cow health and welfare.

2.3 Energy balance and fertility

There is a strong association between nutrition, energy balance, and fertility. A state of severe negative energy balance (NEB) postpartum negatively influences the reproductive system on several levels: the regulating hormonal feedback system, the quality of the oocyte and the early embryo, the environment in oviducts and uterus, and also the immune system, with effects on uterine involution.

Dairy cows are rarely able to eat the amount of feed needed to keep up with the enormous acceleration in milk production during early lactation, and thus the cow ends up in NEB (Figure 3). The uncoupling of the somatotropic axis in early lactation (Renaville et al. 2002) means that the dairy cow compensates for the lack of energy intake by mobilizing body reserves in the form of fat and muscle mass, resulting in body condition loss (Roche et al. 2009). Body condition change is an indirect measure of energy balance in dairy cows (Butler 2003) and has been linked to health and reproductive outcomes. The NEB is manifested by several biomarkers, such as increased non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) levels (Ospina et al. 2013), and in decreased concentrations of insulin-like growth factor-1 (IGF-1) in blood, as well as an increased proportion of long-chain fatty acids, such as stearic (C18:0) and oleic (C18:1 cis-9) acid in milk (Churakov et al. 2021; Ntallaris et al. 2023). Although physiological, all these changes may involve some amount of stress and increase the risk of disease for the cow (Figure 4). As reviewed by Pascottini et al. (2022), a cow's ability to adapt to the challenges of the transition period affects both her health and her fertility.

The association between severe NEB and reduced reproductive performance is well known (de Vries & Veerkamp, 2000; Pascottini et al. 2022). Hypoglycemia is associated with inhibited gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus, impairing the luteinizing hormone (LH) pulse (Roland & Moenter 2011; Pascottini et al. 2022). The resumption of cyclicity after calving is dependent on the LH pulse pattern. Increased LH pulse frequency is linked to the capacity of the dominant follicle to produce estradiol, which is needed at sufficient levels during pro-estrus (Boer et al. 2010), together with IGF-1 bioavailability, to induce a GnRH and LH surge thereby inducing ovulation (Crowe et al. 2014). Estradiol is also crucial for estrus behavior (Pfaff 2005). The first ovulation after calving is generally "silent", but a corpus luteum that produces progesterone is formed, making progesterone an important biomarker in milk of resumption of ovarian cyclicity (Tarekegn et al. 2019; Ntallaris et al. 2023). Milk yield has been found to be negatively correlated with estradiol concentration, estrus expression, and duration of estrus (Lopez et al. 2004). Further, estrus intensity has been shown to be connected to conception rate, with cows expressing stronger estrus signs having a greater chance of conception (Nyman et al. 2016).

Several studies have linked high NEFA levels to detrimental effects on different reproductive functions. Serum NEFA levels may be reflected in oviductal fluid (Jordaens et al. 2017) and follicular fluid (Leroy et al. 2004). High NEFA levels have also been linked to impaired oocyte development (Van Hoeck et al. 2011; Ruebel et al. 2022), and to affect early embryo physiology by decreasing the number of cells and increasing the proportion of apoptotic cells in the blastocyst (Van Hoeck et al. 2011). Civiero et al. (2021) found that cows with lower energy balance had a longer interval from calving to onset of luteal activity (measured by progesterone in milk), and to first observed heat, but found no association between energy balance and conception rate at first insemination. Moreover, body condition loss in early lactation has been linked to delayed ovulation (Opsomer et al. 2000), reduced embryo quality, and decreased number of pregnancies per insemination (Carvalho et al. 2014).

Extending the VWP, and thereby postponing the first insemination, gives the cow a chance to regain her energy balance before insemination, which could improve reproductive performance. An overview of indicators of reproductive performance used in this thesis is provided in Figure 5.

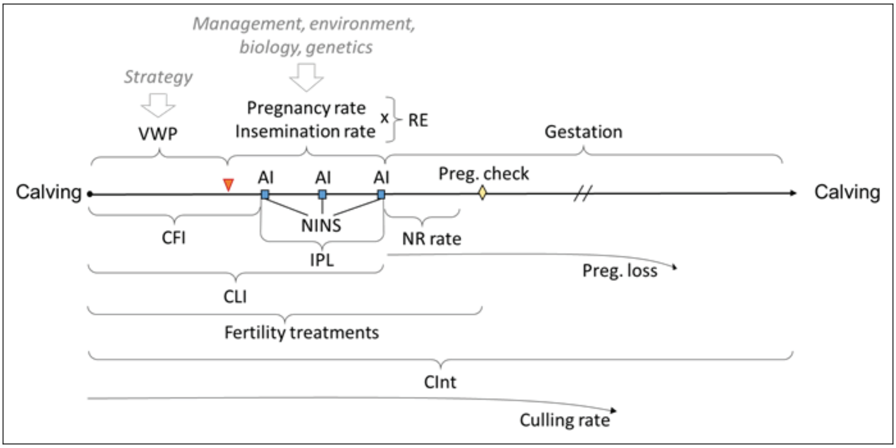

Figure 5. Indicators of reproductive performance in relation to time between two calvings, voluntary waiting period (VWP), reproductive efficiency (RE, equal to pregnancy rate, *i.e.*, conception rate × insemination rate), artificial insemination (AI), pregnancy check (Preg. check), calving to first insemination interval (CFI), calving to last insemination interval (CLI), insemination period length (IPL), number of inseminations (NINS), non-return (NR) rate, pregnancy loss (Preg. loss), culling rate refers to culling due to reproductive disorders, and calving interval (CInt). Modified after Löf (2012).

2.4 Review of the literature on voluntary waiting period

2.4.1 Retrospective studies

Early studies of CInt length have commonly been retrospective. The effect of CInt on average milk yield during the CInt was already being discussed 100 years ago, when Hammond and Sanders (1923) and Sanders (1927) concluded that the CInt should not be shorter than 12 months. Subsequent studies by Matson (1929) and Gaines and Palfrey (1931) found that an important factor determining milk yield in the current lactation was length of the previous CInt. Furthermore, Matson (1929) found that lifetime yield was increased with longer CInt and depressed by very short intervals. Gaines and Palfrey (1931) concluded that if desired, the CInt could be extended to 18 months without affecting the average yield over several CInt. On examining lactation records for around 3000 cows in Sweden, Johansson and Hansson (1940) confirmed that an increase in CInt has a positive effect on yield in the subsequent lactation. They also concluded that the optimal CInt for primiparous cows was about 14 months, for second parity cows around

13 months, and for cows in later lactations around 12 months, and that cows with persistent lactations could be allowed a longer CInt.

A later study by Louca and Legates (1968) found that days open is positively correlated with milk yield for primiparous cows, and negatively correlated for multiparous cows, and suggested that a 13-month CInt is optimal for primiparous cows and a 12-month CInt for multiparous cows. Weller and Folman (1990) confirmed that the optimum days open is longer for primiparous than for multiparous cows.

The advantage of retrospective studies is the possibility of involving a large number of animals or lactations based on pre-determined criteria. The disadvantage is that it is seldom possible to draw unbiased conclusions regarding the effect of extended VWP on fertility, as extended VWP is not generally practiced and the VWP for individual cows is rarely recorded. Therefore, it is generally not possible to distinguish a voluntary extension of the VWP from poor fertility, *e.g.*, inability of the cow to show estrus or to conceive. This may lead to the somewhat biased conclusion that extended CInt is the cause of poor fertility (Sawa & Bogucki, 2009; Mellado et al. 2016). It is possible to draw conclusions about correlations between factors, but perhaps less so regarding causality in this type of study.

A study by Römer et al. (2020) divided cows into different milk yield groups and compared the relationship between CFI and NINS, and CFI and first service conception rate (FSCR). They found that for cows with a yearly yield of <7,000 kg, short CFI is correlated with shorter first to successful insemination interval and lower NINS, but for high-yielding cows, with >12,000 kg yearly milk yield, the correlation is reversed. CFI could be used as a proxy for VWP, but is biased in that cows not showing estrus in time, or not being inseminated due to, *e.g.*, disease, will have extended CFI for reasons other than purely voluntary delay of the first insemination. This means that only cows showing estrus and being inseminated in time may have short CFI, whereas long CFI groups may contain cows with longer VWP and cows with a longer CFI than intended.

2.4.2 Simulation studies

Most simulation studies of extended CInt have investigated the relationship to economics and optimization of VWP, days open, or CInt. The strength of these studies is that they are flexible and can account for several factors. A drawback is that they are by definition highly dependent on the input data selected. Many simulation studies are based on retrospective data and on information based on conventional VWP, traditionally aiming at 12- to 13-month CInt.

Many simulation studies have concluded that the CInt should be as short as possible (James & Esslemont, 1979; Olds et al. 1979; Strandberg & Oltenacu, 1989; Inchaisri et al. 2011; Steeneveld & Hogeveen, 2012). However, Holmann et al. (1984) found 13 months to be optimal, while a study by Reyes et al. (1981) taking weight loss and differential feeding practices into consideration found that income over feed cost for different milk yields did not differ between a 13- and 15-month CInt regime. Dijkhuizen et al. (1985) investigated how long it is profitable to keep inseminating a cow with poor fertility and concluded that in high-yielding young cows, it may be profitable even up to 8-9 months after calving, leading to a CInt of up to 18 months.

More recent simulation studies, using input from customized retrospective studies on intentional extended CInt (Gaillard et al. 2016; Clasen et al. 2019; Kok et al. 2019; Lehmann et al. 2019), have concluded that extending the VWP for first parity cows, followed by conventional VWP for multiparous cows, is generally the most profitable option.

However, using data and assumptions based on conventional or customized retrospective VWP regime when modeling extended CInt runs the risk of introducing similar bias as in retrospective studies, *e.g.*, assuming fertility is similar or worse in cows with extended CInt, leading to a circular argument. To break free of such arguments, experimental studies on extended VWP are important, both for the results they provide *per se* and to provide input to simulation models.

2.4.3 Customized retrospective studies

Two previous studies have investigated customized VWP (Lehmann et al. 2014, 2016, 2017; Burgers et al. 2021b). Both these studies used retrospective data collected from commercial herds practicing extended VWP, but with criteria for VWP extension varying between herds. Consequently, there was no predetermined VWP for each cow and the herd manager could choose when to inseminate the cows, which risks creating similar bias as in retrospective studies. Cows in both studies were allocated to different groups based on either CInt or CFI, and with both these measures it is difficult to distinguish cows with an extended VWP from cows not showing estrus and thereby ending up with longer CFI or CInt.

The study by Lehmann et al. (2014, 2016, 2017) involved four commercial herds in Denmark practicing extended CInt. The authors concluded that the cows receiving extended CInt (of 17 or 19 months) were able to produce the same amount of energy-corrected milk (ECM) per feeding day. However, they observed that not all cows could maintain milk production for an extended lactation and that DPL was increased by 3-5 days. Burgers et al. (2021b) performed their study in 13 Dutch commercial dairy herds managed for extended CInt. On dividing the cows into CFI groups, they found that cows in the short CFI group had longer CInt than expected. Moreover, more than 50% of cows in the group with longer CInt received more than one insemination, indicating that they were not planned for a long CInt extension, while a longer CInt was also related to higher NINS. However, no correlation between NINS and CFI was found. Cows with CInt between 364 and 531 days had the highest yield.

To avoid potential drawbacks from extended VWP, identifying cows able to maintain milk yield for an extended lactation is crucial. Burgers et al. (2021b) found that high-yielding primiparous cows had the highest milk yield per day with a CFI of more than 196 days. Lehmann et al. (2017) found that ECM yield in early lactation (second and third test milking) could be used to predict cows ending up with a high lactation yield. When characterizing cows with extended CInt and high or low milk yield per CInt day, Lehmann et al. (2017) found that cows with high lactation yield had high peak and dry-off ECM yield, and greater lactation curve persistency between 60 and 305 days in milk (DIM). Moreover, these cows had higher NINS and a smaller proportion of the cows had high BCS at dry-off.

2.4.4 Random-controlled studies

There are several examples of random-controlled studies on extended VWP length, most dealing with the topics of milk production and fertility, although some also discuss health, culling, and economics. Most of these studies are based on results from one or two research herds, giving greater control over recording and better compliance with the research protocol. However, the drawback is that results from one research herd may not be as generalizable as those in studies performed on multiple commercial herds. As shown in Figure 6, some studies have reported results for primiparous and multiparous cows separately (Schindler et al. 1991; Ratnayake et al. 1998; Arbel et al. 2001; Österman & Bertilsson, 2003; Burgers et al. 2021b). This makes the results more relevant for this thesis, as for some variables, such as persistency, there are differences between primiparous and multiparous cows.

Randomized studies on the effect of extended VWP on milk yield have found that 305-d yield (Schneider et al. 1981; Burgers et al. 2021a) and whole lactation yield (Rehn et al. 2000; Österman & Bertilsson, 2003) are higher for cows with extended VWP. Previous findings on milk yield per day in the CInt, DPL, milk yield at dry-off, milk yield at the beginning of the subsequent lactation, FSCR and NINS, are presented in Figure 6.

Concerning health, some randomized studies on extended VWP have reported a number of disease treatments (Niozas et al. 2019a; Burgers et al. 2021a; van Knegsel et al. 2022), but no conclusive results have been presented. The impact of extended VWP on SCC has also been described (Österman et al. 2005; Niozas et al. 2019a; Ma et al. 2022). Ma et al. (2022) observed slightly higher SCC for cows with extended VWP (200 compared with 50 days) during the first six weeks of the next lactation, but apart from that, no clear effects of VWP treatment have been reported.

Culling rate and number of cullings have also been reported in previous studies. Larsson and Berglund (2000) found a higher frequency of culled cows during the CInt in cows with conventional compared with extended VWP. However, Niozas et al. (2019a) found an overall tendency during the whole study for a higher percentage of culled cows in the extended VWP group.

Compliance

In randomized controlled trials, compliance with the intended treatment is an important factor to consider. Compliance measures the proportion of participants intended to receive a treatment that actually receive the treatment. In cases of poor compliance, there is a risk of bias if many participants do not receive the treatment due to a factor that might influence the results.

A possible way to get around this is by performing an "intention-to-treat" analysis, whereby all cows randomized to each treatment are included in the analysis (Mansournia et al. 2017). This answers the question of what the total effect would be if the treatment were to be applied to all cows.

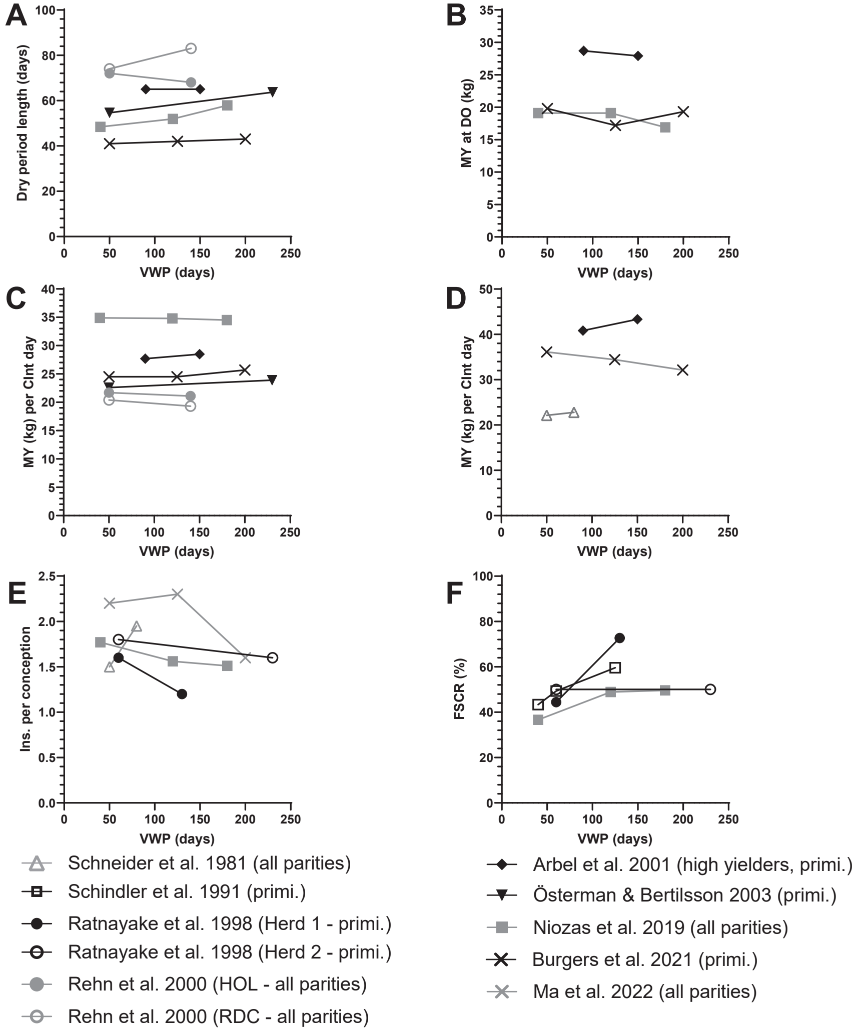

Figure 6. Summarized results from randomized studies on extended voluntary waiting period (VWP). A) Dry period length, B) milk yield at dry-off, C) milk yield per calving interval (CInt) day, D) milk yield per day in early next lactation, E) number of inseminations per conception, and F) first service conception rate (FSCR). Results from primiparous cows (primi.) or studies reporting results for all parities together (all parities). Rehn et al. (2000) reported results for Red Dairy Cattle (RDC) and Holstein (HOL) cows separately and Ratnayake et al. (1998) reported results for two herds separately (Herds 1 and 2). Modified from van Knegsel et al. (2022).

However, if cows not receiving the intended treatment are also included in the analysis, the actual treatment effect is diluted. To answer the question of what the actual treatment effect is, a "per protocol" analysis can be made, where only cows receiving the intended treatments according to the research protocol are included. This reveals the effect of the treatment on cows actually receiving the treatment.

2.4.5 Studies of voluntary waiting period in seasonal calving systems

A number of studies on extended VWP have been conducted in countries with seasonal calving and all-year-round grazing systems (Kolver & Roche, 2005; Kay et al. 2007; Kolver et al. 2007; Auldist et al. 2010). The motive, in addition to avoiding breeding at the time of lowest energy balance, is to achieve more even milk production throughout the year. To sustain seasonal calving, an extreme extension of the VWP of about a year is usually applied, in combination with different management of year-around grazing. Therefore, results for systems with seasonal calving are presented separately in this section. Such studies have found that reproductive performance increases in seasonal calving (Kolver & Roche, 2005; Kolver et al. 2007) and that milk production can be sustained for an extended lactation of up to 650 days (Kolver & Roche, 2005) with 75-100% of yearly milk solids (Kolver et al. 2007). Kay et al. (2007) investigated potential predictors of cows suitable for extended lactations and found that BCS in late previous lactation, previous lactation milk yield, and early current lactation information about blood metabolites (NEFA and glucose) and hormones (insulin and IGF-1) may be promising indicators.

The cheese-making properties of milk from cows undergoing extreme extensions of VWP have also been investigated. Auldist et al. (2010) found that milk from the second half of the lactation in cows with extended lactations of up to 22 months contained more milk solids and yielded more cheese per 100 kg milk than milk from cows with 10-month lactations, without compromising cheese-making properties. These results were confirmed by a study by Maciel et al. (2016) in a Danish research herd comprising cows with an 18-month CInt.

2.5 Description of the knowledge gap

As shown above, extended CInt, days open, and VWP have been the subject of numerous studies of different types, but no large-scale randomized study on multiple high-yielding commercial herds has been performed in recent years. Only one previous experimental study (Jarman et al. 2020) has monitored primiparous cows with an extended VWP in the first lactation through a following second lactation without extended VWP, which can be expected to be promising based on results from previous retrospective and simulation studies (see section 2.4.1 and 2.4.2). The study by Jarman et al. (2020) was performed on a seasonal calving system, practicing year-around grazing, and extending the VWP by 8 months.

In Sweden, the two most common breeds, both commonly represented in Swedish herds, are Swedish Holstein (HOL, 57%) and Swedish Red Dairy Cattle (RDC, 37%). HOL cows are known to have higher milk yield, while RDC cows usually have higher fat and protein concentrations in the milk. Although potential interactions may exist, few studies have investigated the interaction between VWP and breed (Rehn et al. 2000).

Due to the high individual variation between cows regarding milk yield and persistency, further investigations of individual adaption and customization of the VWP have been proposed in reviews of extended lactations (Sehested et al. 2019; van Knegsel et al. 2022). As mentioned, customized VWP has been investigated previously, but not using a randomized study design that could shed light on the matter, especially regarding results on reproductive performance not fully captured in retrospective studies.

3. Aim of the Thesis

The overall aim of this thesis was to investigate the effects of randomized and customized extensions of the VWP on milk production, fertility, health, and culling in primiparous dairy cows.

Specific objectives were:

- In a randomized-controlled study, to investigate the effect of VWP extension in primiparous cows on milk production, fertility, health, and culling during a first lactation with VWP intervention and a following second lactation without VWP intervention.
- To investigate potential interactions between VWP and breed.
- In a study of customized VWP in primiparous cows, to compare milk production, fertility, health, and culling of cows expected to be suited for extended VWP and randomized to receive either a conventional or extended VWP, and of cows expected to be suited for, and receiving, a conventional VWP.
- To explore compliance with the research protocol and how it affects culling rate and disease incidence.

4. Materials and Methods

4.1 Experimental design

Field trials were performed in commercial dairy herds, examining the effect of extended VWP on milk production, fertility, health, and culling, with the work divided into two studies (Figures 7 and 8). The intention was to use information from the first, randomized, study (August 2018 – September 2021) to create selection criteria for the second study (October 2020 – October 2022). The first study comprised a randomized controlled trial with two treatments, conventional and extended VWP, where the cows were followed through their first lactation with VWP intervention, and during their following second lactation without VWP intervention. The results were used to design the second study, examining the effect of customized extended VWP, where cows expected to be suited for extended VWP were randomized to receive a conventional or an extended VWP, and cows not expected to be better suited for extended VWP were all assigned to a conventional VWP.

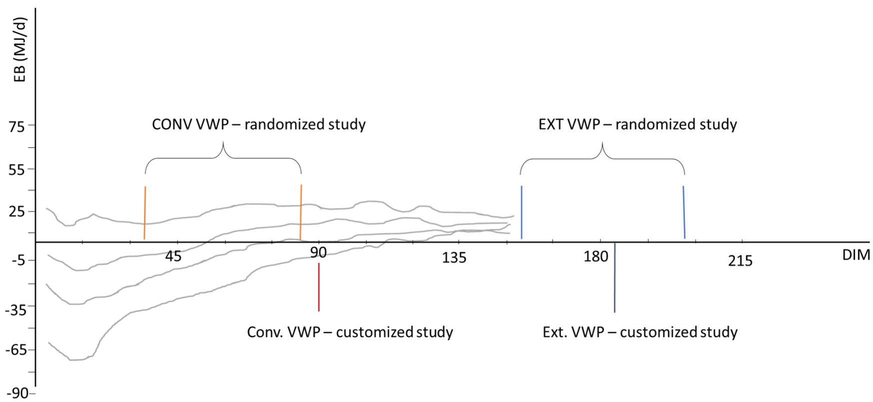

Figure 7. Schematic presentation of experimental design, with planned voluntary waiting period (VWP) interventions in the randomized study described in Papers I and II (conventional, CONV, and extended, EXT VWP), and in the customized study described in Papers III and IV (conventional, Conv., and extended, Ext. VWP). The grey lines represent quartiles of energy balance (EB) of primiparous cows, modified from Civiero et al. (2021). DIM = days in milk.

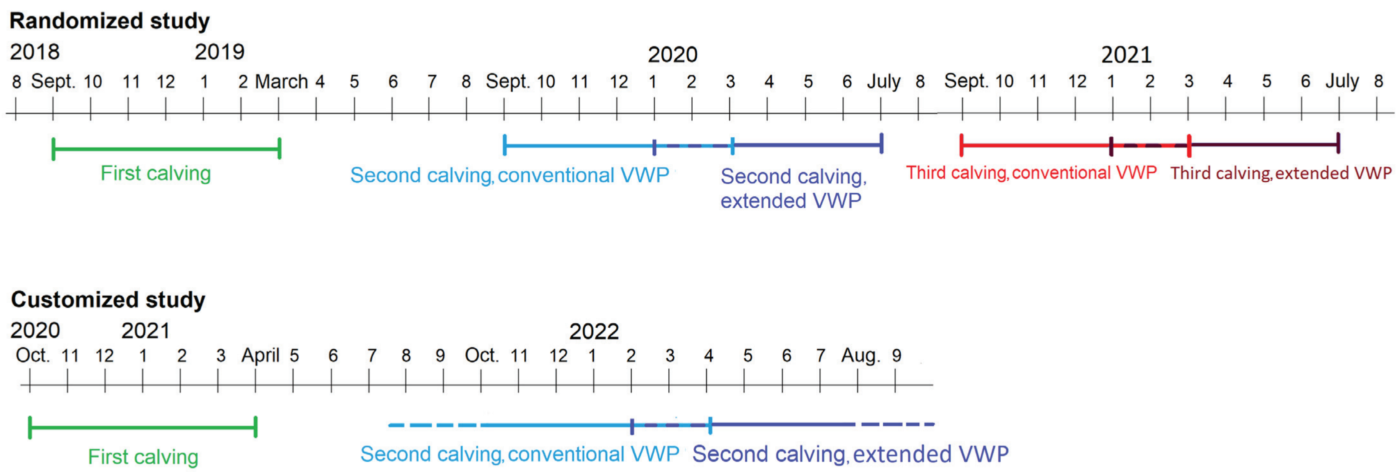

Figure 8. Timeline of the randomized and customized study of extended voluntary waiting period (VWP) on which this thesis is based.

4.2 Herd inclusion

Recruitment of herds meeting the inclusion criteria (Figure 9) was based on information from the Swedish National Dairy Herd Recording Scheme (SNDRS) for the production year 2016/2017. The herds were located in southern Sweden and, to facilitate transfer of herd information, they had to be connected to the SNDRS, which includes 76% of Swedish cows and is operated by a farmers' association called Växa Sverige. In the initial randomized controlled trial, 16 herds were included, of which 14 also participated in the customized extended VWP study, together with four additional herds recruited for that study. Regarding the main characteristics of the included herds, average yearly milk yield was 11,400 kg (range: 9,800 to 13,400 kg), CInt was 12.6 months (range 12-14 mo), and reproductive efficiency was 35% (range 25-45%).

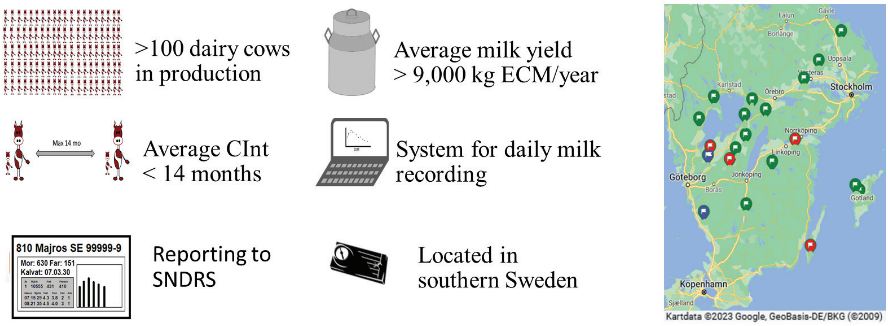

Figure 9. Herd inclusion criteria and geographical location within southern Sweden of participating herds (blue = participated in the randomized study, green = both studies, red = customized study). ECM = energy-corrected milk, CInt = calving interval, SNDRS = Swedish National Dairy Herd Recording Scheme.

4.3 Cow selection and intervention

4.3.1 Randomized study (Papers I and II)

Cow recruitment for the randomized study started within one month of 1 September 2018 on all farms and continued by recruiting all dairy heifers of the HOL or RDC breed (defined as Swedish Red, Danish Red, and Swedish Ayrshire) calving in the herds within six months from the starting date. A total of 533 cows (Paper I) and 531 cows (Paper II, two cows excluded due to incomplete fertility data) were followed during their first lactation with a VWP intervention. They were randomly allocated by odd or even ear number to a conventional (35-85 d) or extended (155-205 d) VWP treatment, with the aim of having a CInt of 12 or 16 months, respectively (Figures 7 and 8). A VWP range was used to define the expected range of first insemination for each treatment, with the same range (50 d) applied for both treatments, and to allow for variation between herds.

Of the cows that had a second calving, 42 cows in one herd were part of another VWP intervention study during their second lactation and were therefore excluded from the results, leading to 379 cows being followed through their second lactation in Paper I. In Paper II, only cows with a full lactation were followed through the second lactation (n = 357) (Figure 10). There was no VWP intervention during the second lactation and herd managers could decide when they wished to start inseminating the cows.

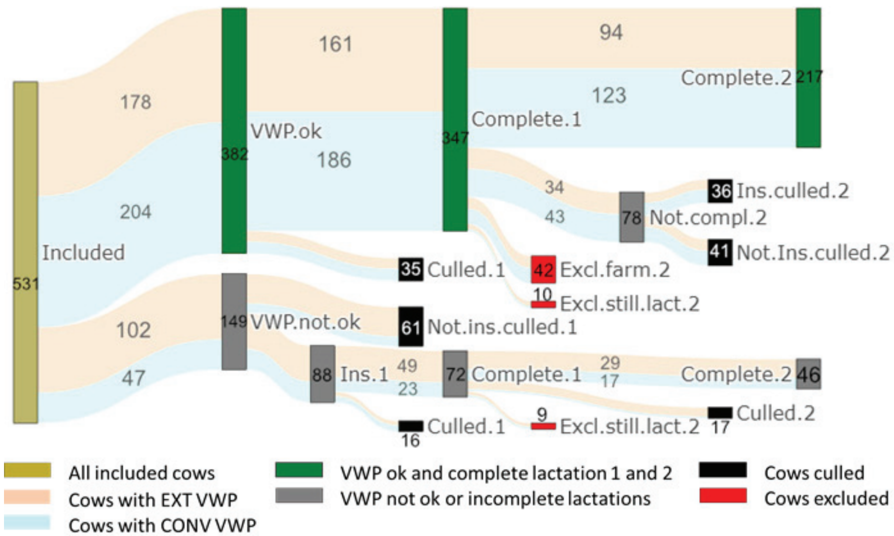

Figure 10. Schematic presentation of the flow of animals in the randomized study described in Paper II.

4.3.2 Customized study (Papers III and IV)

In the customized study, 530 HOL and RDC heifers having their first calf between 1 October 2020 and 31 March 2021 were enrolled in Paper III. In Paper IV, 17 cows that did not complete their first CInt were excluded, leaving a total of 513 cows. Cows expected to be suited for an extended VWP, based on three selection criteria (described below), were randomly allocated to either an extended (ExtExt) or conventional (ExtConv) VWP treatment. Participating farmers were offered and encouraged to do genomic testing of heifers that could potentially be enrolled in the trial. This was done to obtain the heifers' genomic persistency index (PI) as defined by the Nordic Cattle Genetic Evaluation (2022), which was used as the first selection criterion. Of the genomically tested cows with a PI value in November 2020 (78%), the 10% with the highest PI (which meant PI >111) were expected to be suited for extended VWP. For the remaining cows with no registered PI or with PI ≤111, an assessment based on the second and third selection criteria was made before 40 DIM. The second criterion was cows that had a difficult calving (including, *e.g.*, twins), or a disease event, reported by the farmer during early lactation. These cows were expected to need a longer recovery period before the first insemination. The third criterion was higher daily milk yield during 4-33 DIM than the herd average, calculated at the start of the trial and updated during the study. This criterion was applied to the remaining healthy cows.

All cows meeting at least one of the criteria were randomly assigned to either an extended VWP of ≥185 DIM (ExtExt treatment) or a conventional VWP of ≤90 DIM (ExtConv treatment). Cows not meeting any of the three criteria, and thus not expected to be suited for an extended VWP, were all assigned to a conventional VWP (ConvConv treatment). The numbers of cows selected per criteria, and breed distribution in the VWP treatments, are presented in Figure 11. The study design with cows receiving the planned VWP is shown in Figure 12.

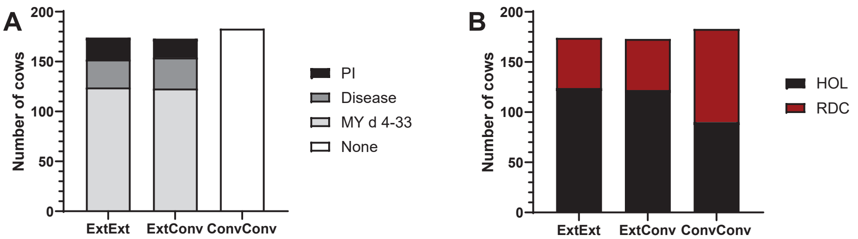

Figure 11. A) Number of cows selected based on each selection criterion and B) breed distribution in the three voluntary waiting period (VWP) treatments (ExtExt, ExtConv, ConvConv) in Paper III (n = 530).

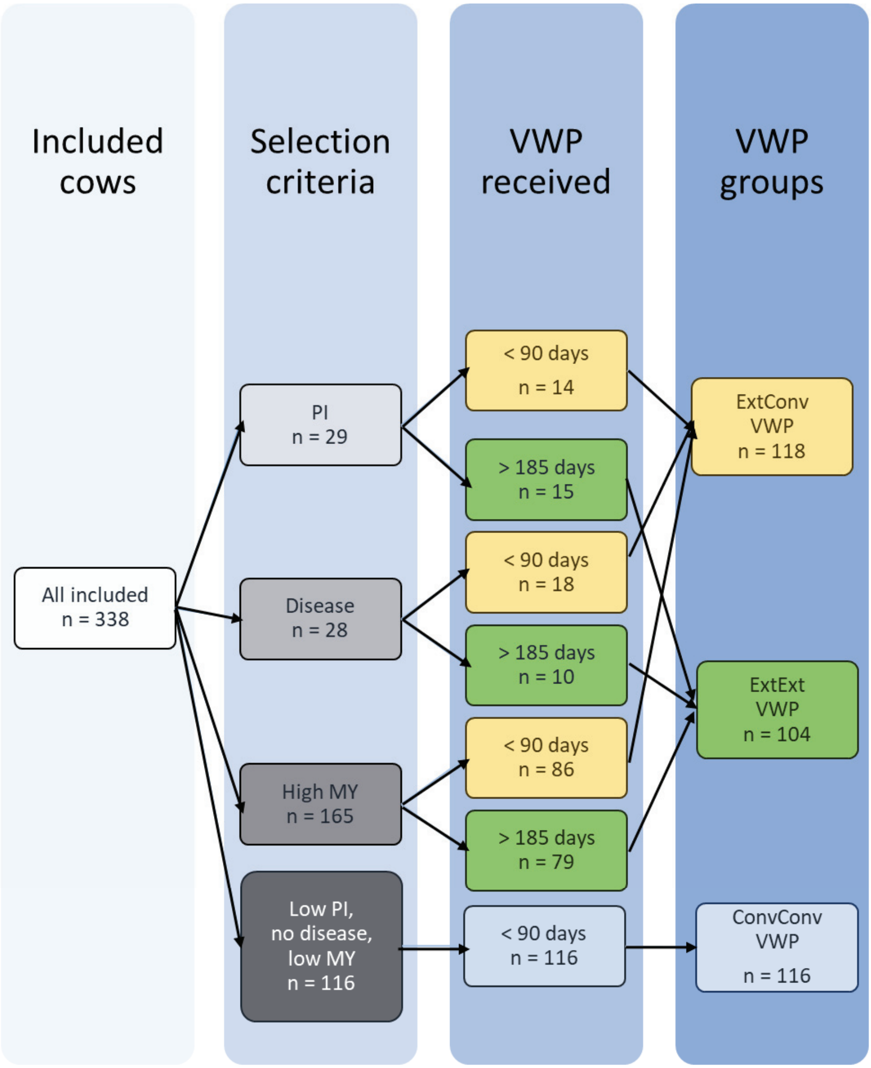

Figure 12. Schematic flow diagram of the study design in Paper III, with number of cows allocated to the different voluntary waiting period (VWP) treatments, number of included cows fulfilling the three selection criteria for extended VWP (high persistency index (PI), difficult calving or disease postpartum, and/or higher than average yield (MY) in early lactation); and number of cows fulfilling each criterion and randomized to and receiving the ExtExt and ExtConv treatments. Cows not fulfilling any criterion for extended VWP were allocated to the ConvConv treatment (Figure 1 in Paper III).

4.4 Calculation of variables studied

Data for Papers I and II covering the period August 2018 – September 2021 and data for Papers III and IV covering the period October 2020 – October 2022 were obtained from the SNDRS. The data included information such as breed, PI, calvings, estrus intensity, inseminations, diseases, SCC from monthly test milkings, test-day yields, 305-d lactation yields, dry-off dates, culling dates, and culling reasons for cows in the participating herds. Information on daily milk yield from the herds' milking systems was also collected. Additionally, if the planned VWP treatment was not followed, farmers were asked to report the reason. For Papers I and II, data for two consecutive lactations were obtained. For Papers III and IV, during the cow inclusion period farmers were asked to report difficult calvings or disease events in early lactation on a weekly to monthly basis. Calculation of the parameters measured is described in Tables 2 and 3 in the Appendix to this thesis.

4.4.1 Variable inclusion criteria

Three variable inclusion criteria defining which cows were to be included in each analysis were applied (Table 1). The first criterion was cows complying with the assigned VWP ± 10 days, accounting for the "per protocol" effect, *i.e.*, the "pure" treatment effect only including cows that received the intended treatment. For cows in Papers I and II, this meant that the EXT cows should have received their first insemination after calving at between 145 and 215 DIM (n = 178), and CONV cows should have received their first insemination after calving at between 25 and 95 DIM (n = 204). For cows in Papers III and IV, it meant a minimum CFI of 175 d in the ExtExt treatment and a maximum CFI of 100 days in the ConvConv and ExtConv treatments (Paper III, n = 399; Paper IV, n = 382). However, this variable inclusion criterion was not used in the calculation of compliance with the assigned treatments, or of "intention-to-treat" effect on culling rate. In those analyses, all cows were included, in order to account for potential bias due to lack of compliance.

The second variable inclusion criterion used was a complete CInt, meaning that the cow had a second calving (or third regarding lactation 2 variables) before the end of data collection (September 2021 in Papers I and II, October 2022 in Papers III and IV). In Papers I and III, only cows with complete CInt (Paper I: lactation 1, n = 421, lactation 2, n = 265; Paper III:

n = 396) were used for the analysis of all variables, as calculation of many outcome measures required a complete CInt. When analyzing SCC variables (Papers II and IV), only cows with complete CInt (Paper II: lactation 1, n = 419, lactation 2, n = 263; Paper IV: n = 396) were included.

An additional variable inclusion criterion, "sufficient daily milk yield records", was applied in Papers I and II. To ensure sufficient information, this was set to no more than 40 daily milk yields in the beginning of lactation and 60 daily yields at the end of lactation missing, and less than 50% total missing daily yields (Paper I: lactation 1, n = 430, lactation 2, n = 198). This inclusion criterion was used to ensure accurate calculation of lactation length, DPL, DPL category, milk yield before dry-off, and for the lactation curve diagrams. In Paper II, the criterion of sufficient daily yield records, defined as no more than 10 of the initial 40 daily yields missing (n=524) and no more than 50% of total yields missing (n = 512), was applied in calculation of mean daily yield between 4-33 DIM and 4-145 DIM.

4.4.2 Statistical analysis

Organization of data was initially done in Microsoft Excel 2016, with further organization and statistical analysis performed using R software (R Core Team 2022; RStudio Team 2022). GraphPad Prism (2023) and R software were used for data visualization. All statistical models except the grouped analysis of NINS, disease incidence, and culling rate were mixed models and included VWP treatment (two levels in Papers I and II; three levels in Papers III and IV) and breed (two levels) as fixed factors, and herd as a random effect (16 and 15 levels in first and second lactation, respectively, in Papers I and II; 18 levels in Papers III and IV). The interaction between VWP treatment and breed was also included as a fixed factor and removed from the models if not significant. The confidence level was set at $p<0.05$, and tendencies were generally not considered or discussed. The emmeans function in R (Lenth et al. 2023) was applied for *post hoc* tests. In Papers III and IV, *p*-values for differences between the treatment groups were calculated.

Linear mixed models were used for continuous variables, such as milk yield variables with approximate normal distribution. They were fitted by restricted maximum likelihood using the lmer function in the lme4 package in R (Bates et al. 2022), and the results are presented as least squares means ± the standard error of the mean. For the binary variables, such as DPL category, FSCR, pregnancy loss, SCC <100,000 cells/mL, and compliance, generalized binomial linear mixed models fitted by Laplace approximation from the glmer function in the lme4 package were used (Bates et al. 2022). For hypothesis testing of the models, Analysis of Deviance was used. The type II Wald Chi-square test was used to determine which of the fixed factors were significant. The results are presented as percentage and proportion of cows (n/N, where n is the number of cows in each DPL category, with first service conception, pregnancy loss, SCC <100,000 cells/mL, or complying with the assigned treatment, and N is the total number of cows in each analysis within each VWP treatment).

Regarding NINS, disease incidence, and culling rate, the results were analyzed at group level, where one value was calculated for each treatment within each farm (Paper II: n = 32 subgroups in lactation 1, n = 30 subgroups in lactation 2; Paper IV: n = 51 subgroups). In Paper IV, not all treatments were represented in one herd, which was therefore excluded from these analyses. Breed was not included as a factor in the models, as not all treatment-breed combinations were represented on all farms. Due to underdispersion in the count data, a negative binomial generalized linear model was applied, using the glmmTMB function in R (Brooks et al. 2017).

Table 1. Number of cows meeting each variable inclusion criterion in in the two voluntary waiting period (VWP) treatments in papers I (and II, numbers in parenthesis), (EXT, CONV) and in the three VWP treatments (ExtExt, ExtConv, ConvConv) in Papers III (and IV, numbers in parenthesis). CInt = calving interval

	Papers I and II, randomized study							Papers III and IV, customized study			
	Lactation 1			Lactation 2				Lactation 1			
	EXT	CONV	Total	EXT	CONV	Total		ExtExt	ExtConv	ConvConv	Total
I	n = 281	n = 252	n = 533	n = 188	n = 191	n = 379	**III**	n =174	n =173	n =183	n = 530
II	(n=280)	(n=251)	(n=531)	(n=171)	(n=186)	(n=357)	**IV**	(n = 167)	(n = 169)	(n = 177)	(n = 513)
Breed											
Holstein	181 (181)	146 (145)	327 (326)	115 (108)	109 (106)	224 (214)		124 (121)	122 (118)	90 (90)	336 (329)
Red Dairy Cattle	100 (99)	106 (106)	206 (205)	73 (63)	82 (80)	155 (143)		50 (46)	51 (51)	93 (87)	194 (184)
Inclusion criteria											
No. of inseminated cows	235 (234)	237 (236)	472 (470)	147 (145)	161 (161)	308 (306)		153 (148)	163 (159)	150 (147)	466 (454)
VWP according to plan	179 (178)	205 (204)	384 (382)	139 (128)	168 (166)	307 (294)		124 (120)	141 (139)	134 (132)	399 (391)
Complete CInt	211 (210)	210 (209)	421 (419)	125 (123)	140 (140)	265 (263)		132 (132)	134 (134)	130 (130)	396 (396)
VWP according to plan + complete CInt	162 (161)	187 (186)	349 (347)	96 (94)	123 (123)	219 (217)		104 (104)	118 (118)	116 (116)	338 (338)

5. Main Findings

5.1 Milk yield, calving interval, lactation length, and dry period length

5.1.1 Randomized study (Paper I)

Extending the VWP of primiparous cows led to higher 305-day and whole lactation yield compared with cows with conventional VWP (Figure 13A). However, there was no difference in milk yield per day in the first CInt or during the first two CInt combined (Figure 13B) (Paper I). During the second CInt, 305-d yield, whole lactation yield, and milk yield per day were higher for the EXT cows (11,778; 12,527; and 32.6 kg) than for the CONV cows (11,061; 11,659; and 30.9 kg) (p<0.01) (Figures 13A and B).

Calving interval, lactation length, and DPL were all longer for cows with extended compared with conventional VWP during the first CInt (Figure 13C). Milk yield at dry-off was lower for EXT compared with CONV cows during the first CInt (24.0 vs. 25.9 kg) (p<0.001) (Figure 13C). Moreover, the proportion of cows with a dry period of more than 70 days was higher for EXT compared with CONV cows (20% vs. 9%) (p=0.002). However, there was no difference between the treatments in terms of any of these parameters during the second CInt.

5.1.2 Customized study (Paper III)

The ExtExt cows in Paper III had lower milk yield before dry-off compared with the ExtConv cows (24.9 vs. 28.3 kg) (p<0.001), while there was no difference in DPL and milk yield per day in the CInt (Figures 13D and F). Further, the ExtExt cows had higher 305-d and whole lactation yield compared with the ExtConv cows (Figure 13E), longer lactation, and higher persistency between the 3rd and 8th test milking (-0.026 vs. -0.037 kg/d (p=0.001). However, there was no difference in persistency between 3rd test milking and dry-off or in DPL between these treatments (Paper III).

The ExtExt and ExtConv cows had higher 305-d yield, whole lactation yield, and milk yield per day in the CInt than the ConvConv cows. The

ExtExt cows also had higher persistency between the 3rd and 8th test milkings compared with the ConvConv cows (0.043 kg/d, p=0.001). Moreover, the ExtConv cows had higher milk yield at the last test milking before dry-off than cows in the ConvConv treatment (Figure 13F). However, there was no difference in persistency measured between the 3rd test milking and dry-off, or in DPL, between any of the three treatments (Paper III).

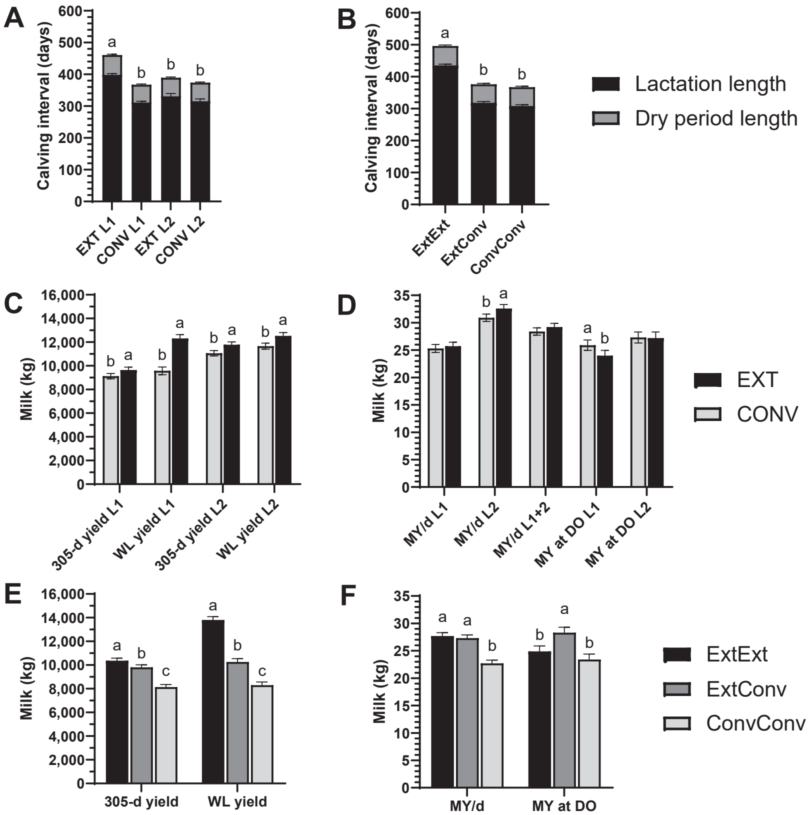

Figure 13. A) 305-d and whole-lactation (WL) yield, B) milk yield per day in the calving interval (MY/d) and at dry-off (MY at DO), and C) calving interval, lactation length, and dry period length in lactation 1 (L1) and lactation 2 (L2) in the extended (EXT, n =161) and conventional (CONV, n = 186) treatment in Papers I and II. D) Calving interval, lactation length, and dry period length, E) 305-d yield and WL yield, and F) MY/d and MY at DO for cows in the extended (ExtExt, n = 104), conventional suited for extended (ExtConv, n = 118), and conventional suited for conventional (ConvConv, n = 116) treatments in Papers III and IV. Different letters above bars (a, b, c) indicate significant differences (p<0.05) between treatments.

5.2 Fertility

5.2.1 Randomized study (Paper II)

Reproductive performance was better for cows in the EXT compared with the CONV treatment in Paper II, with higher FSCR, lower NINS, and shorter insemination period length during the first lactation (Figures 14 and 15). Additionally, a higher proportion of cows in the EXT treatment had a higher estrus intensity score in the first lactation (Paper II). Moreover, CFI, but not CInt, was longer for the EXT cows during the second lactation (Paper II) (Figure 13C). However, there was no effect of VWP on estrus intensity, FSCR, or NINS during the second lactation.

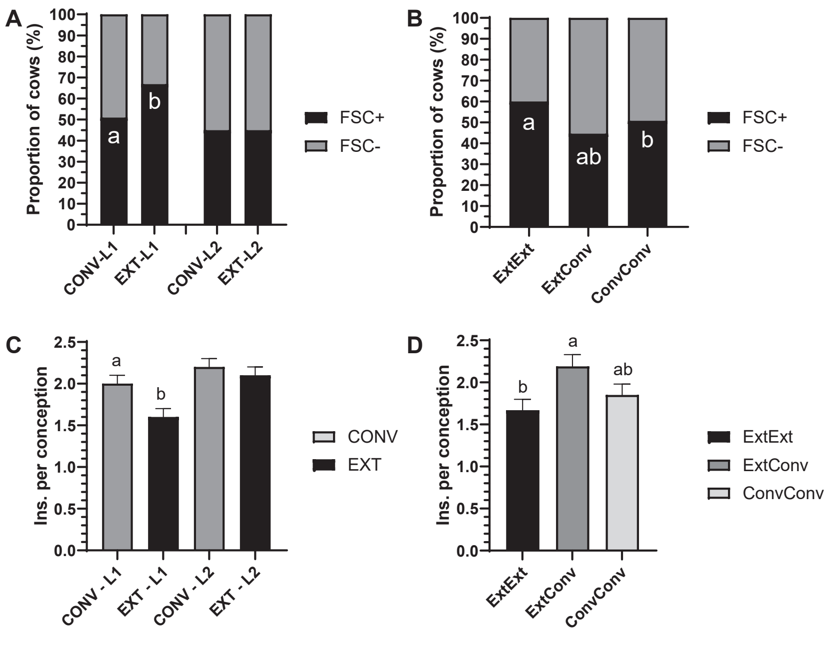

Figure 14. Proportion of cows with first service conception (FSC+) and of cows that did not conceive after the first insemination (FSC-) in A) the extended (EXT, n = 178) and conventional (CONV, n = 204) treatment in lactation 1 (L1) and lactation 2 (L2) in Paper II and B) in the extended (ExtExt, n = 120), conventional suited for extended (ExtConv, n = 139), and conventional suited for conventional (ConvConv, n = 132) treatments in Paper IV. Number of inseminations per conception (NINS) in C) EXT and CONV treatment in L1 and L2 in Paper II, and D) in the ExtExt, ExtConv, and ConvConv treatments in Paper IV. Different letters above and within bars (a, b) indicate significant differences (*p*<0.05) between treatments within each lactation.

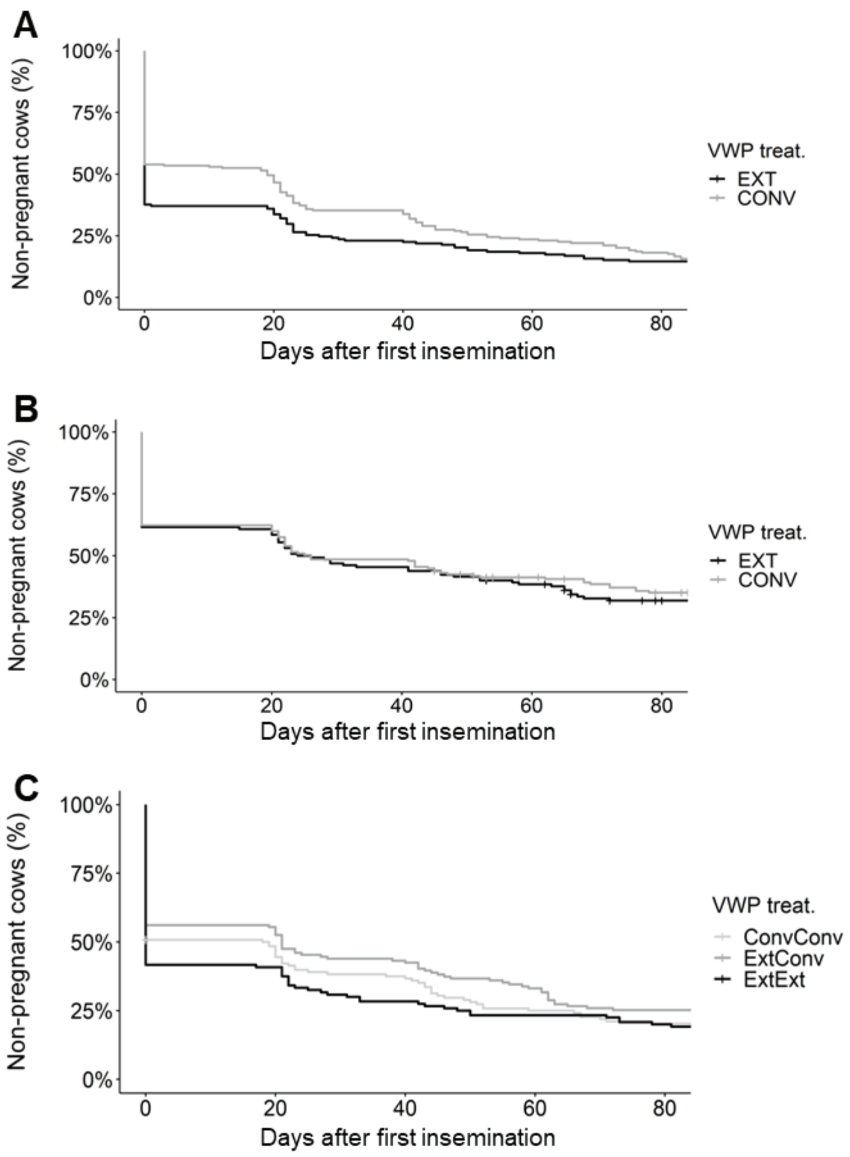

Figure 15. Proportion of non-pregnant cows per day after first insemination, presented descriptively, for cows in A) lactation 1 and B) lactation 2 in the extended (EXT, n = 178) and conventional (CONV, n = 204) treatment in Paper II, and C) for cows in the extended (ExtExt, n = 120), conventional suited for extended (ExtConv, n = 139) and conventional suited for conventional (ConvConv, n = 132) treatments in Paper IV.

5.2.2 Customized study (Paper IV)

Cows expected to be suited for extended VWP and randomized to receive an extended (ExtExt) compared with a conventional VWP (ExtConv) had better reproductive performance, with higher FSCR, lower NINS, and shorter insemination period length (18 vs. 34 d) (p=0.004) (Figure 15). However, there was no difference between the ExtExt and ConvConv treatments, or between the ExtConv and ConvConv treatments, for any of these parameters, and no difference between any of the treatments regarding estrus intensity.

5.3 Health, culling, and compliance

5.3.1 Randomized (Paper II) and customized study (Paper IV)

There was no significant difference in culling rate (Figure 16) or disease incidence per time at risk between cows in any of the treatments in Papers II and IV, except that a higher disease incidence was found in Paper IV for the ExtConv compared with the ConvConv treatment (22 vs. 9 disease cases per 100 cow-years in the study) (p=0.02) (Paper IV).

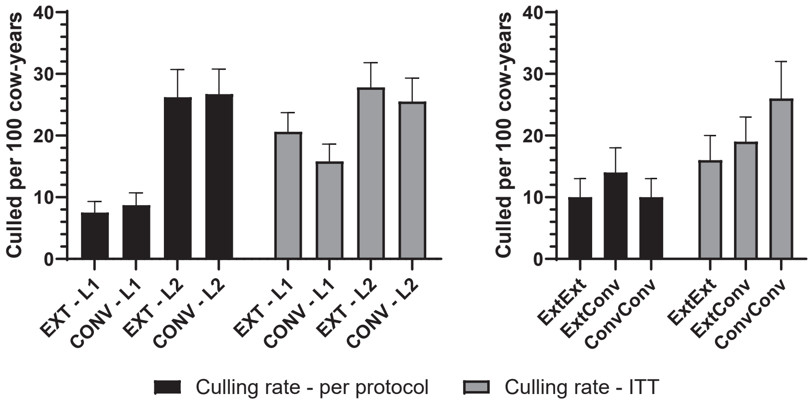

Figure 16. Culling rate per 100 cow-years analyzed for all cows following the intended voluntary waiting period (VWP) treatment ("per protocol", black bars) and for all cows included ("intention-to-treat", grey bars) in A) the extended (EXT, n = 251) and conventional (CONV, n = 280) treatment in lactation 1 (L1) and lactation 2 (L2) in Paper II and B) in the extended (ExtExt, n = 167), conventional suited for extended (ExtConv, n = 169), and conventional suited for conventional (ConvConv, n = 177) treatments in Paper IV.

The only interaction between breed and VWP observed was that of the proportion of cows with healthy SCC. Regarding all other parameters investigated, both breeds (HOL and RDC) responded equally to the VWP treatments in both studies (Papers II and IV).

In Paper II, there was lower compliance with the plan in the EXT compared with the CONV treatment (65% vs. 83%) (p<0.001). However, in Paper IV there was no significant difference in compliance between the three treatments (Figure 17).

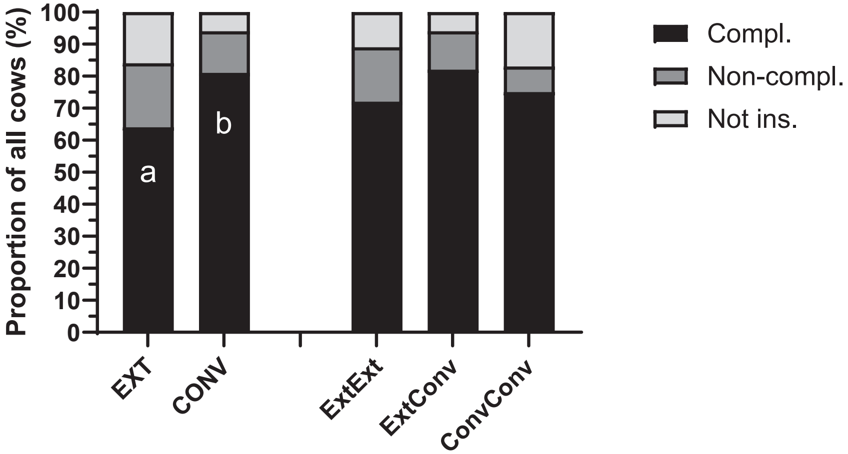

Figure 17. Proportion of cows inseminated in compliance with the assigned voluntary waiting period (VWP) treatments in (left) Paper II (n = 531) and (right) Paper IV (n = 513).

6. General Discussion

6.1 Milk production

6.1.1 Calving interval and lactation length

The extended VWP treatments in both studies resulted in extended CInt, as planned. In Paper I, where cows with a CFI from 145 days were included, this resulted in a mean CInt of 15.2 months (462 d) in the EXT treatment. However, the aim was to have a CInt of 16 months in the extended VWP treatment, so the VWP in the customized study, the VWP was further extended by a month. In Paper III, where cows with a CFI from 175 d were included, this led to a CInt of 16.3 months (496 d). The shorter-than-planned CInt in Paper I was probably partly due to the improved fertility in this treatment, in combination with an eagerness among herd managers to inseminate their cows. When asked for feedback about the trials during farm meetings arranged during both the randomized and customized study, some herd managers reported that the extended time before conception had led to a larger number of cows in estrus in the herd, which some managers perceived as bothersome, and a few reported that cows were injured while expressing estrus behavior. Other herd managers reported that the extended CInt gave them time to see and learn the estrus cycle of individual cows, making it easier to time estrus with insemination.

In the conventional VWP treatments, the CInt was slightly longer than the intended 12 months (12.1 mo in the CONV treatment in Paper I, 12.4 mo in the ExtConv treatment in Paper III). This might be partly explained by the herd managers not being in a hurry to inseminate these cows, as cows with CFI of up to 95 d were included in the randomized study (Paper I) and cows with CFI of up to 100 d were included in the customized study (Paper III).

Another explanation is that the cows in the ExtConv treatment were expected to be better suited for extended VWP, and some of them had calving difficulties and diseases in early lactation that may have impaired their reproductive performance (Ribeiro et al. 2016). In support of the hypothesis that the cows in the ConvConv treatment in Paper III would be better suited for a conventional VWP, this treatment achieved the planned CInt of 12.0 mo. While CFI was slightly longer in the second lactation for cows in the EXT compared with the CONV treatment in Paper I, there were no significant difference in CInt or lactation length.

Although there were some differences in DPL, the lactation length largely followed the CInt length in all treatments, as intended, (Figure 13C and D), implying that the primiparous cows generally had sufficient persistency to maintain an extended lactation (Burgers et al. 2021a). This meant that the proportion of dry days in the CInt was lower in the two extended VWP treatments.

6.1.2 Dry period length and dry-off yield

In the randomized study, DPL was 6.4 days longer and there was a higher frequency of cows with DPL of more than 70 d in the EXT (20%) compared with the CONV (9%) treatment (Paper I). In the customized study, on the other hand, there were no significant differences in DPL between the treatments (Paper III). This supports the hypothesis that cows selected for the customized ExtExt treatment were better suited for extended lactation as regards running a lower risk of having a long dry period, thereby reducing the risk of possible detrimental effects on fertility, health, and culling (Pinedo et al. 2011; Andrée O'Hara et al. 2020; Guadagnini et al. 2023). As most cows in the ExtExt treatment were selected based on early lactation yield, this also supports findings in previous studies that high-yielding cows are more suitable for extended VWP (Lehmann et al. 2017; Burgers et al. 2021b).

High milk yield at dry-off has previously been linked to impaired udder health (Rajala-Schultz et al. 2005), and to factors associated with decreased welfare, such as increased udder pressure and stress (Bertulat et al. 2013). There is no consensus regarding the best method for cessation of milking (Gott et al. 2017; Wieland et al. 2023), but a favorable approach seems to be to aim for lower yield before dry-off, as suggested by Wieland et al. (2023). In Papers I and III, milk yield at the last test milking 50-20 d before dry-off was used as an alternate measure for milk yield before dry-off. In Paper I, cows in the EXT treatment had lower milk yield at dry-off than cows in the CONV treatment. This was also the case on comparing the ExtExt and ExtConv treatments in Paper III, and is in line with findings by Arbel et al. (2001) and Niozas et al. (2019a). Compared with the ConvConv treatment, milk yield before dry-off in the ExtExt treatment did not differ (Figure 13F), despite the ExtExt cows having a higher yield in early lactation, likely explained by their increased lactation length.

6.1.3 305-d and whole lactation yield

For completeness and to make the results more generalizable and useful, milk yield data were expressed both as milk yield and as ECM yield in Paper I and III (when available). The ECM yields were generally slightly higher than the kg milk values, but a similar difference between VWP treatments was observed for these two variables. However, for the two dairy cow breeds reflected in the data, the results diverged during the first lactation, as the RDC cows produced milk with higher fat and protein concentrations. This resulted in a greater difference between ECM yield and uncorrected milk yield than for the HOL cows, and in turn led to a breed difference regarding uncorrected milk yield, but no difference between the breeds in any of the yield variables (305-d yield, whole lactation yield, or milk yield per day in the CInt) measured as ECM yield.

In both the randomized and customized studies, the extended VWP treatment led to higher whole lactation yield compared with the conventional treatments during the first lactation. This was not surprising, as cows in the extended VWP treatment had longer lactations. The 305-d lactation yield was increased with the extended compared with the conventional treatments in all cases, in line with previous findings (Lehmann et al. 2016; Burgers et al. 2021b). This might be explained by the delayed effect of pregnancy on milk yield (Hammond & Sanders, 1923; Strandberg & Lundberg, 1991) as seen in Figure 18. Cows in the ConvConv treatment had lower yield than cows in the ExtConv treatment in Paper III, which was expected due to the selection for lower early lactation yield. On comparing the lactation yields of the EXT cows in Paper I and the ExtExt cows in Paper III, it was found that the ExtExt cows had considerably higher whole lactation (16%) and 305-d lactation (9%) yield, which was expected as they were selected based on yield, and also as they had a one month longer lactation.

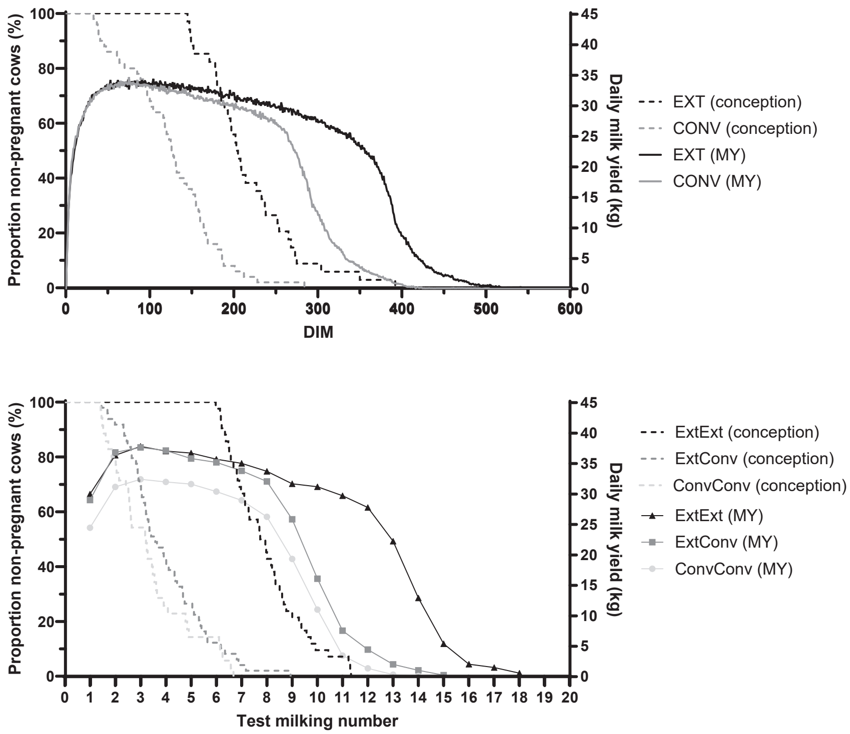

Figure 18. Lactation curves showing daily milk yield (MY) and proportion non-pregnant cows (dashed lines), A) for the two VWP treatments in the randomized study (Paper I and II) per day in milk (DIM) (modified after Figure 2 in Paper I), and B) for the three VWP treatments in the customized study per test milking (approximately 30 days apart) (modified after Figure 2 in Paper III).

6.1.4 Persistency

Persistency is crucial for maintaining an extended lactation and is the reason why primiparous cows are often considered especially well-suited for extended VWP. It was therefore investigated specifically in this thesis, with genomic PI being used as one of the selection criteria in Papers III and IV. However, there was no difference in PI between the treatments, likely because there were relatively few cows selected based on their PI (n = 41). Persistency measured as the decline in milk yield per day between test milking 3 and 8 was higher in the ExtExt treatment compared with the two conventional treatments (Paper III). As mentioned previously, this was probably due to the delayed effect of pregnancy. Contrary to findings by

Burgers et al. (2021a), persistency between test milking 3 and dry-off did not differ between the groups. Persistency was not analyzed statistically, but is visualized in Figure 18B.

6.1.5 Milk yield per day in the calving interval

Although it is interesting to discuss lactation length, DPL, lactation yield, and persistency, an outcome measure that summarizes and represents all these parameters is the milk yield per day in the CInt. This is therefore generally the most useful yield parameter for farmers, and for comparisons between studies (Lehmann et al. 2016).

During the first lactation, there was no difference, regarding either milk yield or ECM yield per CInt day, between the EXT and CONV treatments in Paper I, or between the ExtExt and ExtConv treatments in Paper III. These results are in line with previous findings relating to extended VWP in primiparous cows (Österman & Bertilsson, 2003; Niozas et al. 2019a; Burgers et al. 2021b). Unsurprisingly, milk yield per day in the CInt was lower for cows selected for the ConvConv treatment than in cows in the two other treatments, which were partly selected based on higher yield.

6.1.6 Milk production in the subsequent lactation

In the second lactation in the randomized study, all milk yield and ECM variables were higher for the cows that received the extended VWP treatment in their previous lactation (Paper I). In addition, milk yield per day in the CInt was 6% higher. However, when the first and second lactation yields were combined, although the average daily yield for cows in the EXT treatment was numerically (0.8 kg/day) higher, the difference was not statistically significant. A possible explanation for the higher yield in lactation 2 is that with a conventional VWP, the cows are still growing and are not fully developed during their second lactation. Hence, if the time to the second calving is extended, the cows would have more time to grow, and might need less energy for growth in the second lactation (Lehmann et al. 2016).

The association between extended CInt and higher yield in the subsequent lactation has been known for almost 100 years (Matson, 1929; Gaines & Palfrey, 1931). Several retrospective studies have found CInt in a previous lactation to be correlated with higher yield in the current lactation, with Bar-

Anan and Soller (1979) finding the effect to be greatest in higher-yielding herds.

In a previous randomized study on the effect of extended VWP, Jarman et al. (2020) compared grazing cows randomized to an 8-month extension of the VWP in a seasonal calving system and cows with 12-month CInt. They followed the conventional cows for three lactations and the extended VWP cows for the extended VWP and a following 12-month VWP. They found that cows with an 8-month extension of the VWP, and two instead of three lactations during the same period, yielded the same amount of milk solids during the period. Moreover, those cows yielded more milk solids and had a longer second lactation and better reproductive performance during their first lactation than cows in the conventional VWP treatment. However, they gained more weight and body condition until the second calving and required more concentrate feed than the conventional VWP group to maintain the same lactation length. Österman and Bertilsson (2003) examined the effect of two consecutive extended CInt of 18 months compared with three 12-month conventional lactations and found that cows in the 18-month treatment had shorter dry periods during their second compared with their first extended CInt.

A few randomized studies have investigated milk yield at the beginning of the lactation immediately following an extended CInt. Findings by Arbel et al. (2001) and Lehmann et al. (2016) are in line with the results in the present study, with increased average daily yield during the first 150 and 80 days, respectively. On the other hand, Burgers et al. (2021a) observed lower yield for cows in the extended VWP treatments in the next lactation, although they followed the cows for a shorter period (42 d) in the following lactation.

6.2 Fertility

With extended VWP, cows have a longer time to improve their energy balance before the first insemination (Figure 7), which may be beneficial for fertility (Chen et al. 2015). In both the randomized and customized studies in this thesis, reproductive performance in the form of NINS, FSCR, and insemination period length was improved for the cows in the extended VWP treatments compared with the CONV treatment in Paper II and the ExtConv treatment in Paper IV. This was in line with the results reported by Niozas et al. (2019b) and might partly be explained by the finding by Ma et al. (2022)

that a higher proportion of cows with extended VWP have normal ovarian cycles at the end of the VWP.

6.2.1 Association between fertility and milk yield

The better fertility of the ExtExt cows compared with the ExtConv cows in Paper IV is in agreement with results reported by Madouasse et al. (2010) and Bedere et al. (2018), who found high yield to be associated with reduced reproductive performance. Additionally, Römer et al. (2020) observed that with increasing CFI, high-yielding cows had shorter insemination period length and lower NINS. In the same study, the CFI for lower-yielding cows was not associated with fertility. Hence, the lack of difference between ConvConv and ExtExt in Paper IV indicates that both treatments may have received a suitable VWP from a fertility perspective. This might explain the contrasting results in previous studies, where average yearly yield was lower and cows with extended VWP had poorer reproductive performance (Schneider et al. 1981) or where there were no differences between the extended and conventional VWP treatments (Ratnayake et al. 1998). However, the association between yield and reproductive performance is debated (LeBlanc 2010; Bello et al. 2012; Bedere et al. 2018) and is probably complex and dependent on several factors (*e.g.*, feed and management).

6.2.2 Association between health and fertility

The association between early lactation disease and reproductive performance is well documented (Ribeiro et al. 2016; Carvalho et al. 2019; Bogado Pascottini et al. 2020; Pinedo et al. 2020). The hypothesis tested in Paper IV was that cows with difficult calvings and diseases in early lactation would be suited for an extended VWP, as they would have more time to recover before the time of insemination (Inchaisri et al. 2011).

As the ConvConv cows did not have any disease in early lactation, they were expected to have better fertility than the ExtConv cows, but the difference between these treatments was not significant. Conversely, there was no difference in disease incidence between the ExtExt and the ExtConv cows, but there was a difference in reproductive performance (Paper IV). These results might be explained by the relatively low number of cows selected due to disease or calving difficulties in the extended VWP treatments.

6.2.3 Estrus intensity and second lactation calving to first insemination interval

Regarding estrus intensity, the results in Papers II and IV are inconsistent. In Paper II, estrus intensity improved for cows in the extended VWP treatment, which was in line with the other results on reproductive performance. However, in Paper IV there was no significant difference and the trend was the opposite. No plausible explanation for these results have been found. However, estrus intensity is a subjective measure and the reporting varies greatly between farms. Cows with stronger estrus intensity have been found to have a higher chance of conception (Nyman et al. 2016), which support the results in Paper II but not in Paper IV, where FSCR was higher for the ExtExt cows than the ExtConv cows but there was no difference in estrus intensity. A possible explanation for higher FSCR despite no difference in estrus intensity, is that some farmers reported that, for cows with extended VWP they had more time, and thereby repeated opportunities to get to know the estrus cycles of individual cows, which might have improved conception rate.

In the second lactation in Paper II, the EXT cows had longer CFI than the CONV cows (86 vs. 74 d). The reason for this is unknown, but it is possible that farmers were more willing to extend the CFI for these cows, as they knew they had coped with a previous extended CInt. However, the increase in CFI did not affect the length of the second CInt significantly (386 vs. 377 d).

6.3 Health

6.3.1 Disease incidence

As diseases are most common in the transition period, it might be assumed that less frequent transition periods would result in a lower incidence of disease at both cow and herd level. However, there was no difference in disease incidence between any of the treatments, except in Paper IV, where higher disease incidence per 100 cow-years in the study was found in the ExtConv treatment compared with the ConvConv treatment. This was not surprising, as the cows in the ExtConv treatment were partly selected due to early lactation disease, and Carvalho et al. (2019) found that cows with one early lactation disease have a higher disease incidence rate after 21 DIM than cows without early lactation disease.

However, there was no significant difference in disease incidence between the ExtExt and the ConvConv treatments in Paper IV, or between the ExtExt or ExtConv treatments. One explanation might be that primiparous cows generally have lower disease incidence than multiparous cows (Figure 4). Moreover, the number of reported disease events was low. At the same time, there is a large variation in disease incidence between different herds (Växa Sverige 2023a). Together, this might have contributed to low statistical power. Further studies, possibly meta-studies using data from several experiments, are needed to determine the impact of VWP on disease incidence.

Previous conclusions regarding disease incidence in extended CInt are scarce (Niozas et al. 2019a; Burgers et al. 2021a; van Knegsel et al. 2022). Burgers et al. (2022) did not find any difference in the total number of veterinary treatments between cows receiving different VWP, whereas Ratnayake et al. (1998) found a lower number of treatments for ovarian disorders in cows with extended VWP. This is consistent with findings by Ma et al. (2022) of a higher number of normal estrus cycles in cows receiving an extended VWP treatment.

6.3.2 Udder health

Regarding udder health, a SCC of less than 100,000 cells per mL milk was assumed to represent cows with a healthy udder (Waller et al. 2020). In Paper II, an interaction between VWP treatment and breed was found for SCC. A larger proportion of RDC cows in the EXT treatment had low SCC at the end of lactation 1, and the opposite was true at the end of the second lactation, with a lower proportion of RDC cows in the EXT treatment having low SCC. In Paper IV, there was no difference between the treatments, which was slightly unexpected as cows selected due to early lactation disease might have been expected to have increased SCC. However, this was not the case, probably due to the low number of animals with reported disease.

Another hypothesis tested was that, as the cows in the extended VWP treatment had lower dry-off yield, which is known to be beneficial for udder health (Rajala-Schultz et al. 2005), they would have improved SCC in the following lactation. However, that hypothesis was not confirmed by the results in Paper II and was contradicted by results in Ma et al. (2022), who found an increase in SCC at the beginning of the subsequent lactation for cows with extended VWP. Other studies have reported no effect of VWP on SCC (Österman et al. 2005; Niozas et al. 2019a).

6.4 Culling

Compared with the Swedish average culling rate of 26% for primiparous cows and 36% for multiparous cows (Växa Sverige 2023b), the culling rate in all studies in this thesis was generally lower in all treatments except ConvConv in Paper IV, where it was 26%.

The culling rate was analyzed both "per protocol" and "intention-to-treat", and expressed as number of culled cows per 100 cow-years at risk in each treatment. This was done to investigate potential bias that might arise from the lack of compliance. However, there was no significant difference between the treatments in Paper II or in Paper IV, which is in line with previous findings (Arbel et al. 2001; Burgers et al. 2021a). On the other hand, Niozas et al. (2019a) reported a higher number of cows culled overall due to low productivity, with a VWP of 180 days compared with 40 days.

Although there was a numerical difference in culling rate between some of the treatments analyzed in this thesis, these differences did not prove to be statistically significant. Unsurprisingly, culling rate was generally higher in the "intention-to-treat" than in the "per protocol" analysis, as only cows inseminated were included in the "per protocol" analysis and cows planned to be culled are usually not inseminated. Furthermore, in Paper II the proportion of culled cows was similar in both treatments in the "per protocol" analysis, but in the "intention-to-treat" analysis the proportion of culled cows was larger in the EXT treatment, potentially reflecting the higher risk of culling cows that are not pregnant (Gröhn et al. 1998). However, this is only speculative, as there were no significant differences between the treatments.

In Paper IV, there was a large apparent difference in culling rate in the ConvConv treatment between the "per protocol" and "intention-to-treat" analysis, with a considerably higher proportion of cows culled among those assigned to the treatment compared with those receiving it. This numerical difference might be explained by lower early lactation yield, which might have caused cows assigned the ConvConv treatment to have a higher risk of being culled (Owusu-Sekyere et al. 2023). However, the differences between treatments were not significant in any of these analyses.

The reported reasons for culling were not analyzed statistically, but are presented descriptively in Figure 19. The culling reason that appeared to explain the largest part of the variation in culling rate between the treatments was culling due to low milk yield. In Paper II, cows randomized to the EXT treatment were more frequently reported to be culled due to low yield during lactation 1 in contrast to lactation 2. Not surprisingly, in Paper IV culling due to low yield was more common in cows assigned the ConvConv treatment in "intention-to-treat" analysis.

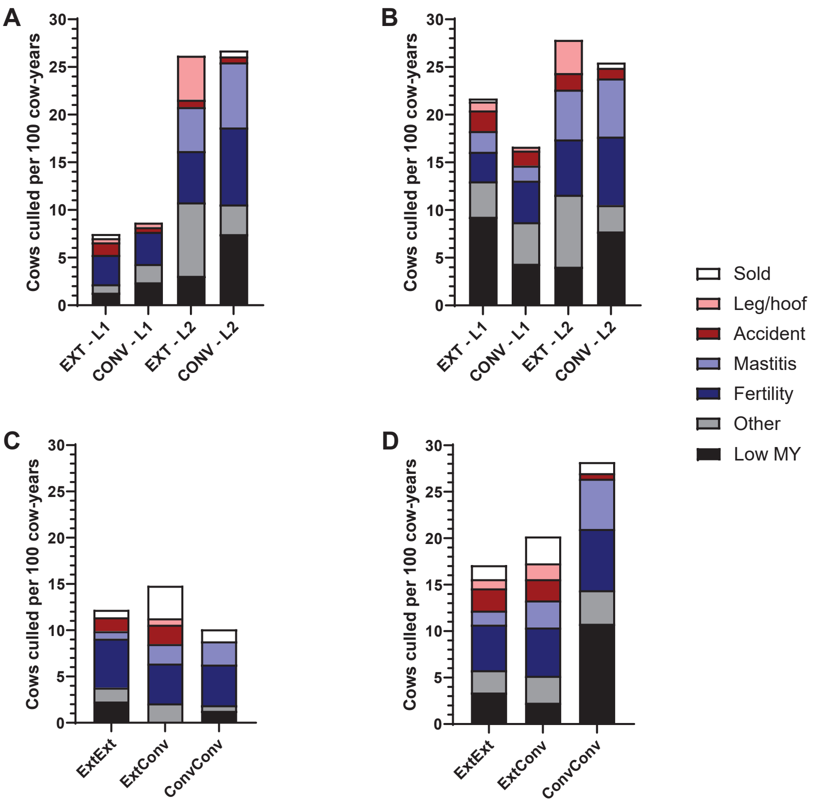

Figure 19. Reported culling reasons in the voluntary waiting period (VWP) treatments tested in Paper II (n = 531) as analyzed (A) "per protocol" and (B) for all cows included "intention-to-treat", and in Paper IV (n = 513) as analyzed (C) "per protocol" and (D) "intention-to-treat").

6.5 Breed

As HOL cows generally have higher milk yield than RDC cows (Växa Sverige 2023a), and yield appears to affect the results of extended VWP (Lehmann et al. 2017; Römer et al. 2020), a specific objective in this thesis was to investigate potential interactions between treatment and breed. Both HOL and RDC cows are commonly represented in Swedish herds, so potential interactions might have important future implications.

As also mentioned in section 6.3.2, the only analysis in which the interaction between VWP treatment and breed was significant was for udder health in the last test milking of each lactation in Paper II. A larger proportion of RDC cows in the EXT compared with the CONV treatment had low SCC at the end of lactation 1, while the opposite was true for lactation 2. Meanwhile, there was no difference between the treatments in either lactation for HOL cows. The reason for this remains unexplained, and in Paper IV these results were not repeated, as no interaction between treatment and breed was found for SCC. This implies that the results should be interpreted with caution, and further studies are needed to draw any conclusions on the matter.

Overall, with the exception of SCC in Paper II, there were no interactions between treatment and breed, which implies that the results in this thesis appear to be valid for both breeds. For this reason, only treatment effects are presented in Papers III and IV.

6.6 General considerations and limitations

6.6.1 Limitations

Several management factors may differ between commercial dairy herds, such as feeding strategies, milking systems, housing, and staff, to mention a few. This may be both a strength and a weakness. It is a strength because the results may be more generalizable than if the study had been performed on a single herd, and a weakness because variability is introduced, which in some cases may conceal treatment effects. The completeness and reliability of data reported to the SNDRS may also vary between herds. As a way to account for variation between herds, herd was included (where possible) as a random factor in the statistical models in this thesis. It was also assumed that, as cows with extended and conventional treatments were represented in all herds, they received the same treatment regarding estrus detection, insemination technique, and so forth.

However, some management routines might have been affected by the intervention *per se*, *i.e.*, the VWP. Cows that are not pregnant generally run a higher risk of culling than non-pregnant cows (Gröhn et al. 1998), which puts the cows allocated to extended VWP at risk for a longer period during the CInt as conception is delayed. However, this might also be argued to be a treatment effect and an advantage for the herd manager, as the decision on which cows to inseminate and which to cull is postponed, and more information about the cow's performance is available, possibly leading to more informed replacement decisions.

Although not statistically tested, it appeared that a higher proportion of cows in the EXT compared with the CONV treatment in Paper I were culled due to low milk yield during the first lactation. This might have affected the results on milk yield in the second lactation, and thus the higher yield in the second lactation might have been an indirect rather than a direct effect of the VWP extension.

Furthermore, the fact that selection of cows for Papers III and IV was based on three different criteria complicates interpretation of the results, especially as the number of cows selected based on PI and disease or calving problems was relatively low. Moreover, as the cows assumed not to benefit from an extended VWP received a conventional VWP, there was no treatment consisting of cows expected not to be suited for, but subjected to, an extended VWP. However, this was done in Papers I and II, although not in a deliberate manner, as all cows were randomly allocated to both treatments.

6.6.2 Compliance

Compliance was defined as the proportion of cows randomized to each VWP treatment that were actually inseminated according to the plan. As the trials were performed in multiple commercial herds, there was limited possibility to influence compliance, and compliance varied between the herds (Figure 1 in Paper I). This was expected as management and several other factors also varied between the herds.

Compliance was highest in the CONV treatment in Paper I and the ExtConv treatment in Paper III. This was expected, as these cows were inseminated according to conventional management routines familiar, to the herds. In the ExtConv in contrast to the ConvConv treatment, many cows were selected based on high yield or high PI, which may have increased the probability of them being inseminated (Warner et al. 2022).

The lowest compliance was seen in the extended VWP treatments in both studies. This could be explained partly by it being a new management routine, with many reported mistakes (Figure 20) where cows were inseminated earlier than planned (as seen in Figure 1 in Paper II). The ConvConv treatment in Paper IV had intermediate compliance compared with the other treatments. This might partly be caused by the treatment containing lower-yielding cows, running a higher risk of not being inseminated at all (Warner et al. 2022). On the other hand, it contained cows expected to be more suited to this treatment, and additionally, it is the conventional routine that most farmers are familiar with.

6.6.3 Reported causes for lack of compliance

Some reasons for cows not complying with the intended treatments were reported by the farmers. However, despite efforts to obtain complete information, the most common reason for lack of compliance was "unknown" (Figure 20), which means that the results should be interpreted with caution. However, as they might influence the main results to some extent, and although incomplete, may give additional information, they are discussed further.

Mistakes were the second most commonly reported reason for the extended VWP treatments in both studies. This might be due to the change in management routine regarding VWP. The reported "mistakes" in the ExtExt treatment should not have affected the results, as if they were pure mistakes it could be assumed that they were random. Disease/accident was the third most commonly reported reason for lack of compliance in the ExtExt and the ExtConv treatments, which could also have been expected as some of these cows were selected due to calving problems and diseases in early lactation, in contrast to the ConvConv treatment. However, in Paper II disease/accident was more frequently reported for the cows randomized to the EXT treatment compared with the CONV treatment. Another possible reason is that due to the extended VWP treatment, these cows had more time to become sick or injured before the first insemination.

The second most commonly reported reason for cows in the ConvConv treatment not being inseminated according to plan was "low yield", which is not surprising as these cows were selected for the treatment due to their lower early lactation yield. However, the culling and lack of compliance due to low yield in the ConvConv treatment might have caused milk yield for this treatment to be overestimated. "Fertility issues" was the second most reported reason in the CONV treatment in Paper II and in the ExtConv treatment in Paper IV. This was also in line with the expectations, as cows in the ExtConv treatment, were expected to be better suited for extended VWP, to give them more time to recover after calving and regain cyclicity. Impaired fertility was the third most common reason for lack of compliance in the ConvConv treatment. Not complying with the planned conventional treatments due to "impaired fertility" in cows might also have led to slight overestimation of the fertility results in this treatment, as cows with late onset of estrus were excluded.

To test for bias due to lack of compliance in Paper II, milk yield in early lactation was compared between cows not complying with the extended and conventional VWP treatment. There was no difference between the cows receiving each treatment in early lactation, indicating that this was not the case. However, comparing milk yield for days 4-145 for cows in the extended VWP treatment complying with and not complying with the treatment revealed higher yields for cows receiving the planned treatment. One explanation might be the higher incidence of culling due to low yield in this treatment (Figure 19) (Paper II).

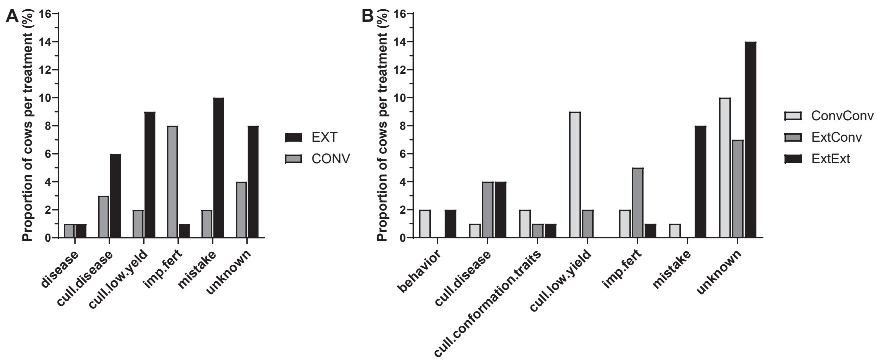

Figure 20. Proportion of cows per treatment with different reported reasons for lack of compliance in A) the extended (EXT) and conventional (CONV) VWP treatments in Paper II, and B) in the extended (ExtExt), conventional suited for extended (ExtConv), and conventional suited for conventional (ConvConv) treatments in Paper IV. For cows culled lacking a reported reason, the culling reason was assumed to also be the reason for lack of compliance.

7. Conclusions

The overall conclusion from the work in this thesis was that primiparous cows subjected to extended VWP before first insemination, had improved reproductive performance and maintained milk production per day in the calving interval, while there was no effect of VWP length on disease incidence or culling rate. There was lower compliance with the planned VWP treatment for cows randomized to receive an extended compared with a conventional VWP.

Specific conclusions were that:

- Extending the VWP led to increased 305-d and whole lactation yield in both the first lactation and the subsequent lactation without VWP intervention.
- Extension of the VWP in the first calving interval led to a higher milk yield per day during the second calving interval.
- Milk yield before dry-off was lower for cows with extended VWP.
- Dry period length was extended for cows receiving a randomized extended VWP, but not for cows receiving a customized extended VWP.
- Extension of the VWP led to improved reproductive performance.
- Extension of the VWP did not affect culling rate and disease incidence.
- Both RDC and HOL cows responded equally to the VWP treatment regarding milk production, fertility, disease incidence, and culling rate.
- Compliance was lower for cows randomized to an extended VWP, but not for cows with a customized extension of the VWP.

8. Practical implications and future perspectives

The results presented in this thesis can be used as decision support for dairy advisors and in commercial high-yielding dairy herds to adjust VWP length to improve fertility and reduce the number of transition periods while maintaining milk production, without effects on disease incidence and culling for primiparous cows.

Extended VWP is a way of increasing flexibility in management, and thereby resilience, in dairy herds. Having the alternative to increase the VWP of some animals in the herd may reduce laborious events around dry-off and calving, and increase the proportion of animals in late lactation, thereby decreasing dependence on costly feed ingredients. It might also be a way of shifting calving to a more suitable time of the year, if desirable. From an animal welfare perspective, reducing the frequency of transition periods and associated stressful events may be beneficial for the dairy cow. Reduced yield at dry-off may also be important for animal welfare, as high yield at dry-off is associated with reduced welfare in the form of increased stress and udder pressure, causing pain and posing a risk of mastitis.

However, extended VWP might not be suitable for all cows or herds. For example, low-yielding cows have lower ability to maintain milk yield per day in the CInt in an extended lactation, and thereby have higher body condition at dry-off. Extended VWP might not be as well suited for low-yielding herds, herds depending on calves for meat production, expanding herds with a high need for replacement heifers, and herds breeding animals aiming at high genetic progress, as the number of calves and thereby replacement heifers would be diminished. Strategic use of sexed semen and embryo transfer could overcome that issue, while using beef semen for cows with less promising genetic merit may be a way of increasing the value of the calves. Moreover, available housing must fit the changes in herd dynamics following an altered VWP management strategy. A higher proportion of lactating cows and fewer young stock would alter the demand for housing, which needs to be considered.

Finally, the modern dairy cow has a tremendous genetic potential for milk production and also for improvement in reproductive performance. By optimizing the VWP management strategy for the individual cow and herd, allowing flexibility may be a way to take better advantage of this potential.

And finally, some ideas for future research:

- Further development of existing economic models using results from this thesis may improve predictions, potentially providing a useful decision support tool for commercial farms.
- More research is warranted to develop, compare and evaluate predictors in early lactation of cows suited for extended VWP.
- Multiparous cows, with their higher disease incidence and higher peak yield, may also gain from an extended VWP. However, on average they show lower lactation persistency, therefore it is highly relevant to develop prediction tools that support implementation of customized VWP in these cows.
- Long-term randomized and follow-up studies are needed to shed more light on the effect of VWP on longevity and lifetime production.
- Disease incidence and culling rate should be studied in long-term studies, or potentially meta-studies, with randomized extension of the VWP.

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Popular science summary

A conventional 12-13 month calving interval for dairy cows has traditionally been seen as most beneficial from an economic perspective, due to highest milk production in early lactation. This thesis contain results from two studies of extended calving interval, in dairy cows, during their first lactation. In the first study, the cows were randomized to receive either a conventional 12 months' calving interval or an extended 16 months' first calving interval, and monitored during two lactations. The second study investigated cows, selected to and expected to be suited for, a longer calving interval. Cow selection was based on genomic potential for persistency, calving problems or diseases, and high milk yield in early lactation.

Cows receiving an extended calving interval maintained milk yield per day in the first calving interval, and in the first and second calving interval combined. They also had lower milk yield before dry-off which may be beneficial for their udder health and welfare. Furthermore, for cows with an extended calving interval, the reproductive performance was improved as a higher proportion of the cows got pregnant at the first insemination, fewer inseminations were needed per pregnancy and they had shorter interval between first and successful insemination. However, an extended calving interval had no effect on disease or culling frequency. Cows randomized to an extended calving interval in the first study had longer dry period than cows with a conventional calving interval. However this was not true in the second study.

The results in this thesis shows that extended calving intervals may be a viable alternative management strategy in high-yielding dairy herds, utilizing the potential for milk production and fertility of modern dairy cows, and thereby increasing flexibility and resilience in the herds.

Populärvetenskaplig sammanfattning

Ett kalvningsintervall på 12-13 månader har länge ansetts vara mest ekonomiskt fördelaktigt för mjölkkor. En förlängning av kalvningsintervallet innebär att det blir längre mellan de påfrestande övergångsperioderna med sinläggning, kalvning och ny laktation, både för enskilda kor och på gårdsnivå. I denna avhandling presenteras resultat från två studier, en där korna lottades till förlängt kalvningsintervall, där korna följdes under två laktationer, och en studie där kor som förväntades passa för ett förlängt kalvningsintervall valdes ut baserat på genetiska meriter, hög mjölkmängd i tidig laktation samt besvärliga kalvningar eller sjukdom i tidig laktation.

Kor med förlängt kalvningsintervall behöll samma mjölkmängd per dag i kalvningsintervallet under första, och ökade mjölkmängden per dag under andra laktationen jämfört med kor med traditionellt kalvningsintervall. De hade även lägre mjölkmängd vid sinläggning vilket är positivt för juverhälsa och djurvälfärd. Dessutom förbättrades kornas fruktsamhet, fler blev dräktiga vid första insemineringen efter kalvning och det behövdes färre inseminationer för att korna skulle bli dräktiga. Hälsoläget var gott och varken sjukdomsfrekvens eller utslagningsfrekvens påverkades. Korna som lottats till ett förlängt kalvningsintervall i första studien hade en längre sinperiod, men det gällde inte för korna i den andra studien som valts ut för att de förväntades passa för ett förlängt kalvningsintervall.

Resultaten i denna avhandling visar att förlängda kalvningsintervall kan vara en möjlig alternativ strategi för högproducerande besättningar som bättre utnyttjar de moderna mjölkkornas fruktsamhet och stora potential för mjölkproduktion. Att utöka repertoaren av möjliga skötselrutiner ökar flexibiliteten vilket även kan bidra till ökad resiliens i besättningarna.

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Appendix

Table 2. Description and definition of milk production variables studied in this thesis (modified after Table A1 in Paper I)

Variable	Description/calculation
Calving interval (CInt)	Number of days between two consecutive calving dates.
Last day of lactation	The dry-off date recorded in SNDRS was used as the last day of lactation if this occurred later than the date of the last test milking and if the date of the last test milking occurred later than the date of the last recorded daily yield. Otherwise, the date of either the last recorded daily yield or the last test milking was used, whichever was the latest.
Lactation length	The interval between calving and the last day of the lactation.
Dry period length (DPL)	CInt minus lactation length.
DPL category	"Short", defined as less than 40 days, "moderate", defined as 40-70 days, or "long", defined as more than 70 days. In the analysis, the number of cows in the short and long DPL subgroups were compared with the number of cows with moderate DPL.
Daily milk yield (DMY)	Sum of individual milk record yields (kg) from the milking systems for a given day in milk (DIM). Outliers were defined as DMY values more than two standard deviations from the two-week mean and removed. Missing values between recorded values were interpolated based on existing DMY. Missing DMY at the end of lactation were calculated based on the mean lactation curve for all cows in each VWP group with sufficient DMY at the end of lactation for first and second lactation, respectively. Daily yields were used to calculate the lactation curves for each VWP group.
MY d 4-33 and 4-145	The mean milk yield (kg) day 4-33 and d 4-145 after calving, calculated from daily milk recordings made by the milking systems in the herds.
ECM yield	Calculated according to the equation by Sjaunja et al. (1990): kg ECM = kg MY * ((38.3 * fat (g/kg) + 24.2 * protein (g/kg) + 783.2) / 3140)

305-d yield	Data on 305-d lactation milk, fat, and protein yields retrieved from SNDRS.
Whole-lactation yield	Whole-lactation milk, fat, and protein yields calculated based on test milkings, using the Test Interval Method (Sargent et al. 1968). Dry-off dates reported to SNDRS were used to define end of lactation in these calculations.
MY before dry-off	Daily milk yield at the test milking between 50 and 20 days before dry-off. If the cow had more than one test milking in this period, the first of these milkings was used.
Milk and ECM yield per day in each CInt and for 2 CInt combined	Whole-lactation yield divided by the number of days in the calving interval, and for two CInt combined, the sum of the two whole-lactation yields, divided by sum of the days in two CInt.
Phenotypical persistency between 3rd and 8th test milking	Change in milk yield (kg per d) between the 3rd and 8th test milking.
Phenotypical persistency between 3rd test milking and dry-off	Change in milk yield (kg per d) between the 3rd test milking and dry-off.

Table 3. Description and definition of fertility variables studied in this thesis (modified after descriptions in Paper II)

Variable	Description/calculation
Voluntary waiting period (VWP)	Defined as the days between calving and when the cow is eligible to receive the first insemination.
Estrus intensity	Estrus intensity at first insemination in each lactation, rated on an ordinal scale from 0-5 (with 0 representing no signs of estrus and 5 representing strong estrus signs). Scores 0 to 2 and scores of 4 and 5 were merged, as they have the same biological and practical relevance (Nyman et al. 2016).
Calving to first insemination interval (CFI)	Calving to first insemination interval in days.
Reason why, if planned VWP was not followed	Reported by the farmers and categorized as: Disease/accident, Impaired fertility, Low milk yield, Mistake or Unknown. For cows not inseminated at all during the lactation, the reported culling reason was assumed to be the cause of the lack of compliance, if no other cause was reported.
First service conception rate (FSCR)	Proportion of cows with conception at first insemination divided by all inseminated cows each lactation. Conception at first insemination was defined as cows that had a complete CInt and calculated to have conceived at first insemination, or cows that had a positive pregnancy diagnosis after the first registered insemination.
Inseminations with positive pregnancy diagnosis	Number of inseminations with confirmed positive pregnancy diagnosis performed after the insemination.
Insemination that led to calving	DIM for the last insemination in the interval of 280 +/- 14 days before the next calving.
Insemination period length	The interval in days between the first insemination and the insemination that led to calving.
Total number of inseminations	Number of recorded inseminations during the lactation. All recorded inseminations were counted, even when given at an interval of a few days during the same estrus.
Number of conceptions	A conception is defined as either a positive pregnancy diagnosis or a cow that later had a calf. Repeated conceptions for the same cow during the same lactation were ignored.
Number of inseminations per conception (NINS)	Total number of inseminations divided by the total number of conceptions, calculated for each lactation.

Pregnancy loss	Defined as a cow with a 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 by a negative pregnancy diagnosis.
Calving interval (CInt)	Number of days between two consecutive calving dates.
Disease incidence	Disease incidence was calculated as total number of disease events per lactation and treatment divided by 100 cow years in each studied CInt (time at risk). During the first lactation, records of disease before the VWP intervention starting at 25 DIM (Paper II) and 33 DIM (Paper IV) were excluded.
Disease categories	Recorded diseases were divided into eight categories: Mastitis, Subclinical mastitis, Reproductive disorders, Leg/hoof lesion, Puerperal paresis, Accident/trauma, Metabolic, and Other.
Somatic cell count (SCC)	SCC values were available in the test milking data. A binomial variable representing cows with SCC <100.000 (Y/N) was calculated for the last test milking of each cow in each lactation.
Culling rate	Calculated as number of cullings per 100 cow-years in the study for each herd and VWP treatment subgroup each lactation.
Culling reasons	Reasons for culling divided into seven categories: Accident, (Impaired) Fertility, Leg/hoof disorder, Low milk yield, Mastitis, Other, or Sold (reported as a culling reason in the SNDRS).
Time in trial	Calculated for each CInt as the number of days in the CInt or time between calving and culling if the cow was culled during the lactation.
Grouped analysis of NINS, disease incidence, and culling rate	Calculated in a grouped analysis, per herd and VWP treatment, resulting in one value per herd and treatment subgroup for each lactation (Paper II: n = 32 subgroups in lactation 1, n = 30 subgroups in lactation 2; Paper IV: n = 51 subgroups).

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A randomized study on the effect of extended voluntary waiting period in primiparous dairy cows on milk yield during first and second lactation

A. Edvardsson Rasmussen,[1]* K. Holtenius,[1] R. Båge,[2] E. Strandberg,[3] M. Åkerlind,[4] and C. Kronqvist[1]

[1]Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Box 7024, 750 07 Uppsala, Sweden
[2]Department of Clinical Sciences, Swedish University of Agricultural Sciences, Box 7054, 750 07 Uppsala, Sweden
[3]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Box 7023, 750 07 Uppsala, Sweden
[4]Växa Sverige, Box 288, 751 05 Uppsala, Sweden

ABSTRACT

Extending the voluntary waiting period (VWP) for primiparous cows can have a positive impact on fertility without a negative impact on milk production per day in the calving interval (CInt). We investigated the effect of extended VWP during first lactation on milk yield (MY) during 2 consecutive lactations in primiparous cows. The study involved 16 commercial herds in southern Sweden. A total of 533 Holstein and Red dairy cattle (Swedish Red, Danish Red, Ayrshire) dairy cows were randomly assigned to a conventional 25 to 95 d VWP (n = 252) or extended 145 to 215 d VWP (n = 281). Data on calvings, inseminations, and test-day yields were retrieved from the Swedish Milk Recording System. Cows with VWP according to plan and completing 1 or 2 CInt with a second or third calving were included in the data analysis. Whole lactation and 305-d energy-corrected milk (ECM) yield were higher for the extended VWP group than the conventional VWP group in both the first lactation (12,307 vs. 9,587 and 9,653 vs. 9,127 kg ECM) and second lactation (12,817 vs. 11,986 and 11,957 vs. 11,304 kg ECM). We found no difference between the VWP groups in MY per day during the first CInt or during the first and second CInt combined, although MY per day during the second CInt was around 1.5 kg higher for cows with extended VWP than for cows with conventional VWP. Thus extended VWP for primiparous cows can be used as a management tool without compromising MY.
Key words: extended calving interval, extended lactation, lactation length, milk production

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*Corresponding author: anna.edvardsson.rasmussen@slu.se

INTRODUCTION

The effect of voluntary waiting period (**VWP**), defined as the period between calving and when the cow is permitted to receive the first insemination, on milk production and economics has been examined in several previous studies but with inconclusive results. Randomized controlled studies have generally found extended VWP to be more economically favorable relative to conventional VWP (Arbel et al., 2001; Burgers et al., 2021a), than previous simulation studies (Strandberg and Oltenacu, 1989), simulation studies based on data from voluntary extension of the VWP (Gaillard et al., 2016), and studies based on retrospective data (Holmann et al., 1984; Inchaisri et al., 2011). Since the time of those studies, average yearly milk yield (**MY**) of dairy cows has increased dramatically. Moreover, retrospective studies risk including data from involuntarily extended calving intervals (**CInt**) due to poor fertility or poor fertility management, in contrast to studies with planned extension of VWP (Niozas et al., 2019a; Rehn et al., 2000; Burgers et al., 2021b). Irrespective of study design, there is general agreement that extended VWP and longer CInt are more beneficial for primiparous cows with high yield and more persistent lactations than for multiparous cows with less persistent lactations (De Vries, 2006; Lehmann et al., 2019; Römer et al., 2020).

Planned extended VWP has been shown not to affect milk production per CInt day during the first lactation (Österman and Bertilsson, 2003; Niozas et al., 2019a; Burgers et al., 2021a) or to increase it (Arbel et al., 2001). Milk yield during early lactation after a previous lactation with extended VWP is reported to be higher (Arbel et al., 2001; Lehmann et al., 2016) or similar (Burgers et al., 2021a) to that after a previous lactation with conventional VWP. In a 3-yr study, Österman and Bertilsson (2003) monitored 1 group of cows with 3 consecutive 12-mo CInt and another group with 2 consecutive 18-mo CInt and found no difference between the groups in ECM yield per day of CInt during the second lactation.

A planned extended VWP can have several consequences. For example, improved fertility has been reported in terms of number of services per conception (Niozas et al., 2019b; Larsson and Berglund, 2000), interval between first and successful insemination (Larsson and Berglund, 2000), and first service conception rate (Niozas et al., 2019b). It may also be a strategy to decrease the overall time within their lifetime that dairy cows spend in the sensitive period around calving and dry-off (Burgers et al., 2021a) when the incidence of many diseases is highest (Ingvartsen et al., 2003). This lowers the labor requirement related to transition periods such as calving events and dry-offs and also lowers the proportions of calves, recruitment heifers, and dry cows in the herd (Lehmann et al., 2019). Extending the VWP may also lead to lower dry-off yields (Niozas et al., 2019a), which can decrease the risk of mastitis during the transition period (Rajala-Schultz et al., 2005) and has been linked to longer productive life (Römer et al., 2020). Extended VWP and CInt have been associated with an increased dry period length (Rehn et al., 2000) and higher BCS at the end of lactation, especially in multiparous cows (Burgers et al., 2021a). In a simulation study, an extended lactation for primiparous cows followed by conventional 10-mo lactations was identified as one of the most profitable alternatives (Gaillard et al., 2016). However, to our knowledge, no randomized controlled study to date has monitored primiparous cows with extended VWP in their first lactation and through a complete second lactation with no VWP intervention.

The 2 most common dairy breeds in Sweden are Swedish Holstein and Swedish Red dairy cattle. Holstein cows have higher MY, while Red dairy cattle produce milk with higher fat and protein concentrations (Växa Sverige, 2021). Our objective in this study was to investigate the effects on milk production in these 2 breeds in commercial herds during a first lactation with conventional or extended VWP and a second lactation without any VWP intervention.

MATERIALS AND METHODS

Study Design and Herd Selection Criteria

A randomized controlled trial focusing on conventional and extended VWP was carried out on 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). A total of 218 farmers were invited to join the study, based on data from the year 2016 to 2017 obtained from the Swedish national dairy herd recording scheme (**SNDRS**) managed by Växa Sverige. The inclusion criteria were: herds with >100 dairy cows in production and herd-average milk production of at least 9,000 kg ECM/cow per year, mean CInt less than 14 mo, and a system for daily milk recording. Nineteen herds agreed to participate, but 1 herd dropped off during the study due to miscommunication and 2 herds were excluded after analysis of insemination data due to poor compliance with the research protocol. Data from the second lactation in 1 herd were excluded because that herd was also involved in another study on extended VWP in multiparous cows.

Herd Description

The 16 participating herds had mean herd size of 165 cows (range 102 to 305), mean yearly milk production, defined as the total produced yield in the herd during the year divided by the mean number of cows in the herd during the same year, of 10,623 kg ECM (range 9,000 to 12,623 kg ECM), and mean CInt of 12.7 mo (range 11.8 to 13.8 mo) during the previous year (2016 to 2017). The herds had either automated milking systems (n = 12) or parlors (n = 4) and the cows were fed a partial mixed ration (n = 14) or TMR (n = 2). The cows were categorized as Holstein (**HOL**), Red dairy cattle (**RDC**, defined as Swedish Red, Danish Red, or Swedish Ayrshire cattle), or cross/other breeds, with a cow considered purebred if the dam and sire were of the same breed. Based on the SNDRS data for 2017 to 2018, the breed distribution in the herds was: HOL mean 50% (range 5 to 97%), RDC mean 41% (range 2 to 90%), and cross/other mean 9% (range 0 to 34%).

Cow Inclusion, Exclusion, and Intervention Regarding the Voluntary Waiting Period

The study period started within 1 mo of September 1, 2018, in all herds. Only purebred HOL and RDC heifers calving within 6 mo of the starting date of each herd were recruited for the study. The primiparous cows selected for inclusion were allocated by odd or even ear tag number to either a control group with conventional VWP of 35 to 85 d or a test group with extended VWP of 155 to 205 d. No intervention in VWP was made during the second lactation. Cows lacking insemination data or not having a complete first lactation before the end of data collection in September 2021 were excluded (n = 5).

Data Collection, Description, and Calculation of Variables

Data on breed, calvings, inseminations, 305-d lactation yields, test-day yields, and dry-off dates during 2 consecutive lactations between August 2018 and September 2021 were obtained from the SNDRS. Information about daily MY and number of milkings for individual cows was obtained from the herds' milking systems. All variables included and the way in which they were calculated are presented in Appendix Table A1. Supplemental files (documentation, metadata, and so on) are published in the Swedish National Data Service catalog (Edvardsson Rasmussen et al., 2022). Research data cannot be openly published due to restrictions in the agreement with the principal owner of the data, Växa Sverige. The figures were made using GraphPad Prism version 9.5.0 (GraphPad Prism, 2022).

Variable Inclusion Criteria

Three inclusion criteria were used, depending on the variable under investigation. These were: VWP according to plan, complete lactation, and sufficient daily MY records.

Voluntary Waiting Period According to Plan and Complete Lactation. The cows were considered to have VWP according to plan when the instruction regarding number of days from calving to the first insemination interval was followed, allowing a deviation of 10 d from the plan. Thus for cows in the conventional group, VWP of 25 to 95 DIM was considered to comply with the plan, while for the cows in the extended VWP group, VWP of 145 to 215 DIM was considered to be in compliance. Cows that had their second or third calving before the end of data collection in September 2021 were considered to have a complete first or second lactation, respectively. Both these inclusion criteria were applied for all variables studied.

Sufficient Daily Milk Yield Records. Daily MY records were considered sufficient if there were less than 50% missing daily yield records in total, no more than the first 40 d of lactation missing, and no more than the last 60 daily yields in the lactation missing. These inclusion criteria were applied for lactation length, dry period length (**DPL**), DPL category, MY at test milking 50 to 20 d before dry-off, and lactation curve calculations. For cows with between 2 and 60 daily yield records missing in the end of lactation, the DPL and DPL category was calculated as described in Appendix Table A1.

Statistical Analysis

Initial data handling was performed in Microsoft Excel 2016 and statistical analysis was performed in R software (R Core Team, 2014), using R studio version R-4.1.2 (RStudio Team, 2021). The confidence level was set at $P < 0.05$. All models included VWP group (2 levels) and breed (2 levels) as fixed factors and the random effect of herd (16 and 15 levels in first and second lactation, respectively). Interaction between VWP group and breed was also tested as a fixed factor and was removed from the models if not significant.

For continuous variables with an approximate normal distribution of residuals, i.e., MY variables, CInt, lactation length, and DPL, linear mixed models were fitted by restricted maximum likelihood using the lmer function from the packages lme4 and emmeans in R (Bates et al., 2022; Lenth et al., 2022). The results of the models were analyzed with Type III Analysis of Variance with Satterthwaite's method to obtain *P*-values, and the results are presented as least squares means (LSM) ± standard error of the mean (SEM).

Table 1. First and second lactation calving interval, lactation length, dry period length, and milk yield (MY) at test milking (TM) 50 to 20 d before dry-off (DO; LSM ± SEM) for cows with conventional (CONV) or extended (EXT) voluntary waiting period (VWP) for the 2 breeds Holstein (HOL) and Red dairy cattle (RDC)

		VWP group			Breed		
Item	n	CONV	EXT	*P*-value	HOL	RDC	*P*-value
First lactation							
Calving interval (d)	349	368^{b} ± 3.7	462^{a} ± 3.9	<0.001	413 ± 3.7	416 ± 4.4	0.60
Lactation length (d)	320	311^{b} ± 4.2	398^{a} ± 4.3	<0.001	353 ± 4.1	355 ± 5.0	0.67
Dry period length (d)	320	56.2^{b} ± 2.6	62.6^{a} ± 2.6	<0.001	59.8 ± 2.6	59.0 ± 2.9	0.72
MY TM before DO (kg)	285	25.9^{a} ± 0.94	24.0^{b} ± 0.95	<0.001	26.2^{a} ± 0.93	23.7^{b} ± 1.0	0.003
Second lactation							
Calving interval (d)	219	377 ± 6.2	386 ± 6.6	0.13	390^{a} ± 6.6	373^{b} ± 7.4	0.03
Lactation length (d)	127	315 ± 7.8	331 ± 8.7	0.06	332 ± 8.2	314 ± 9.3	0.06
Dry period length (d)	127	58.8 ± 2.1	58.1 ± 2.4	0.75	58.2 ± 2.2	58.7 ± 2.5	0.84
MY TM before DO (kg)	106	27.3 ± 1.0	27.2 ± 1.1	0.93	30.1^{a} ± 1.0	24.4^{b} ± 1.2	<0.001

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

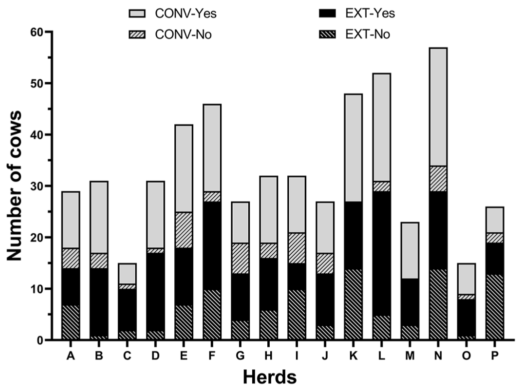

Figure 1. Number of cows that finally received (Yes) or did not receive (No) the planned voluntary waiting period (VWP) in each VWP group conventional (CONV) and extended (EXT) in each herd.

For the binary variable, DPL category, a generalized binomial linear mixed model was fitted by Laplace approximation, using the glmer function in the packages lme4 and emmeans in R (Bates et al., 2022; Lenth et al., 2022). The model was analyzed with analysis of deviance using type II Wald chi-square tests for hypothesis testing. Numbers of cows in the short and long DPL subgroups were compared with the number of cows with moderate DPL.

RESULTS

The initial number of cows recruited for the study was 533, of which 252 cows were included in the control group with conventional VWP and 281 cows were included in the test group with extended VWP. The number of cows per lactation and VWP group following each combination of inclusion criteria is presented in Appendix Table A2, and the number of cows following the planned VWP in each VWP group in each herd is presented in Figure 1. Breed distribution between planned VWP groups in each lactation is shown in Appendix Table A3. The interaction between VWP and breed was not significant and was removed from all models.

Effect of Voluntary Waiting Period on Calving Interval, Lactation Length, and Dry Period Length

For the cows in the extended VWP group, CInt, lactation length, and DPL were all longer during their first CInt than for the group with conventional VWP (Table 1). When comparing the proportion of cows in different DPL categories (short, moderate, or long dry period), we found that the proportion of cows with a long dry period in their first CInt was higher in the extended than in the conventional VWP group. There was no significant difference between the VWP groups regarding the proportion of cows with short DPL (Table 2). During the second lactation, CInt, lactation length, DPL, and DPL category did not differ significantly between the 2 VWP groups (Tables 1 and 2).

Effect of Voluntary Waiting Period on Milk and Energy-Corrected Milk Yield

Whole lactation (**WL**) yield during the first lactation was 28% greater for the extended VWP group than for the conventional group, and the former group also had 6% higher 305-d yield. However, when yield was calculated per day of CInt, there was no difference be-

Table 2. Percent and prevalence (n/N) of short and long dry period in each lactation for cows with conventional (CONV) or extended (EXT) voluntary waiting period (VWP) for the 2 breeds Holstein (HOL) and Red dairy cattle (RDC)

		VWP					Breed				
		CONV		EXT			HOL		RDC		
Item	N_{tot}[1]	%	(n/N)	%	(n/N)	*P*-value	%	(n/N)	%	(n/N)	*P*-value
First lactation											
Short dry period[2]	277	8	(13/158)	5	(6/119)	0.33	5	(9/181)	10	(10/96)	0.10
Long dry period[3]	301	9[b]	(15/160)	20[a]	(28/141)	0.002	13	(25/197)	17	(18/104)	0.35
Second lactation											
Short dry period[2]	111	3	(2/64)	6	(3/47)	0.73	3	(2/66)	7	(3/45)	0.79
Long dry period[3]	122	13	(9/71)	14	(7/51)	0.86	12	(9/73)	14	(7/49)	0.61

[a,b]Values (%) within rows with different superscripts differ significantly ($P < 0.05$).

[1]N_{tot} = total number of cows included in each model.

[2]Less than 40 d dry, n = number of cows with short dry period, N = sum of cows with short and moderate dry period length. Moderate dry period length = (40–70 d, lactation 1: N = 145; lactation 2: N = 62).

[3]More than 70 d dry, n = number of cows with long dry period, N = sum of cows with long and moderate dry period length.

Table 3. First and second lactation 305-d lactation yield, whole lactation (WL) yield, and average milk yield (MY) per day in each calving interval (CInt) and during 2 consecutive CInt (kg, LSM ± SEM) for cows with conventional (CONV) or extended (EXT) voluntary waiting period (VWP) for the 2 breeds Holstein (HOL) and Red dairy cattle (RDC)

		VWP			Breed		
Item	n	CONV	EXT	*P*-value	HOL	RDC	*P*-value
First lactation							
305-d ECM	347	9,127[b] ± 228	9,653[a] ± 230	<0.001	9,488 ± 231	9,291 ± 244	0.28
305-d MY	347	8,882[b] ± 230	9,492[a] ± 233	<0.001	9,474[a] ± 234	8,901[b] ± 249	0.003
WL ECM	349	9,578[b] ± 325	12,307[a] ± 329	<0.001	11,070 ± 331	10,816 ± 352	0.36
WL MY	349	9,279[b] ± 306	11,872[a] ± 311	<0.001	10,926[a] ± 312	10,225[b] ± 335	0.01
Daily ECM	349	26.1 ± 0.75	26.7 ± 0.76	0.15	26.7 ± 0.76	26.1 ± 0.80	0.30
Daily MY	349	25.3 ± 0.73	25.7 ± 0.74	0.30	26.3[a] ± 0.74	24.7[b] ± 0.78	0.004
Second lactation							
305-d ECM	219	11,304[b] ± 241	11,957[a] ± 253	0.002	11,973[a] ± 253	11,288[b] ± 281	0.02
305-d MY	219	11,061[b] ± 226	11,778[a] ± 240	0.001	12,115[a] ± 239	10,723[b] ± 268	<0.001
WL ECM	219	11,986[b] ± 274	12,817[a] ± 294	0.005	12,907[a] ± 289	11,896[b] ± 330	0.008
WL MY	219	11,659[b] ± 253	12,527[a] ± 274	0.003	12,963[a] ± 266	11,223[b] ± 307	<0.001
Daily ECM	219	31.8[b] ± 0.70	33.3[a] ± 0.73	0.01	33.2 ± 0.73	31.9 ± 0.81	0.10
Daily MY	219	30.9[b] ± 0.68	32.6[a] ± 0.71	0.007	33.3[a] ± 0.71	30.2[b] ± 0.79	<0.001
Both lactations combined							
Daily ECM	219	29.2 ± 0.70	30.1 ± 0.72	0.07	30.1 ± 0.72	29.2 ± 0.78	0.21
Daily MY	219	28.4 ± 0.67	29.2 ± 0.69	0.10	30.0[a] ± 0.69	27.7[b] ± 0.75	<0.001

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

tween the groups (Table 3). The lactation curves for first-parity cows in the 2 VWP groups are shown in Figure 2. During the second lactation, the extended VWP group had 5 to 7% higher WL yield, 305-d yield, and yield per CInt day than the conventional VWP group (Table 3). The lactation curves for second-parity cows in the 2 VWP groups are shown in Figure 3. Comparisons of average yield per day during the 2 CInt combined revealed that this did not differ significantly between the 2 VWP groups (Table 3).

Effect of Breed

In their first lactation, HOL cows had around 6% higher MY (305d MY, WL, daily MY) than RDC cows, but ECM yields did not differ significantly between the breeds. During the second lactation, HOL cows had higher WL yield, 305-d yield, and MY per CInt day than RDC cows, but there was no significant difference in ECM yield per CInt day. Average MY per day during the 2 CInt combined was 8% higher for HOL than

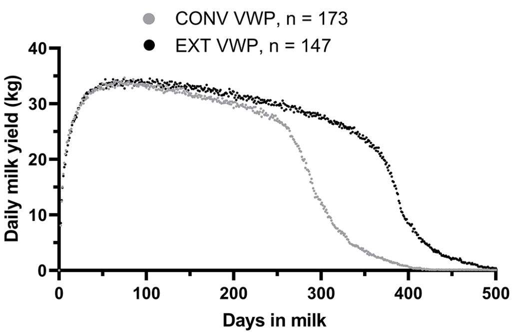

Figure 2. Average daily milk yield per DIM during the complete first lactation for cows with a conventional (CONV) voluntary waiting period (VWP; gray, n = 173) or extended (EXT) VWP (black, n = 147) during their first lactation. Based on data from cows with sufficient daily milk yield records, dried-off cows were included with 0 kg yield per day.

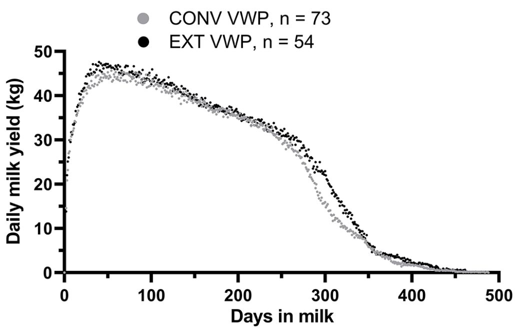

Figure 3. Average daily milk yield per DIM during the complete second lactation for cows with a conventional (CONV) voluntary waiting period (VWP; gray, n = 73) or extended (EXT) VWP (black, n = 54) during their first lactation. Based on data from cows with sufficient daily milk yield records, dried-off cows were included with 0 kg yield per day.

for RDC cows, but there was no difference between the breeds in ECM yield (Table 3).

In terms of CInt, lactation length, DPL, and proportions of cows in different DPL categories (short, moderate, or long dry period), there was no significant difference between the breeds in the first lactation, but during the second lactation HOL cows had 5% longer CInt (Tables 1 and 2). HOL cows had 11% higher MY at the last test milking before dry-off than RDC cows in the first lactation and 23% higher in the second lactation (Table 1).

DISCUSSION

This study investigated milk production during the first lactation with conventional or extended VWP and the following full lactation without a VWP intervention. To our knowledge, no random controlled trial has done this before at a large scale in commercial herds.

The study was performed on 16 relatively high-yielding herds (9,000 to 12,623 kg average yearly milk production) in southern Sweden. These herds had 102 to 305 cows with reasonable average CInt before the study (<14 mo) and varying management routines. As the study was performed on commercial herds, there is a possibility that herd managers may have treated the cows differently depending on the VWP group to which they were allocated as indicated by Figure 1. We checked whether average MY during the first 4 to 33 d of lactation differed between groups of cows not following the planned conventional and extended VWP and found no difference (results not shown). The results thus revealed no indication of obvious systematic bias among cows that did not follow the intended VWP treatment in that regard. Another possible bias related to commercial herds is possible inconsistency in data reported to the SNDRS. To mitigate this problem and other deviations in herd-related management routines, we included herd as a random factor in all statistical models.

As expected, because lactations were longer, extended VWP resulted in higher WL yield, as also reported by Lehmann et al. (2016) and Burgers et al. (2021b). Moreover the 305-d yield was higher during the first lactation, probably due to a delay in the negative effect of pregnancy on MY (as seen in Figure 2). Our results for ECM yield per day in the first CInt, which were generally not different between VWP groups, correspond well to previous findings for primiparous cows on ECM per CInt day (Österman and Bertilsson, 2003; Niozas et al., 2019a; Burgers et al., 2021a) or in extended VWP (Arbel et al., 2001; Lehmann et al., 2016).

On comparing MY per day during the second CInt, the cows with extended VWP in their first lactation produced around 1.6 kg more milk per day than the cows with conventional VWP. As mentioned, we found few other random controlled studies investigating the effect of extended VWP on the subsequent lactation and none following a complete second lactation without a VWP intervention. Österman and Bertilsson (2003) studied 2 consecutive CInt, both either conventional (12 mo) or extended (18 mo) and found that the difference between the groups in terms of WL yield increased during the second lactation, with higher yield in cows with extended CInt, but no difference between the groups on comparing ECM per CInt day in either lactation. In studies on early subsequent lactation, Arbel et al. (2001) and Lehmann et al. (2016) observed increased yield per day during the first 80 and 150 d, respectively, for cows with extended VWP in their first lactation. In contrast, Burgers et al. (2021a) found decreased MY per day during the first 42 d in the following lactation for cows with extended VWP in their first lactation, but no difference in fat- and protein-corrected milk. Those authors concluded that 42 d might be too short a period for cows to show their potential for higher yield.

According to Lehmann et al. (2016), a possible reason for the observed higher second lactation yield in cows with extended VWP might be that these cows are more mature in early second lactation, meaning that they may need less energy for growth and can allocate more energy to milk production instead. However, our observations indicated that a higher proportion of cows allocated to extended VWP were culled due to low milk production during the first lactation (data not shown). Thus, it is possible that the observed higher yield in the second lactation was an artifact related to a higher rate of culling of cows with lower yield potential.

From a farmer's perspective, it is interesting to compare average yield per day during the first and second lactations combined. We found no significant difference between the VWP groups, which suggests that the long dry period for cows with extended VWP in their first lactation was compensated for by higher total yield in both lactations or a lower proportion of dry days.

As a consequence of extended VWP, both CInt and lactation length were prolonged in the first lactation, but there was no difference between the VWP groups in the second lactation. Regarding DPL, we found a higher frequency of extended VWP cows in the long DPL category, in line with previous findings (Österman and Bertilsson, 2003; Lehmann et al., 2016). Milk yield at the last test milking before dry-off, which was investigated here as a measure of dry-off yield before the application of dry-off routines, was 1.9 kg lower in the extended VWP group than in the group with conventional VWP in the first lactation, in agreement with findings in Niozas et al. (2019a).

It is well established that HOL cows generally have higher ECM yield than RDC cows, but in the present study there was no such difference between the breeds in their first lactation. This was supported by unpublished data indicating that the difference in ECM yield between the breeds was less prominent during their first lactation (E. Strandberg, Swedish University of Agricultural Science, Uppsala, Sweden, personal communication, 2022). In addition, there was no interaction between breed and VWP, which suggests that both breeds are equally suited to extended VWP. However, HOL cows produced more milk before dry-off than RDC cows, so reduced MY before dry-off may be an incentive to extend VWP for HOL cows. Additionally, we found that HOL cows had a longer second CInt than RDC cows. This may be because the HOL cows had higher yield and farmers might therefore be more willing to delay the first insemination for these cows. It may also be because higher yield is linked to longer first-to-successful insemination interval (Römer et al., 2020).

The results for CInt and lactation lengths and WL yields were consistent with the lactation curves derived from daily MY in both lactations (Figure 2 and Figure 3), although the curves were based on fewer animals because only lactations with sufficient daily yield recordings were included. In the first lactation (Figure 2), an effect of pregnancy on daily yield was observed from about 150 DIM for the conventional VWP group, in line with findings by Österman and Bertilsson (2003).

Our results support previous claims that extended VWP in primiparous cows does not have a negative impact on individual milk production (Rehn et al., 2000; Kok et al., 2019; Lehmann et al., 2019). And we found that cows with extended VWP had fewer unproductive days: 13.5% (62.6 out of 462 d) vs. 15% (56.2 out of 368 d) of the first CInt than cows with conventional VWP (Table 1). Therefore, it may be beneficial to extend VWP, especially when taking account of other potential advantages such as improved fertility (Larsson and Berglund, 2000; Niozas et al., 2019b), possibly better health due to fewer transition periods with high disease frequency (Ingvartsen et al., 2003), lower MY at dry-off (Rajala-Schultz et al., 2005; Niozas et al., 2019a), and a longer productive life (Gaillard et al., 2016; Römer et al., 2020). However, when applying extended VWP in practice, consideration has to be given to what this means for herd dynamics. Longer VWP leads to more lactating cows, a lower need for recruitment heifers, fewer dry cows, and fewer calves born (Lehmann et al., 2019).

CONCLUSIONS

Cows with extended VWP during their first lactation had higher 305-d and whole lactation yield than cows with conventional VWP, not only in the first lactation when VWP differed for the 2 groups, but also in their second lactation without a VWP intervention. We found no difference between the VWP groups regarding yield per CInt day in the first CInt or in the 2 CInt combined. In the second CInt, yield per day was higher for cows with extended VWP during their first lactation. Regarding breed, HOL cows generally had higher MY than RDC cows, but ECM yield did not differ between the breeds during the first lactation and there were no interactions between VWP and breed. These results improve understanding of the effect of extended VWP on milk production during 2 consecutive lactations and can have implications for management decisions regarding VWP for primiparous cows. To conclude, extending the VWP for primiparous cows can be used as a management tool without compromising MY.

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ORCIDS

A. Edvardsson Rasmussen https://orcid.org/0000-0003-3026-9847
K. Holtenius https://orcid.org/0000-0003-1739-263X
R. Båge https://orcid.org/0000-0003-1413-6913
E. Strandberg https://orcid.org/0000-0001-5154-8146
M. Åkerlind https://orcid.org/0000-0002-3538-7036
C. Kronqvist https://orcid.org/0000-0003-3005-1004

APPENDIX

Table A1. Variables used in the analysis and the method of calculation

Variable	Description/calculation
305-d yield	Data on 305-d lactation milk, fat, and protein yields retrieved from the Swedish national dairy herd recording scheme (SNDRS).
Calving interval (CInt)	Number of days between 2 consecutive calving dates.
Daily milk yield (DMY)	Sum of individual milk record yields (kg) from the milking systems for a given DIM. Outliers were defined as DMY values more than 2 SD from the 2-wk mean and removed. Missing values between recorded values were interpolated based on existing DMY. Missing DMY at the end of lactation were calculated based on the mean lactation curve for all cows in each VWP group with sufficient DMY at the end of lactation for first and second lactation, respectively. Daily yields were used to calculate the lactation curves for each VWP group.
Dry period length (DPL)	Calving interval minus lactation length.
DPL category	Short defined as less than 40 d, moderate defined as 40 to 70 d, or long defined as more than 70 d.
ECM yield	Calculated according to the equation by Sjaunja et al. (1990): kg ECM = kg MY × {[38.3 × fat (g/kg) + 24.2 × protein (g/kg) + 783.2]/3,140}
Lactation length	The interval between calving and the last day of the lactation (see below).
Last day of lactation (used to calculate lactation length and dry period)	The dry-off date recorded in SNDRS was used as the last day of lactation if this occurred later than the date of the last test milking *and* if the date of the last test milking occurred later than the date of the last recorded daily yield. Otherwise, the date of either the last recorded daily yield *or* the last test milking was used, whichever was the latest.
Milk and ECM yield per calving interval day	Whole lactation yield divided by calving interval.
Milk and ECM yield per day during 2 calving intervals	Sum of the 2 whole lactation yields, divided by sum of the 2 calving intervals.
Milk yield (MY) at test milking before dry-off	Daily milk yield at the test milking between 50 and 20 d before dry-off. If the cow had more than 1 test milking in this period, data for the test milking closest to 50 d before dry-off were used.
Whole lactation yield	Whole lactation milk, fat, and protein yields calculated based on test milkings, using the test interval method (Sargent et al., 1968). Dry-off dates reported to SNDRS were used to define end of lactation in these calculations.

Table A2. Number of cows with conventional (CONV) or extended (EXT) voluntary waiting period (VWP) following the different inclusion criteria and combinations of inclusion criteria applied in data analysis[1]

	Lactation 1			Lactation 2		
Inclusion criteria	CONV VWP (n = 252)	EXT VWP (n = 281)	Total lact. 1 (n = 533)	CONV VWP in lact. 1 (n = 191)	EXT VWP in lact. 1 (n = 188)	Total lact. 2 (n = 379)
VWP according to plan (VWP OK)	205	179	384	168	139	307
Complete lactation (compl.)	210	211	421	140	125	265
Daily milk yields OK (MY OK)	207	223	430	107	91	198
<50% missing daily yields	228	245	473	129	113	242
<40 missing daily yields in early lactation	248	278	526	160	131	291
<60 missing daily yields at the end of lactation	219	235	454	129	122	251
VWP OK + compl.	187	162	349	123	96	219
VWP OK + compl. + available 305-d yield	186	161	347	123	96	219
VWP OK + compl. + MY OK	173	147	320	73	54	127
VWP OK + compl. + MY OK + available test milking yield 50–20 d before dry-off	151	134	285	59	47	106
VWP not OK + MY OK	25	63	88	12	20	32

[1]Lact. = lactation.

Table A3. Number of cows divided by breed [Holstein (HOL) or Red dairy cattle (RDC)] with a planned conventional (CONV) or extended (EXT) voluntary waiting period (VWP) in the first and second lactations

	CONV		EXT		Total	
Lactation	HOL	RDC	HOL	RDC	HOL	RDC
First lactation	146	106	181	100	327	206
Second lactation	109	82	115	73	224	155

II

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

A. Edvardsson Rasmussen[1], R. Båge[2], K. Holtenius[1], E. Strandberg[3], Claudia von Brömssen[4], M. Åkerlind[5], and C. Kronqvist[1]

[1]Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management, P.O. Box 7024, 750 07 Uppsala, Sweden. [2]Swedish University of Agricultural Sciences, Department of Clinical Sciences, P.O. Box 7054, 750 07, Uppsala, Sweden. [3]Swedish University of Agricultural Sciences, Department of Animal Breeding and Genetics, P.O. Box 7023, 750 07 Uppsala, Sweden. [4]Swedish University of Agricultural Sciences, Department of Energy and Technology, Unit of Applied Statistics and Mathematics, P.O. Box 7032, 750 07 Uppsala, Sweden. [5]Växa Sverige, P.O. Box 7024, 750 07 Uppsala, Sweden.

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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 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 (HOL) and Red Dairy Cattle (RDC) breeds. Data from the Swedish national dairy herd recording scheme (SNDRS) collected between August 2018 and September 2021 were used in the analysis, including records on breed, calvings, estrus intensity, inseminations, disease, somatic cell count (SCC), 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 to 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 to the conventional VWP treatment. Regarding disease incidence rate or culling rate expressed as number of events per cow-time in the study, there were no differences between the cows receiving the two VWP treatments in any lactation. Calving to first service interval (CFI) 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, higher FSCR, and lower NINS, during the first lactation. However, there was 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 two 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.

Keywords: 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), give cows better possibilities to regain their energy balance, which may improve fertility (Butler, 2005). Estrus intensity, first service conception 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, e.g., 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 days 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, Niozas et al. (2019a) reported higher overall culling and culling due to low productivity in cows with 180-d compared with 40-d VWP, while 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.

Randomized-controlled studies may have inherent biases relating to degree of compliance with the planned treatments, choosing either to report effects 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. Further, an aim was to identify potential bias due to possible differences in compliance between cows randomized to the two VWP treatments, compliance and the "intention-to-treat" effect of extended VWP on culling rate were analyzed.

Materials & 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 below.

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 CInt of less than 14 months, 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.

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

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

The cow inclusion period in each herd started within 1 month of 1 September 2018 and continued for 6 months 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 months. 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 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. 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. 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 days in milk (DIM) at the last insemination in the interval 280 ±14 days 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 as NINS makes less sense on individual basis, NINS was calculated as total number of inseminations divided by total number of conceptions, resulting in one 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, also when 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 by a negative pregnancy diagnosis.

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 two 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 VWP (n = 251)	EXT VWP (n = 280)	Total lact. 1 (n = 531)	CONV VWP (n = 186)	EXT VWP (n = 171)	Total lact. 2 (n = 357)
VWP according to plan (VWP OK)[1]	204	178	382	166	128	294
Complete lactation (compl.)	209	210	419	140	123	263
VWP OK + compl.	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 according to plan during lactation 1, during lactation 2 there was no VWP intervention and the farmers were free to inseminate the cows whenever they choose.

The estrus intensity score used was that recorded for the first insemination in each lactation. It was rated on an ordinal scale from 0-5 (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 as 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-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 VWP intervention start at 25 DIM were excluded. Recorded diseases were divided into 8 categories: mastitis, subclinical mastitis, reproductive disorders, leg/hoof lesion, puerperal paresis, accident/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 a healthy udder (Jashari et al., 2016).

Mean milk yield on day 4-33 for cows that were not inseminated according to the planned treatment, and on day 4-145 after calving for cows with a planned extended VWP treatment, was calculated from daily milk data recorded by the milking systems in the herds. 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/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.

Statistical analyses

Microsoft Excel 2016 was used for initial data organization and GraphPad Prism version 9.5.0 (GraphPad Prism, 2022) was used to visualize estrus intensity, culling reason, and disease incidence ate. R software (R core team, 2022) and R studio version R-4.1.2, (R studio, 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 above. 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-square tests were used to determine which of the fix 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 LSM ±SEM. Differences in disease incidence rate, NINS and culling rate, between cows receiving the two 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 number of cows of the two 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). There were no differences in estrus intensity scores between cows receiving the two different VWP treatments during lactation 2 (Figure 2).

In the first lactation, FSCR was higher (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 days, P<0.001) for cows receiving the extended compared with the conventional VWP treatment. However, there was 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 days for cows receiving the extended and conventional VWP treatment, 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 to conventional VWP treatment (Table 4). We found no interaction between VWP treatment and breed for any of the fertility traits considered (data not shown).

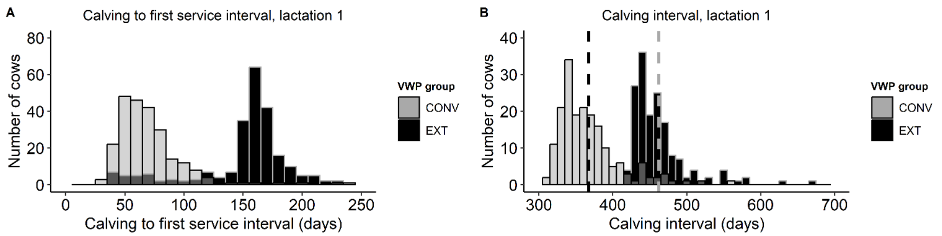

Figure 1. Distribution of A) interval between calving and first insemination, for cows randomized to each treatment ("intention to treat"), and receiving a first insemination, with conventional (grey, n = 236) and extended (black, n = 234) VWP treatment, and B) distribution of calving interval, and mean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

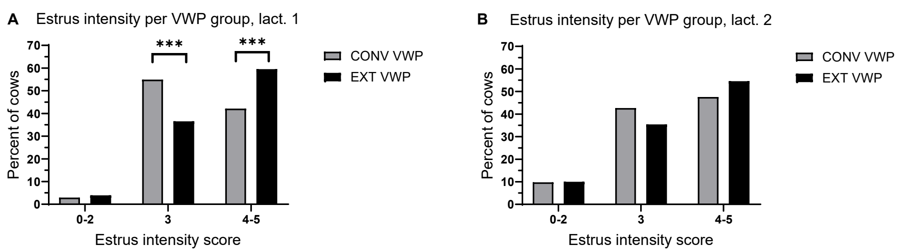

Figure 2. Estrus intensity scores at first insemination on a scale from 0-5, where 5 represents the strongest estrus intensity, and with scores 0-2 and 4-5 merged (*** = P < 0.001). Displayed as proportion of cows receiving the intended conventional (CONV) or extended (EXT) voluntary waiting period (VWP) treatment with each estrus intensity score in A) lactation 1 (n = 382) and B) lactation 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. Presented as proportion of cows (% 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 treatment in each lactation, and n_{tot} is total number of cows in each analysis*

		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†
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 row with different superscripts differ significantly (P< 0.05).
†P = 0.0503

Table 4. *Number of inseminations per conception (NINS) in lactation 1 and 2, and calving to first insemination interval (CFI) in lactation 2. 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 least squares means ±SEM for cows with conventional (CONV) or extended (EXT) VWP*

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

[a–b] Mean values within row with different superscripts differ significantly (P< 0.05).
[1]Result of the intervention and therefore not tested.

Health records

We found no difference between the VWP treatments or breeds regarding proportion of cows with SCC <100,000 cells/mL 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 there was an interaction between VWP treatment and breed in both lactations. For HOL cows, there was no difference between cows receiving different VWP lengths, but at the end of 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). In the end of the second lactation, on the other hand, 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). There was 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 in both lactations combined (17.7 ±5 vs 18.3 ±5, P = 0.87) (Table 6). There was also 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 second lactation (26.2 ±4.1 vs 26.7 ±4.5, P = 0.93) (Tables 6 and 7).

Disease incidence rate per disease category is illustrated in Figure 3, while culling rate per culling reason category for cows receiving extended or conventional VWP is shown in Figure 4.

Intention-to-treat analysis and culling

There was no difference in milk yield per day during the first 4-33 days 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). On the other hand, for cows allocated to an extended VWP, but not following their planned VWP treatment, i.e. that failed to comply, the mean yield during 4-145 DIM was 8% lower than for cows that were following the planned extended VWP treatment, i.e. succeeded to comply (23.5 vs. 28.4 kg/day, 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 = 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.

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 somatic cell count (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) VWP treatment within the breeds Holstein (HOL) and Red Dairy Cattle (RDC)*

		HOL					RDC					
		CONV		EXT		HOL	CONV		EXT		RDC	Interaction
SCC at last TM	n_{tot}	%	(n/N)	%	(n/N)	P-value	%	(n/N)	%	(n/N)	P-value	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 row with different superscripts differ significantly (P< 0.05).

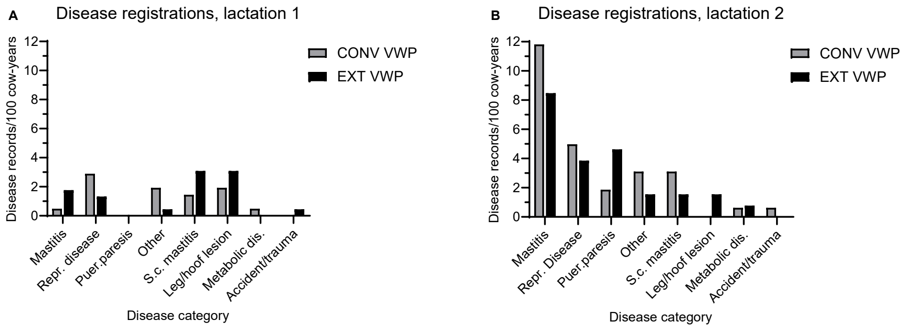

Figure 3. Disease incidence rate for each disease category per 100 cow-years in the study for cows receiving an extended (EXT; lactation 1, n =178; lactation 2, n = 128) and conventional (CONV; lactation 1, n = 204; lactation 2, n = 166) voluntary waiting period, respectively, during A) lactation 1 and B) lactation 2, for cows receiving the intended VWP. Diseases recorded on day 0-25 in lactation 1, before the start of the intervention, were excluded. The results are presented descriptively.

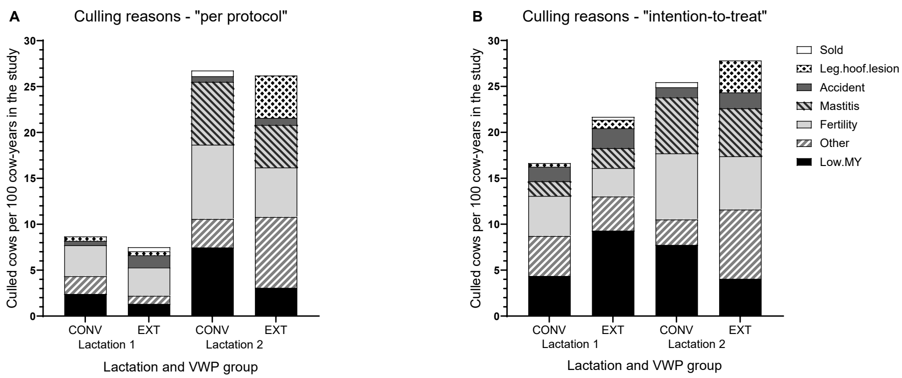

Figure 4. Reported culling reasons, presented as number of culled cows per culling reason category and 100 cow-years in the study in each lactation, for A) cows receiving their planned conventional (CONV) or extended (EXT) voluntary waiting period (VWP) treatment, “per protocol”, (lactation 1, n = 382;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

Discussion

This randomized-controlled study investigated the effect 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 to retrospective, observational, field studies. In the latter case, it is often unknown weather ta 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 making inclusion of more cows possible.

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 there was no difference in CInt during the second lactation (Edvardsson Rasmussen et al., 2023). During the second lactation, there was no VWP intervention 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.

There are several possible reasons for the diverging results in previous randomized studies of fertility in 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-day 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 around 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, while 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 non-esterified fatty acid levels in the blood. The increased non-esterified 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., 2023), 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 weeks before dry-off for multiparous cows receiving an extended VWP of 200 d, compared to 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 weeks 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 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 than 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.

Table 6. *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) VWP treatment. Diseases and cow time in study recorded for day 0-25 in lactation 1, before the start of the intervention, were excluded*

	"Per protocol"		"Intention to treat"	
Variable and lactation	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

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 to 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 there was no difference 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, while 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 the disease incidence is highest) per unit time (Ingvartsen, 2003). Moreover, a lower dry-off yield, as was found in lactation 1 for cows with extended VWP (Edvardsson Rasmussen et al., 2023), have been connected to a reduced risk for mastitis at dry-off (Rajala-Schults et al 2005). However we found no difference in disease incidence rate between cows receiving the two different VWP treatments in either lactation, or in both lactations combined.

Compliance and Intention-to-treat analysis

Since 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 herds (Edvardsson Rasmussen et al., 2023). Compliance was lower for the extended than for the conventional VWP treatment. An 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, since a new routine increases the requirement for precision in 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, due to "mistakes", and for the conventional VWP treatment due to "fertility issues".

Table 7. *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 treatment. The results were calculated per herd and voluntary waiting period (VWP) treatment subgroup (number of subgroups lactation 1, n = 32, and lactation 2, n = 30). The values presented are least squares means ±SEM*

	Per protocol				Intention to treat			
Culling rate	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

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 there is a higher risk of culling a non-pregnant cow than 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 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. During 4-33 DIM, we found no difference in average daily yield between the VWP treatments, which indicates 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 only allowing comparison between cows with planned extended VWP treatment), we found higher yield for cows receiving, in contrast to not receiving, an extended VWP. Additionally, there was 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 the difference in compliance, we performed intention-to treat analysis for the culling rate. We found no difference in culling rate between the two VWP treatments, either for cows randomized to “intention to treat” or 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

Since 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, etc. were comparable for cows receiving the two different VWP treatments, as both treatments were represented in each herd. However, some routines might have been affected by the intervention, e.g., by differences in management regime 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), and combining these with the current results, it appears that for primiparous cows with an extension of VWP to 145-205 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 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 two different VWP treatments in either lactation. Compliance with the planned VWP treatment was lower for cows with extended compared to 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 indicate that further research, for example on the customization of VWP for individual cows, might give room for further improvements.

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Acta Universitatis Agriculturae Sueciae

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In this thesis, randomized and customized extensions of the voluntary waiting period before first insemination after calving in primiparous dairy cows was investigated. The results showed that fertility was improved, and milk yield per day in the calving interval was maintained, while there was no effect on disease frequency or culling rate, indicating that extended voluntary waiting periods may be a viable alternative management strategy in high yielding dairy herds, increasing flexibility and thereby resilience in the herds.

Anna Edvardsson Rasmussen received her postgraduate education at the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences. She obtained her veterinary degree in 2012 at the faculty of Veterinary Medicine and Animal Science, SLU.

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