The Significance of Sustainable Breeding and Management Programs on Reproductive Performance in Norwegian Red Cows

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CONTENTS

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Oslo, December 2009

Randi Therese Garmo

Randi Therese Garmo

LIST OF ABBREVIATIONS

AI: Artificial Insemination

C-LA: Commencement of Luteal Activity

CFAI: Interval from Calving to First Artificial Insemination

CLAI: Interval from Calving to Last Artificial Insemination

DMI: Dry Matter Intake

DOV: Delayed Ovulation

EB: Energy Balance

FUM: Norwegian Feeding value per Unit of Milk

GEE: General Estimating Equation

HMP: High Milk Production (Paper II)

HI: High Index (Paper II)

IGF-1: Insulin like Growth Factor type 1

LH: Luteinizing Hormone

LMP: Low Milk Production (Paper II)

lnWIM: Natural logarithm of Weeks In Milk

LS-means: Least Square-means

NDHRS: Norwegian Dairy Herd Recording System

PAG: Pregnancy Associated Glycoproteins

PCL: Persistent Corpus Luteum (yellow body)

SCC: Somatic Cell Count

SD: Standard Deviation

WIM: Weeks In Milk

LIST OF PAPERS

The thesis is based on the following four papers, which will be referred to by their Roman numerals.

Paper I: R. T. Garmo, A.O. Refsdal, K.Karlberg, E.Ropstad, A.Waldmann, J.F. Beckers, and O. Reksen.

Pregnancy Incidence in Norwegian Red Cows Using Nonreturn to Estrus, Rectal Palpation, Pregnancy Associated Glycoproteins and Progesterone.

J. Dairy Sci. 2008. 91:3025-3033.

Paper II: R. T. Garmo, E.Ropstad, Ø. Havrevoll, E. Thuen, H. Steinshamn, A.Waldmann, and O. Reksen.

Commencement of Luteal Activity in Three Different Selection Lines for Milk Yield and Fertility in Norwegian Red Cows.

J. Dairy Sci. 2009. 92:2159-2165.

Paper III: R. T. Garmo, A. D. Martin, E. Thuen, Ø. Havrevoll, H. Steinshamn, E. Prestløkken, Å. Randby, M. Eknæs, A. Waldmann, and O. Reksen. 2009.

Characterization of Progesterone Profiles in fall-calving Norwegian Red Cows

J. Dairy Sci. 2009. 92:4919-4928.

Paper IV: R. T. Garmo, S. Waage, B. I. F. Henriksen, S. Sviland, O. Østerås, and O. Reksen. 2009.

Reproductive Performance, Udder Health, and Antibiotic Resistance in Mastitis Bacteria isolated from Norwegian Red Cows in Conventional and Organic Farming.

Submitted to Acta Vet Scand.

SUMMARY

The breeding program for the Norwegian Red breed over the last 35 years has included fertility, health and functional traits in addition to milk yield and other production traits. Currently, the demands placed on individual cows are increasing particularly in regards to milk yield, dry matter intake, fertility, and longevity. The overall goal for the four studies included in this thesis was to assess the reproductive performance in modern Norwegian Red cows.

In the first field study, the 60 d non return rate, pregnancy incidence, calving rate and differences between non return rate and pregnancy incidence following first artificial insemination (AI) were investigated. None of the animals were treated with reproductive hormones prior to AI. The proportion of cows not returning to estrus was 72.5%, the pregnancy incidence 6 wk after AI was 63.7%, and the calving rate was 57.2%. The difference between non return rate and pregnancy incidence was higher for older cows compared with younger cows and heifers. Parity number did not affect the likelihood of pregnancy when heifers were excluded from the analysis, but heifers had higher pregnancy incidence than cows. The interval to first AI was not associated with pregnancy incidence, which could be due to a relatively long voluntary waiting period (85.3 d). Milk yield was also not associated with the pregnancy incidence. The moderate milk yield in Norwegian Red cows and inclusion of non return rate as a fertility trait in the Norwegian breeding program over the past 35 years are probably important reasons for such good reproductive performance as seen in this study.

Multiple ovarian cycles before first AI after calving have shown to reduce the number of AI per pregnancy. Hence, early reestablishment of ovarian activity and onset of luteal activity that usually occurs 4-5 d after first ovulation post partum are important factors for good reproductive performance in dairy cows. Consequently, in some countries recent attention has focused on breeding for early onset of luteal activity. Onset of luteal activity after calving and relationships with the occurrence of persistent corpus luteum (yellow body) and delayed ovulation were evaluated in clinical trials conducted in the dairy herd at the Norwegian University of Life Sciences. The onset of luteal activity after calving was investigated in cows selected for low milk yield, high milk yield, or the combination of high milk yield and fertility. Cows selected for high milk yield produced more, and had a longer interval to onset of luteal activity than cows selected for low milk yield. Whereas, time to onset of luteal activity in cows that had been selected for both milk yield and fertility was between the two other selection lines. Consequently, the increase in the interval from calving

to onset of luteal activity by selection for high milk yield can be reduced, at least partially, by inclusion of fertility in the breeding program. The energy balance after calving was negatively related to onset of luteal activity, but could not explain the whole difference between the selection lines. Hence, the impairment in reproductive efficiency following selection for high milk yield must be attributable also to factors other than negative energy balance caused by high milk production. Further research is necessary to investigate which factors other than negative energy balance affect the phenotypic difference in days to onset of luteal activity between cows selected for low or high milk production. The occurrence of a persistent corpus luteum and delayed ovulation after calving was lower in the study population of Norwegian Reds than reported for most other dairy populations. Cows experiencing a persistent corpus luteum after calving had shorter intervals from calving to onset of luteal activity. Hence, the inclusion of onset of luteal activity as the only fertility trait in a breeding program may be unwise because of the increased risk of the occurrence of a persistent corpus luteum, rather than continuous cyclic activity, in animals that resume luteal activity too quickly after calving. The occurrence of a persistent corpus luteum was not related to the pregnancy rate after first AI, but cows experiencing a persistent corpus luteum were more likely to be inseminated during the luteal phase. The likelihood of AI during the luteal phase was higher in the study population than in the general population of Norwegian Red. This may, at least partly, explain why the occurrence of persistent corpus luteum was associated with the likelihood of AI during the luteal phase.

When reproductive performance and udder health were compared in conventional and organic dairy farming systems, the interval from calving to first AI was shorter in conventional cows, although no differences were seen in the interval from calving to last AI or the calving interval. Conventionally managed cows were younger, milked more, and received more concentrates than organic cows. Organic cows had lower milk somatic cell count than conventional cows. This difference between the management systems persisted after adjustment for age and milk yield in the statistical models. Higher levels of concentrates are now fed in organic dairying than a decade ago. This is probably an important factor for the higher reproductive efficiency seen today in organic farms compared with previous studies. Consequently, the Norwegian Red is shown to be a sustainable breed that adapts well to both conventional and more extensive production systems, such as organic farming.

SAMANDRAG

Fruktbarheit, helse og brukseigenskapar har vore inkludert i avlsprogrammet for Norsk Rødt Fe (NRF) dei siste 35 åra. I dagens mjølkeproduksjon har krava for den individuelle kua med hensyn på mjølkeproduksjon, fôrinntak og fertilitet auka. Det overordna målet for dei fire studiane i denne avhandlinga var å vurdere fertiliteten til dagens NRF-kyr som eit resultat av eit bærekraftig avlsprogram.

I det første feltstudien undersøkte vi 60 d ikkje omløpsprosent, drektigheitsprosent og kalvingsprosent etter første inseminasjon (AI) hjå kyr og kviger som ikkje var hormonelt behandla føreåt med tanke på å framkalle brunst. Ikkje omløpsprosenten var 72.5%, medan drektigheitsprosent 6 veker etter AI og kavlingsprosenten var 63.7% og 57.2%. Differansen mellom ikkje omløpsprosent og drektigheitsprosent var større hos eldre kyr samanlikna med dei yngre og kviger. Det var ikkje samanheng mellom laktasjonsnummer og drektigheit når kvigene ikkje var inkludert i analysane, men kvigene hadde høgare drektigheitsprosent. Sannsynlegheita for drektigheit etter første AI var ikkje assosiert med tid frå kalving til AI, noko som kan skuldast ei forholdsvis langt intervall frå kalving til første AI (85,3 d). Heller ikkje mjølkeytelse var sett i samanheng med drektigheit på første AI. Den forholdsvise moderate mjølkeproduksjonen og avl for fruktbarheit dei siste 35 åra er sannsynlegvis dei viktigaste grunnane for såpass god fertilitet hjå NRF-kyr.

Det er vist at fleire brunstar før første AI, reduserer antalet AI pr drektigheit slik at tidleg igangsetting av ovarieaktivitet (eggstokkfunksjon) etter kalving er viktig for god fruktbarheit. Dette har ført til avl for tidleg igangsetting av ovarieaktivitet i enkelte land. Igangsetting av ovarieaktivitet og førekomst av persisterande corpora lutea (gule legemer) og forsinka eggløsing blei studert i besetninga på UMB ved hjelp av progesteronmåling i mjølk. Igangsetting av ovarieaktivitet blei studert hos kyr i tre grupper avla for låg mjølkeproduksjon, høg mjølkeproduksjon eller kombinasjonen av høg produksjon og fruktbarheit som er representententative for dagens populasjon av NRF-kyr. Kyr avla for høg produksjon mjølka meir og hadde lenger tid til igangsetting av ovarieaktvitet enn kyr avla for låg produksjon, medan kyrne som var avla både for mjølk og fruktbarheit låg mellom dei to andre gruppene. Energibalansen etter kalving virka negativt inn på igangsetting av ovarieaktivitet, men kunne ikkje forklare heile differansen mellom avlsgruppene og igangsetting av ovarieaktivitet etter kalving. Det er altså andre faktorar som virkar inn på ovarieaktiviteten til stades, men desse blei ikkje studert i dette forsøket. Auka intervall frå kalving til igangsetting av ovarieaktvitet ved avl for høgare mjølkeproduksjon kan bli redusert ved å inkludere fertilitet i avlsprogrammet.

Førekomsten av persisterande corpora lutea og forsinka ovulasjonar (eggløsing) var lågare hjå NRF-kyr samanlikna med det fleste andre populasjonar av mjølkekyr. Kyr med eit persisterande corpus luteum etter kalving hadde tidligare igangsetting av ovarieaktivitet. Det er difor grunn til å vere oppmerksam på å unngå avl for tidleg igangsetting av ovarieaktivitet som einaste mål for fruktbarheit i eit avlsprogram pga større risiko for utvikling av persisterande corpus luteum framfor kontinuerleg syklisk aktivitet. Kyr med eit persisterande corpus luteum etter kalving hadde ikkje lågare sannsynlegheit for å bli drektig på først AI, men sannsynlegheiten for AI i midtsyklus var større for desse kyrne. Ei mogeleg forklaring til det siste kan vere at andelen kyr inseminert i midtsyklus i studiepoulasjonen var høgare enn i NRF-populasjonen generelt.

Når vi samanlikna fruktbarheit og jurhelse i økologisk og konvensjonell produksjon såg vi at dei konvensjonelle kyrne blei inseminert tidlegare, medan det ikkje var nokon forskjell mellom tid til siste AI eller kalvingsintervall. Dei konvensjonelle kyrne var yngre, mjølka meir, og blei fôra med meir kraftfôr enn dei økologiske. Økologiske kyr hadde lågare celletal enn dei konvensjonelle kyrne. Celletalet var også lågare for dei økologiske kyrne når vi hadde justert for alder og produksjon i dei statistiske modellane. Høgare kraftfôrandel til økologiske kyr er sannsynlegvis ein viktig faktor for betre fruktbarheit i økologisk landbruk i dag samanlikna med situasjonen for 10 år sidan. Det kan seiast at NRF er ei bærekraftig rase som tilpassar seg dei krava som blir sett i både konvensjonell og økologisk produksjon.

INTRODUCTION

Since the 1970s, milk yield per cow in the dairy industry has increased rapidly because of intense genetic selection, improved management and better nutrition (Lucy, 2001). Economic considerations have been the main driving force behind increased genetic selection for milk yield (VanRaden, 2004). In recent decades, there has been a worldwide decline in the reproductive performance of dairy cows. Potential factors, such as increased milk production and associated negative energy balance, larger herd size and higher inbreeding percentages, have been suggested as reasons for infertility in dairy cows (Lucy, 2001; Butler, 1998). Reproductive performance of dairy cows is shown to influence the profitability of the herd (Louca and Legates, 1968; Gröhn and Rajala-Schultz, 2000; VanRaden, 2004). Poor fertility increases the insemination costs and involuntary culling rate, reduces the percentage of cows in peak lactation and hence the milk yield, such that the herd's profitability decreases (Louca and Legates, 1968).

The Norwegian Red breed

There have been several studies conducted to investigate the relationship between mastitis resistance and selection lines for milk yield in Norwegian Red cows (Heringstad et al., 2003; 2007; 2008). However, there have been no investigations into the relationships between selection lines and fertility in the breed although reproductive efficiency has been included in the breeding program in the last 35 years.

Currently, 95% of 265,000 dairy cows in Norway are Norwegian Reds or Norwegian Red crossbreds (Geno, 2009a). The average 305-d milk yield per cow year (1 cow year = 365 d for a cow in the herd during one year) has increased from 6,190 kg in 2002 to 6,921 kg in 2008 (TINE Rådgiving, 2009). Over the same time period average herd size has increased from 15.3 to 19.8 cow years. In 2007-2008, 314,541 heifers and cows were presented for first AI (Geno, 2009b).

There has been a long tradition of cooperation between the Norwegian breeding and AI association, Geno, and the Norwegian Dairy Herd Recording System (**NDHRS**). Both Geno and the NDHRS are cooperatives owned, and managed, by 13,200 and 11,794 Norwegian dairy farmers, respectively (Geno, 2009b; TINE Rådgiving, 2009). All of these herds are included in the Norwegian Red breeding program managed by Geno. The NDHRS has operated in Norway since 1975. Data for inseminations are routinely reported to Geno by veterinarians and AI technicians and transferred to the NDHRS. Data from individual health records **(Figure 1**), milk analyses, and AI are recorded on an individual basis, together with

Figure 1. Example of individual health record for dairy cows (online version) in the Norwegian Cattle Health Recording System.

HELSETJENESTEN FOR STORFE

Helsekort ku

ku nr 0835

	Fylke	Kommune	Gard
Produsent-nummer	xx	xx	xxxx

Føres av eier/røkter **Sjukdomsregistrering**

Kalving		Fødsel/fruktbarhet					Produksjonssjukdommer										Registrert av veterinær nr/andre	For Husdyrkontrollen
		Tilbakeholdt etterbyrd 326	Børbetennelse 333	Eggstokkcyster 334	Brunst/omløp <u>331,340,341</u>		Mjølkefeber 386	Ketose 385	Indigestion 260	Speneskader 306	Klinisk mastitt Alvorlig/moderat 303	Klinisk mastitt Mild 304	Andre sjukdommer og forebyggende behandling (se kodelista)		<u>Type behandling</u>			Innrapp.
År	Dato	Dato	Dato	Dato	Dato	Kode	Dato	Dato	Dato	Dato	Dato	Dato	Dato	Kode	Kode	Merkn. om sympt., behandl. m.v.	Nummer	Dato
2004	18.09																	
2005	12.10																	
2006	20.10																	
2007	28.10																	
											03.03				<u>2</u>		5310	10.10.08
													07.07	<u>262</u>	<u>9</u>		5310	10.10.08

Fødselsår og dato 01.10.2002 **Kuas nr og navn** 0835 **Slakta/sjøldau/mista/solgt til liv dato** .... **Årsak** ....

calving information, milk yield records, culling information etc. recorded by the farmers. All diseases and most of the health registrations are made by veterinarians, although in recent years courses have been offered for dairy producers to assist them in making some additional health recordings.

Selection of sires

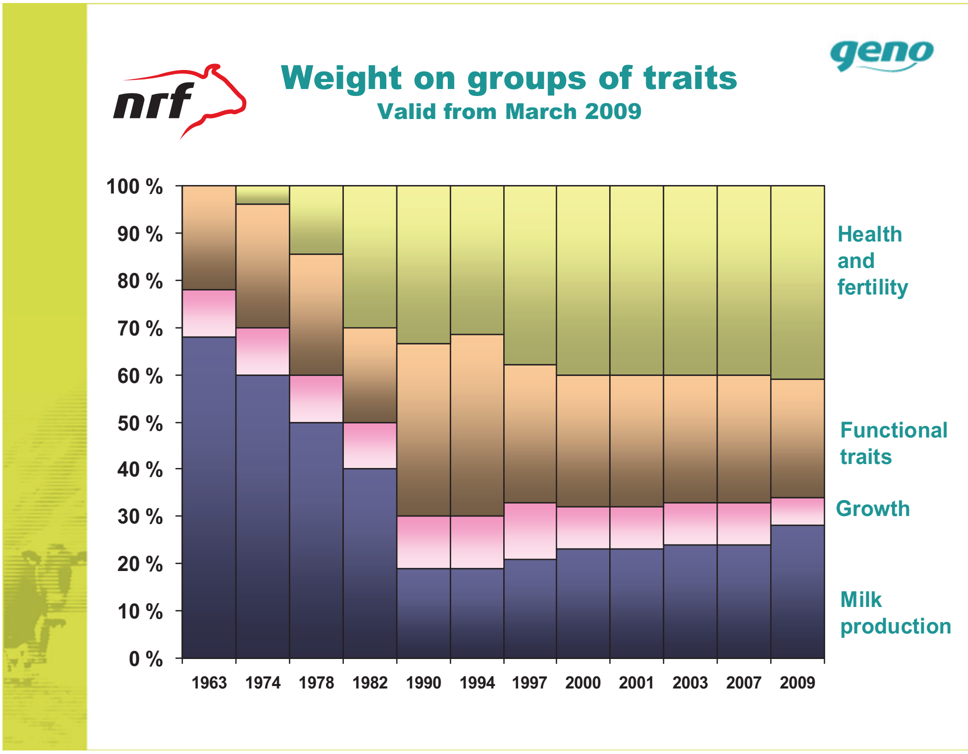
Approximately 330 bull calves are selected from ordinary herds annually, on the basis of predicted breeding values, for testing at the Geno Performance Test Station (Geno, 2009c). The bull calves are evaluated for inclusion into the breeding program on the basis of growth rate, temperament and confirmation parameters in addition to libido and sperm quality. Approximately 130 of these performance tested bulls are selected for semen production and progeny testing. To ensure that these young sires (test bulls) produce enough offspring (daughters) for progeny testing, Geno stipulates that they are used for a minimum of 40% of the AI in each herd. The total merit index for the sires is calculated on the performance of about 250 to 300 daughters. The total merit index has a range from -30 to + 30 with an average value of 0. The 10 to 12 best test bulls are selected as elite bulls based on the progeny test results and individual evaluation. In 2008, the effective population size of the Norwegian Red was 173, inbreeding rate per generation 0.29%, and inbreeding increase per year was 0.06%. The kinship for the elite bull is weighted by 30% in the selection index (Geno, 2009d).

Traits in the total merit index

Currently, 10 traits are included in the total merit index. The following traits (and relative weights) were included in the breeding program in 2008: milk yield (24%), mastitis resistance (22%), fertility (15%), udder (15%), bone confirmation (6%), growth rate (9%), temperament (4%), other diseases: (milk fever, ketosis, retained placenta (3%)), calving ease (1%), and still birth (1%) (Geno, 2009d). Changes of the relative weight in the breeding program for milk production, fertility and health, and functional traits from 1963 to 2009 are given in **Figure 2**.

Female fertility has been included in the total merit index of the Norwegian Red since 1972 (Andersen-Ranberg et al., 2005). The relative emphasis on fertility has been 8 to 15% throughout this period. From 2009 the emphasis on fertility will increase to 18%. Historically, the trait used in selection was the 56 d non return rate of virgin heifers. Since 2002, a combination of 56 d non return rate in heifers and first-lactation cows has been used. Andersen-Ranberg et al. (2005) reported that selection for non return rate has stabilized the non return rate in first lactation cows, but concurrently there has been an undesirable increase in the interval from calving to first AI for first parity cows (Refsdal, 2007). From 2009, the traits used for fertility selection will include the interval from calving to first AI for first to third lactation cows, in addition to 56 d non return rate (Geno, 2009d).

Figure 2. Changes of the relative weights in the Norwegian Red breeding program for milk production, health and fertility, and functional traits from 1963 to 2009.



Mastitis resistance and other diseases have been included in the total merit index since 1978. The relative emphasis on mastitis resistance has been between 7 to 22% and 3 to 9% for other diseases like milk fever and ketosis. The relative emphasis on milk production has decreased from 60% in 1972 to 21% in 1997. In 2009, the relative weight on milk production will be 24% (Geno, 2009d).

Heringstad et al. (2003) reported that it is possible to obtain genetic improvement for clinical mastitis and milk yield simultaneously if the traits are given sufficient weight in selection. It has also been demonstrated that selection against clinical mastitis results in favorably correlated selection responses for ketosis and retained placenta, two of the most frequently reported diseases affecting dairy cows (Heringstad et al., 2007). Non return rate and clinical mastitis are genetically uncorrelated traits, both with antagonistic relationships to milk yield, such that the traits should be included in a breeding program to avoid genetic decline due to selection for increased milk yield (Heringstad et al., 2006).

Reproductive physiology in dairy cows

The mean normal length of the estrous cycle in cows is 21 d with a range of 17 to 24 d (Senger, 2003), whereas the mean duration of estrus is 15 h with a range of 6 to 24 h. The ovulation occurs in late estrus. Fonseca et al. (1983) measured the first cycle post partum to be to 17.1 d, and 21.4 d for consecutive cycles. Royal et al. (2000) reported increased length of estrous cycle from 18.9 d in the 1970s to 22.5 d in the 1990s.

The estrous cycle can be divided into the follicular phase and the luteal phase. The follicular phase is dominated by estrogen produced in the ovarian follicles, whereas the luteal phase that starts after ovulation is dominated by progesterone produced by the corpus luteum. Usually, commencement of luteal activity (**C-LA**) occurs approximately 4 to 5 d after first ovulation post partum, but luteinization of non ovulating follicles can also lead to progesterone production (Corah et al., 1974). In most studies investigating C-LA and progesterone profiles in dairy cows, C-LA is defined to occur when progesterone concentration in plasma or milk exceeds a threshold value. The luteal phase ends with luteolysis (regression of corpus luteum) and decreasing progesterone levels. Luteolysis is induced by $PGF_{2\alpha}$ produced in the endometrium and stimulated by the release of oxytocin from the ovaries and posterior pituitary gland that acts on oxytocin receptors in the endometrium. The precise luteolytic mechanism is not fully understood. The follicular phase commences after the luteolysis. The negative feedback by progesterone on the hypothalamus is removed and gonadotropin releasing hormone is released such that the level of follicle stimulating hormone and luteinizing hormone (**LH**) from the anterior pituitary gland is increasing, promoting follicular development and production of estrogen in the follicles. There are two or three major phases of growth of large follicles during the estrous cycle and the ovulatory follicle is selected approximately 3 d before ovulation (Webb et al., 1999). When the estrogen levels in the dominant follicle peak, the preovulatory LH-surge occurs causing the dominant follicle to ovulate. The ovulation occurs 24 to 30 hours after the LH-surge. In cows, the follicular phase typically lasts for about 4 d, whereas the length of the luteal phase is about 17 d.

Establishment of pregnancy depends on the endocrine communication system between the embryo and mother. The blastocyst produces bovine Interferon τ, a protein that acts on the endometrium to inhibit production of oxytocin receptors, such that oxytocin cannot stimulate $PGF_{2\alpha}$ synthesis and luteolysis (Whates and Lamming, 1995). Sufficient progesterone production in the corpus luteum after fertilization is essential for embryonic development (Mann and Lamming, 1999). The gestation period is approximately 281 d, and the maintenance of pregnancy in cows is dependent on progesterone produced in the corpus luteum and placenta during the whole gestation period.

Decreased pregnancy rate

Traditionally, one calf a year has been the target for good reproductive performance in dairy cows (Louca and Legates, 1968; Call and Stevenson 1985). To achieve this goal, and to avoid decreased production and increased culling rate, the cow has to be pregnant within 90 d after parturition. A decreased pregnancy rate in cows has been reported worldwide (Lucy, 2001). The pregnancy rate to first AI has decreased from 65% in 1951 to 40% in 1996 in New York dairy cows (Butler, 1998) and in 2001 the pregnancy to first AI in US Holstein was at an all time low of 27% (Norman et al., 2009). Since 2002, the pregnancy rate in US Holsteins has stopped declining, and started to improve. In the United Kingdom, the pregnancy to first AI declined from 55.6% to 39.7% between 1975 and 1998 (Royal et al., 2000). To improve fertility in dairy cows, several estrous cycle management systems, involving use of reproductive hormones and timed AI, have been developed (Thatcher et al., 2006). There are few recent studies in which cows were inseminated at spontaneously occurring estrus (Bleach et al., 2004; Andersson et al., 2006; Cairoli et al., 2006) compared with the large number involving induced estrus and timed AI (Lopes-Gatius et al., 2004; Dalton et al., 2005; Howard et al., 2006). The pregnancy rate for timed AI after hormonal induction of estrus is generally lower than AI to a spontaneous occurring estrus, but offer acceptable pregnancy rates without estrus detection in herds with poor breeding management routines (Lucy, 2001).

Non return rate has been regarded as a reasonably accurate indicator of reproductive performance, but it is biased by variation in breeding management and culling practices as well as parity, season of AI, and semen collection (Haugan et al., 2005; Refsdal, 2007). Because only 50% of the cows are pregnancy diagnosed (Reksen et al., 1999a), a reliable estimate of the pregnancy incidence in the Norwegian Red is not available. Internationally, there is increasing interest in importing Norwegian Red semen (Geno, 2009d). Thus, more accurate information about pregnancy incidence and calving rate is required. Additionally, the difference between non return rate and pregnancy incidence may vary between cow parities and should, therefore, be investigated.

Pregnancy diagnosis by rectal palpation and PAG

Pregnancy diagnosis by rectal palpation is the most common way to assess pregnancy. But, pregnancy associated glycoproteins (**PAG**) measured in plasma from around Week 3 of pregnancy can also be used for the same purpose (Zoli et al., 1992). Pregnancy associated glycoproteins constitute a large family of glycoproteins specifically expressed in the outer epithelial cell layer (trophectoderm or chorion) of the placenta (Xie et al., 1997). Plasma PAG concentrations are useful for pregnancy diagnosis 28 d after AI, provided that the time interval between calving and AI is at least 60 d (Haugejorden et al., 2006).

Female Fertility traits

The importance of including fertility related traits in the selection indices has been stressed (Royal et al., 2002; Philipsson and Lindhé, 2003; Chagas et al., 2007), because unfavorable genetic correlations exist between milk yield and reproductive performance (Veerkamp et al., 2001; Royal et al., 2002; Pryce et al., 2004). Currently, female fertility is included in the selection indices of several countries (VanRaden, 2004; Miglior et al., 2005). Fertility traits can be divided into traditional fertility traits and endocrine fertility traits (Royal et al., 2000). Traditional fertility traits, such as calving interval, days to first service, 56 d non return rate, and number of inseminations per conception, have very low heritability (Pryce et al., 1997; Wall et al., 2003; Jamrozik et al., 2005), whereas endocrine fertility traits, which include C-LA, length of first luteal phase, and occurrence of persistent corpus luteum, have moderate heritability (Darwash et al., 1997b; Royal et al., 2002; Petersson et al., 2007). Cows with early C-LA after calving have an increased probability for a short interval from calving to first AI, shorter interval to conception, and higher conception rate such that selection for shorter interval to C-LA has been proposed as a feasible approach to improving fertility (Darwash et al., 1997a; 1997b).

Reproductive performance, milk yield, and energy balance

Multiple ovarian cycles before first AI reduce the number of AI’s per conception (Thatcher and Wilcox, 1973; Darwash et al., 1997a), hence early C-LA is an important constituent of good reproductive performance in dairy cows. High milk yield has been associated with delayed onset of post partum ovarian activity in high yielding cows (Stevenson and Britt, 1979). Other studies, however, have shown no (Villa-Godoy et al., 1987; Patton et al., 2007), or inverse, associations between milk yield and the resumption of ovarian activity (Staples et al., 1990). The nutritional requirements of the cows increase rapidly with milk production after parturition (Butler, 2003) and the negative energy balance (**EB**) in the first 3-4 weeks after calving is highly correlated with milk yield and the interval to first ovulation (Butler and Smith, 1989). The energy balance is most negative in the first and second weeks of lactation (Butler and Smith, 1989). The unfavorable correlation between selection for milk yield and C-LA has been proposed to be caused by a strong association between the length of the anovulatory period post partum and EB (Butler, 2003). Patton et al. (2007) found no associations between milk yield and resumption of ovarian activity, but reported a more negative EB to be associated with later C-LA, and that dry matter intake was the primary component of EB affecting reproduction. Reksen et al. (2001) found that cows with delayed resumption of ovarian activity produced more milk than cows with early and middle responses, suggesting that moderate yielding cows may compensate for energy deficits by decreasing milk yield.

Ovulation of a dominant follicle during estrus is dependent on the reestablishment of LH-secretion from the pituitary gland post partum in addition to estrogen production in the follicle. The physiological state of negative EB post partum impairs the LH-secretion and the ovarian responsiveness to LH, and hence prevents ovulation of the dominant follicle (Butler, 2003). Additionally, low levels of plasma glucose, insulin, and insulin like growth factor I (**IGF-1**) during the period of negative EB are thought to limit the estrogen production of dominant follicles (Butler, 2003). Negative EB early post partum may also impair the oocytes quality during the 80 to 100 d required for follicular development (Britt, 1992).

While the phenotypic correlation between C-LA and EB has been recognized (Reksen et al., 2001; Patton et al., 2007), less is known about the relationship between C-LA and selection for milk yield when phenotypic differences in EB are accounted for. The proportion of the interval between calving and C-LA that is due to differences in EB within and between selection lines can be determined by including EB in the statistical model.

Progesterone profiles post partum and reproductive performance

Several studies have been performed to characterize regular and irregular progesterone profiles in dairy cows post partum (Opsomer et al., 2000; Royal et al., 2000; Shrestha et al., 2004; Petersson et al., 2006; Samarütel et al., 2008). In the United Kingdom, decreased pregnancy rate from 1975 to 1998 has been associated with an increased proportion of atypical progesterone profiles (Royal et al., 2000). Cows with early C-LA have increased probability for early AI, shorter interval to conception, and higher conception rate (Darwash et al., 1997a). However, it has been reported that early C-LA is a risk factor for irregular luteal cyclicity in the form of a persistent corpus luteum (**PCL**) in the Holstein (Opsomer et al., 2000; Royal et al., 2002; Petersson et al., 2007), and that PCL is associated with a decrease in pregnancy to first AI (Royal et al., 2000; Shrestha et al., 2004). Hence, the relationship between time to C-LA, occurrence of PCL and relationship between PCL and pregnancy should be investigated in the Norwegian Red.

Sustainability and reproductive performance

Organic agriculture is defined as a holistic production management system which promotes and enhances ecosystem health, including biological cycles and soil biological activity. The primary goal for organic agriculture is to optimize the health and productivity of inter-dependent communities of soil life, plants, animals and people (FAO and WHO, 2008).

Because the breeding program for the Norwegian Red has taken into consideration fertility, health and functional traits for a long period of time, the breed may be regarded as suitable for organic farming. During the last ten years, three Norwegian studies have been conducted to investigate differences in reproductive performance and herd health (Reksen et al., 1999b; Hardeng and Edge, 2001; Valle et al., 2007) between conventional and organic farming. Reksen et al. (1999b) found lower milk yield and impaired reproductive performance in organic cows, probably due to limited energy intake. Calving season was also different between the management systems. Hardeng and Edge (2001) reported less disease treatment against mastitis, ketosis, and milk fever in organic herds, and no difference in somatic cell count between organic and conventional herds. Valle et al. (2007) reported lower incidence of acute mastitis in organic herds, which could partly be explained by the lower production level on organic farms. Hence, differences in reproductive performance and udder health in organic and conventional farming need to be further elucidated.

OBJECTIVES FOR THE STUDY

1. Estimate of overall and parity specific 60 d non return rate, pregnancy incidence and calving rate to first AI after spontaneous estrus in Norwegian Red cows **(Paper I)**.

2. Assess the relationship between pregnancy and management factors at herd or cow level **(Paper I)**.

3. Compare the accuracy of pregnancy detection by rectal palpation with PAG analysis supported by progesterone measurements **(Paper I)**.

4. Quantify the differences in C-LA, milk yield, and EB between selection lines for low milk yield, high milk yield and high index for both milk yield and fertility. Further, to assess how much of the differences in C-LA could be accounted for by the phenotypic differences in EB **(Paper II)**.

5. Quantify the occurrence of typical and atypical progesterone profiles in Norwegian Red cows **(Paper III)**.

6. Assess the relationship between time to C-LA and the occurrence of PCL and to investigate cow and management specific factors related to the occurrence of PCL **(Paper III)**.

7. Investigate differences in reproductive performance and udder health of Norwegian Red cows in conventional and organic farming **(Paper IV)**.

MATERIALS AND METHODS

Study designs and study populations

All herds in the field studies described in **Papers I** and **IV** in addition to the herd used in **Papers II** and **III** were members of the NDHRS, such that information about calving date, AI date, AI personnel, age at AI, parity, bimonthly somatic cell count, monthly milk yield, monthly concentrate allocation, disease treatment, culling date, culling reason, herd size, herd characteristics, and pedigree could be retrieved from the NDHRS files.

The study described in **Paper I** was a longitudinal field study conducted from October 2004 to October 2005. There were 19 AI technicians and veterinarians in eastern Norway (Oppland, Hedmark, and Akershus) that participated in the study. The technicians and veterinarians were selected for inclusion on the basis of location within a short geographical distance from the Norwegian School of Veterinary Science and Geno. The study included 829 heifers and cows from 308 herds.

The studies described in **Papers II** and **III** were conducted as clinical trials in the dairy herd at the Norwegian University of Life Sciences. The cows were managed in three feeding trials designed to assess the effects of different levels of concentrate and different qualities of roughage on milk yield, energy balance, and reproductive performance. In **Paper II**, milk samples for progesterone measurements were obtained in 268 lactations from 147 cows during the period from 1994 to 2001. In **Paper III**, milk samples for progesterone measurements were obtained in 502 lactations from 302 cows during the periods from 1994 to 2001 and from 2005 to 2008.

The study described in **Paper IV** was a prospective cohort study conducted in 25 conventional and 24 organic herds. The conventional herds were selected to be similar to the organic herds according to the following criteria: localized in the same county or dairy district, similar herd size (± five cow-years), and same barn type (free stall or tied stall). For the investigation of differences in reproductive performance (calving interval, interval from calving to first AI and last AI) between the management systems, 3209 lactations from 2093 cows were included in the analyses. At the herd visits in 2006, quarter milk was sampled from 1010 cows (523 conventional and 487 organic) for bacteriological examination. Data from the herds and individual animals were obtained from the NDHRS from 2005 through 2007.

Statistical analysis

Statistical analyses were performed using SAS, version 9.1 (SAS Institute, Cary, NC) and SPSS, version 15.0 (SPSS Inc., Chicago, IL).

Table1. Overview of statistical analysis used in Papers I through IV.

Main Model	Program	Paper	Outcome	Type of outcome variable	Cluster (random effects)	Correlation structure
Logistic regression	SPSS	Paper I	Pregnancy to first AI (1 or 0)	Dichotomous (1/0)	---	---
General estimating equation (GEE)	SAS, Proc Genmod	Paper I	Likelihood of pregnancy (1 or 0)	Dichotomous (1/0)	Herd (cows within herd)	Exchangeable symmetry
Mixed linear models	SAS, Proc Mixed	Paper II	EB Milk yield Interval to C-LA	Continuous	Repeated within cows, random within concentrate allocation	Compound symmetry
Mixed linear models	SPSS	Paper III	Interval to C-LA	Continuous	Repeated within cows, random within concentrate allocation	Compound symmetry
General estimating equation (GEE)	SPSS	Paper III	Persistent CL, Pregnancy to first AI, First AI in luteal phase	Dichotomous (1/0)	Repeated within cows	Exchangeable symmetry
Mixed linear models	SPSS	Paper IV	CFAI, CLAI, Calving interval	Continuous	Repeated within cow	Compound symmetry
Mixed linear models	SPSS	Paper IV	Test-day milk yield, Somatic cell count Concentrate allocation	Continuous	Repeated within lactation	First order autoregressive
Generalized linear models	SPSS	Paper IV	Mastitis bacteria	Dichotomous (1/0)	---	---

Mixed linear model analyses with repeated measures within cows were performed in **Papers II**, **III**, and **IV**, and repeated measures within lactation in **Paper IV (Table 1)**. Herd was entered as a random effect in the mixed models in **Paper I**, whereas concentrate allocation was entered as a random effect in the mixed models in **Papers II** and **III.** Multiple comparison adjustment for the pair wise difference in least square means was performed using the Bonferroni option. The outcome variables days to C-LA (**Papers II** and **III)**, the interval from calving to first AI (**CFAI**) and last AI (**CLAI**) (**Paper IV**), and somatic cell count (**SCC**) (**Paper IV**) were transformed by their natural logarithm to obtain approximate normality of residuals. The statistical models were evaluated according to the diagnostics described for linear and logistic regression by Dohoo et al. (2003). Statistical significance was considered at $P < 0.05$ for all analyses.

Paper I

The outcome variable, likelihood of pregnancy, was tested against the following explanatory variables both in the univariate logistic regression models and GEE analyses: milk yield, heifer's age, CFAI, parity, season, double AI, housing, personnel performing AI, and herd size. The univariate analyses were performed both simultaneously for all parities and separately for cows and heifers.

Paper II

1) The outcome variables, EB and milk yield for each of the 12 first weeks post partum, were tested against the selection lines for low milk yield (**LMP**), high milk yield (**HMP**), high index for milk yield and fertility (**HI**), using mixed linear models.

2) The outcome variable, days to C-LA, was tested against the following explanatory variables: selection lines for milk yield, parity and EB using mixed linear models.

Paper III

1) The outcome variable, days to C-LA, was tested against the following explanatory variables: milk yield, parity, year, housing, disease treatment before C-LA, and occurrence of PCL, using mixed linear models.

2) The outcome variable, PCL, was tested against the following explanatory variables; milk yield, concentrate allocation, year, parity, housing, DOV, and disease treatment before C-LA, using the GEE approach.

3) The GEE approach was also used to test the outcome variables likelihood of pregnancy to first AI and likelihood of first AI during the luteal phase against the explanatory variable PCL (PCL type I, type II or the combination).

Paper IV

1) The three outcome variables CFAI, CLAI and calving interval were tested against the explanatory variables management, parity, milk yield, barn type, and season, using mixed linear models. The effect of breeding management (natural mating or AI) on calving interval was also assessed. The outcome variables were also assessed with the interactions management system by parity, parity by season, management system by season, barn type by season, barn type by management system, and barn type by parity.

2) Lactation curves for test-day milk yield, test-day SCC and test-day concentrate allocation were constructed. The lactation curves were expressed through inclusion of weeks in milk (**WIM**) and the natural logarithm of WIM (**lnWIM**) as described by Wood (1967) and

Reksen et al. (2007; 2008). The explanatory variables management system and parity were included in the respective models. In addition, test-day milk yield was included in the model expressing the lactation curve for the natural logarithm of SCC. Interactions considered as biologically important such as WIM by management system, WIM by parity, lnWIM by management system, and lnWIM by parity were included in the respective models.
3) The relationship between milk samples positive for mastitis bacteria at the herd visit and the explanatory variables management system, milk yield, and parity were tested using multiple linear regression. Intra-mammary infection was considered to be present when an udder pathogen was isolated from at least one quarter.

For all multivariable tests in **Papers I to IV** the backward elimination procedure was employed. Explanatory variables with $P > 0.05$ were omitted from the models.

Laboratory analysis

Progesterone analysis

Milk samples for progesterone analysis were obtained in a representative manner during the milking in the trials conducted at the Norwegian University of Life Sciences (**Papers II** and **III**). In **Paper I** milk was collected on the day of AI and 21 d after AI by the veterinarian, AI technicians, or the farmers. In **Paper II** milk samples for progesterone analysis were collected three times a week (Monday, Wednesday, and Friday), and in **Paper III** two (Monday and Friday) or three (Monday, Wednesday, and Friday) times a week. Progesterone concentrations in whole milk were measured by an enzyme immunoassay (Waldmann, 1993), modified by using the second antibody coating technique.

PAG analysis

Blood samples for PAG (**Paper I**) were collected approximately six weeks after AI, if the animal did not return to estrus. A heterologous double antibody radioimmunoassay, modified from the method previously described by Zoli et al. (1992), was used to determine PAG concentrations in the plasma of cows.

Bacteriological analysis of milk samples

The quarter milk samples were analyzed for growth of microorganisms (**Paper IV**) in accordance with the official procedure in Norway which is based on the International Dairy Federation guidelines (International Dairy Federation, 1981; 1987).

Energy Requirements

Dry matter intake (**DMI**) (**Paper II**) was calculated as dry matter offered, minus dry matter refused, on three consecutive days in a week. Energy content in dry matter and energy requirements were calculated according to the Norwegian feeding value per unit of milk (**FUM**) system (Ekern, 1991), in which 1 FUM is equal to 6.9 MJ net energy lactation for milk production. Energy intake was calculated as FUM_{intake} = DMI x food FUM concentration. Energy requirements are dependent upon maintenance, growth and milk production according to the following formula (Van Der Honing and Alderman, 1988):

Energy requirements = $FUM_{maintenance} + FUM_{milk} + FUM_{growth}$

Energy balance in FUM was estimated as the difference between energy intake and energy requirements for maintenance, growth in first lactation cows, and milk production.

Selection lines and breeding values

LMP cows

Cows in the LMP selection line were bred to a group of 11 sires that were progeny tested in 1978 and 1979 with estimated breeding values of 92.0 (SD ± 3.7) for milk yield and 102.0 (SD ± 10.6) for fertility.

HMP cows

The HMP cows were sired by the three to four highest ranking proven sires (total 22 sires) for milk production from the most recent group of progeny tested Norwegian Red sires each year. The estimated breeding values for the sires were 111.7 (SD ± 4.1) for milk yield and 102.4 (SD ± 8.3) for fertility.

HI cows

The HI cows were sired by the progeny tested, top-ranked Norwegian Red bulls (elite bulls), both before commencement of the study and until the end of study in 2001. The estimated breeding values for the sires were 109.0 (SD ± 5.6) for milk yield and 102.9 (SD ± 6.9) for fertility.

Definitions of C-LA, Luteal Phase, Inter-ovulatory Interval, DOV, and PCL

C-LA

The interval from calving to C-LA (**Papers II and III**) was defined as the first day of the two consecutive measurements of milk progesterone concentration ≥ 3ng/mL (Royal et al., 2000: Petersson et al., 2007) not earlier than 10 d after calving.

Luteal phase

The length of first luteal phase (**Paper III**) was measured as the time from the first sample of milk progesterone ≥ 3 ng/mL to the last consecutive progesterone sample ≥ 3 ng/mL (Royal et al., 2000).

Inter ovulatory interval

The length of the inter-ovulatory intervals (**Paper III**) was considered to be a measurement of the total estrous cycle length, and was defined as the interval between the milk progesterone rise (≥ 3 ng/mL) in one estrous cycle to the rise of milk progesterone (≥ 3 ng/mL) in the next cycle (Royal et al., 2000).

Delayed ovulation

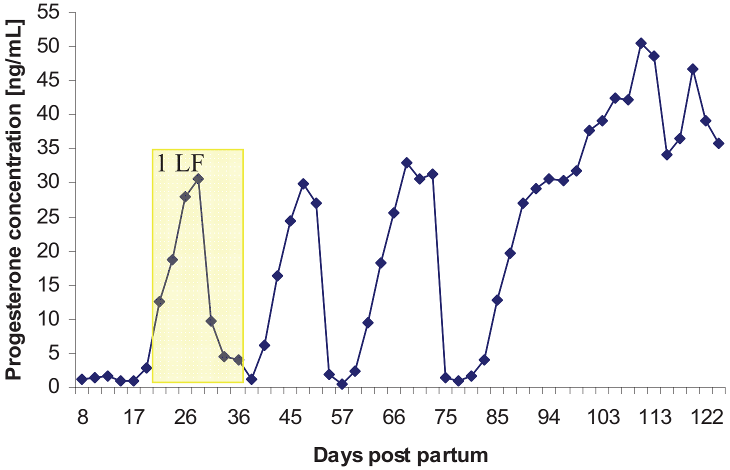
Delayed ovulation type I (**Paper III**) was defined as consistently low progesterone concentration (< 3ng/mL) for ≥ 50 d post partum. Delayed ovulation type II was defined as prolonged inter-luteal interval with milk progesterone measurements < 3 ng/ mL for ≥ 12 d between two luteal phases (Royal et al., 2000: Petersson et al., 2007).

Persistent corpus luteum

Persistent corpus luteum type I (**Paper III**) was defined as delayed luteolysis during the first estrous cycle post partum with milk progesterone ≥ 3 ng/mL for ≥ 19 d, whereas PCL type II was defined as delayed luteolysis with milk progesterone ≥ 3 ng/mL for ≥ 19 d during subsequent estrous cycles prior to AI (Royal et al., 2000: Petersson et al., 2007).

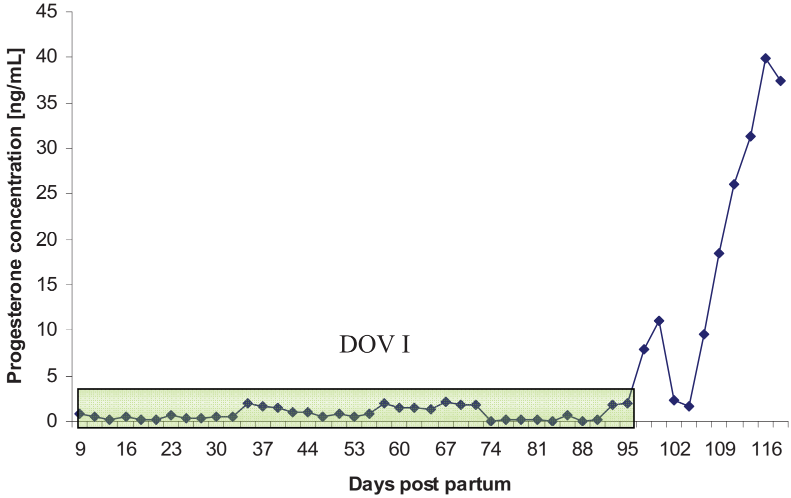
Examples of progesterone profiles

1. Normal profile



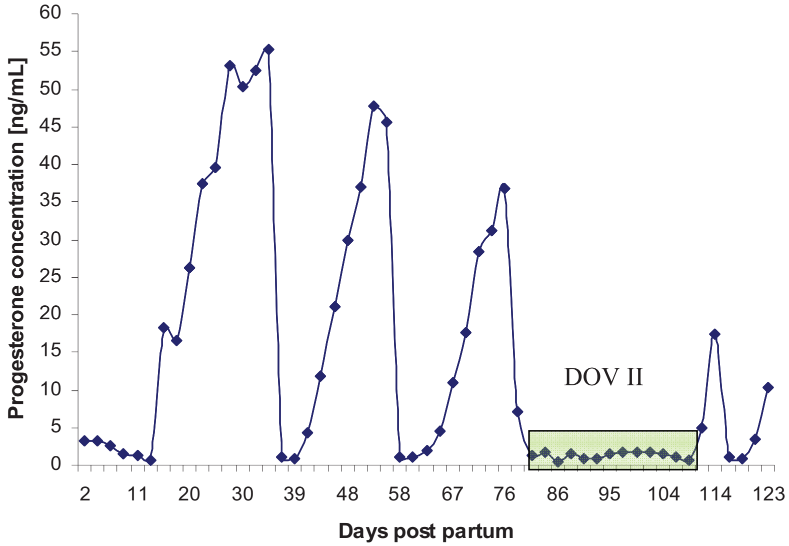
Normal profile with C-LA 21 d post partum. First luteal phase 14 d, second luteal phase 10 d, and third luteal phase 12 d. First inter-ovulatory-interval 18 d, second inter-ovulatory interval 21 d, and third inter-ovulatory interval 21 d. Pregnant after first AI 77 d post partum.

2. DOV type I



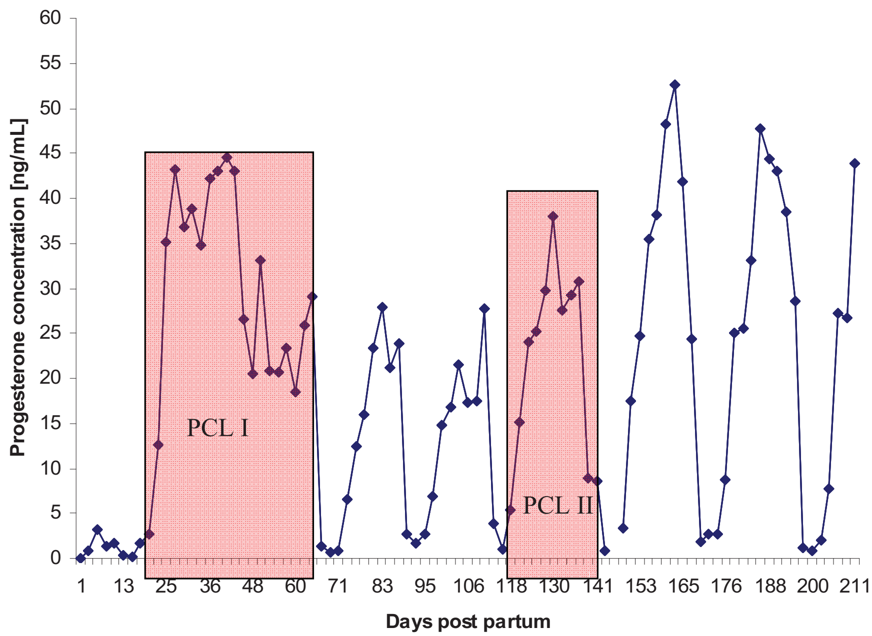
Cow with DOV type I. C-LA 97 d post partum. Pregnant at 103 d post partum.

3. DOV type II



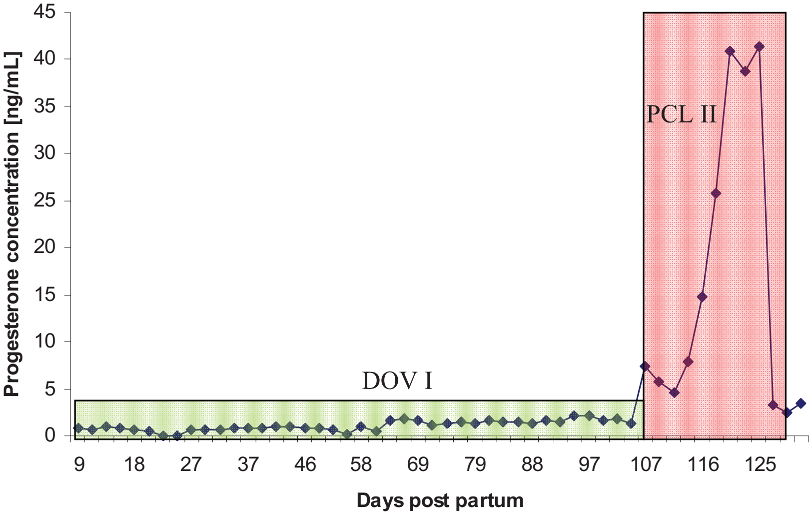
Cow with DOV type II for 28 d.

4. PCL type I and PCL type II



Cow with C-LA 22 d post partum, length of PCL type I 42 d and PCL type II 23 d.

5. DOV type I and PCL type II



Cow with both DOV type I and PCL type I. The interval to C-LA was 107 d and the PCL type I lasted for 21 d.

MAIN RESULTS

Paper I. Pregnancy Incidence in Norwegian Red Cows Using Nonreturn to Estrus, Rectal Palpation, Pregnancy Associated Glycoproteins and Progesterone

The interval from calving to first AI for the cows was 85.3 d. The overall 60 d non return rate after first AI was 72.5%. The corresponding values for heifers, 1st lactation, 2nd lactation, and > 2nd lactation were 76.9, 67.1, 69.9, and 76.2%. Overall pregnancy incidence after first AI was 63.7%. The corresponding values for heifers, 1st lactation, 2nd lactation, and > 2nd lactation cows were 70.0, 58.2, 61.6, and 64.9%, respectively. Overall calving rate to first AI was 57.2%. The corresponding values for heifers, 1st lactation, 2nd lactation, and >2nd lactation were 64.9, 54.3, 54.7, and 53.9%, respectively. The overall difference between 60 d non return rate and pregnancy incidence was 8.8%, whereas the parity specific differences were 6.9, 8.9, 8.3, and 11.3% for heifers, 1st lactation, 2nd lactation, and > 2nd lactation cows, respectively. Parity number did not significantly affect pregnancy to first AI when heifers were excluded from the analysis. Milk yield and CFAI were not associated with the pregnancy incidence to first AI. Pregnancy associated glycoprotein concentrations equal to 2.5 ng/mL gave highest sensitivity (94.3%) and specificity (94.6%) in the assessment of pregnancy. Eight animals with PAG < 2.5ng/mL and classified as pregnant by rectal palpation calved, while 5 animals with PAG ≥ 2.5 ng/mL and classified as non pregnant by rectal palpation also calved.

Paper II. Commencement of Luteal Activity in Three Different Selection Lines for Milk Yield and Fertility in Norwegian Red Cows

The cows included in this study were selected for low genetic merit for milk yield (LMP), high genetic merit for milk yield (HMP), and a combination of high indices for milk yield and fertility (HI). The HMP cows had longer interval to C-LA than LMP cows. The interval from calving to C-LA was 22.5, 30.4, and 27.2 d for LMP, HMP, and HI cows, respectively. The interval to C-LA decreased for the HMP and HI cows after phenotypic adjustment for EB in the model. In a multivariable model that included parity, selection lines, and EB as covariates, the intervals to C-LA were 23.2, 29.7, and 25.6 d for the LMP, HMP, and HI cows, respectively. Cumulative EB during the first 4 weeks of lactation, which itself differed between selection lines, did not fully account for differences in interval to C-LA between selection lines. Thus, the results of the present investigation indicate that selection for milk yield impacts negatively on C-LA over and above the effects caused by concurrent changes in EB. The increase in days to C-LA caused by selection for high yields can be reduced if selection for milk yield is combined with fertility in the breeding program.

Paper III. Characterization of Progesterone Profiles in fall-calving Norwegian Red Cows

Progesterone profiles in Norwegian Red cows were categorized, and associations between the occurrence of irregularities in the profiles and C-LA were investigated. Delayed ovulation type I was present in 14.7%, DOV type II in 2.8%, PCL type I in 6.7%, and PCL type II in 3.3% of the profiles. The occurrence of DOV and PCL in Norwegian Red cows was lower than reported in most other dairy populations. Onset of luteal activity was related to milk yield, parity, and PCL. Cows experiencing a PCL had shorter interval to C-LA post partum. The LS-mean for the interval to C-LA was 24.2 d when PCL type I and II were present, and 29.5 d when PCL type I and II were absent. The likelihood of pregnancy to first service was not affected in cows with a history of PCL when AI was carried out at progesterone concentrations < 3 ng/ml (i.e. during estrus); however, cows that had experienced PCL were more likely to be inseminated during a luteal phase. The frequency of puerperal problems was low and the likelihood of PCL was not associated with disease treatment before C-LA.

Paper IV: Reproductive Performance, Udder Health, and Antibiotic Resistance in Mastitis Bacteria isolated from Norwegian Red in Conventional and Organic Farming.

Reproductive performance, udder health, and antibiotic resistance in udder pathogens in conventional and organic farming were investigated. The interval from calving to first AI was shorter in conventional cows (LS-means: 75.0 vs 77.7 d), whereas there was no difference in the interval to last AI and calving interval between conventional and organic cows. Natural mating was more common in organic farming. The conventional cows yielded more and received more concentrates than organic cows during the whole lactation. There were fewer > 2nd parity cows in conventional farming. The average annual forage: concentrate ratio was 75:25 and 63:37 for organic and conventional herds, respectively. Although adjusted for milk yield and parity, somatic cell count was lower in organic cows than conventional cows. There were a higher proportion of dry quarters at the herd visit in organic herds. There was no difference between conventional and organic cows in quarter samples positive for mastitis bacteria from the herd visit. Milk yield and parity were associated with the likelihood of at least one quarter positive for mastitis bacteria (subclinically infected).

DISCUSSION

In **Paper I**, the pregnancy incidence, non return rate and calving rate in Norwegian dairy cows were investigated. The results showed that the reproductive efficiency in Norwegian Red cows is high compared with previous and comparable studies in dairy cattle (Bleach et al., 2004; Andersson et al., 2006; Cairoli et al., 2006), and superior compared with AI after induced estrus or timed AI (Lopez-Gatius et al., 2004; Dalton et al., 2005; Howard et al., 2006). Even though the animals included in the study were not selected randomly, the cows included in the study may be considered as representative for Norwegian Red cows. This is supported by similar 60 d non return rates in the current study (71.8%) and the average 60 d non return rate in Norwegian Red cows for the same period (72.7%) (Refsdal, 2007). In **Paper I**, 3.6% of the animals were culled due to low fertility ≤ 290 d after first AI. In **Paper IV**, the overall culling rate was slightly higher in conventional herds compared with organic herds.

Differences in C-LA between selection lines

To our knowledge, the experiment in **Paper II** is the first to evaluate and compare C-LA in cows selected for low milk yield, high milk yield, or the combination of high indices for milk yield and fertility. In previous experiments (Westwood et al., 2000; Gutierrez et al., 2006) and population studies (Royal et al., 2000) of differences in reproductive performance between cows with high and low genetic merit for milk yield, contemporary sires with breeding indices for high and low milk yield have been used. By managing the cows in a single herd, phenotypic variation resulting from different management and feeding practices was minimized. The estimated breeding value for fertility was similar for the sires used on the HMP (102.4) and HI (102.9) cows **(Paper II).** However, after adjustment for the phenotypic differences in EB, there was still a difference in days to C-LA of 6.5 and 2.4 d for the HMP and HI cows, respectively, compared with the LMP cows. Hence, the reduction in reproductive efficiency following selection for high milk yield must also be attributable to other factors than negative EB because of high milk production.

It has been shown that cows with a high genetic merit for milk yield mobilize more body tissue in early lactation than cows with average merit for milk yield on a genetic (Pryce et al., 2001), and phenotypic level (Westwood et al., 2000; Pryce et al., 2001). The heritability for body condition score (**BCS**) is high (0.38) (Veerkamp et al., 2001), and there is a strong and negative genetic correlation between BCS and milk yield (Veerkamp et al., 2001; Wall et al., 2003) which may lead to greater negative EB and reduction of fertility in cows selected for high milk yield. Westwood et al. (2000) reported that mean BCS and pattern of BCS changes differed between high and low yielding cows because high genetic merit cows mobilized more body tissue in early lactation than cows with a lower production potential. Fall et al. (2008) and Reksen et al. (2001) have previously reported that organic cows and moderate yielding cows adjust milk production according to energy intake.

Previous phenotypic studies reported that cows with higher plasma concentrations of IGF-1 had an increased likelihood of a shorter interval to C-LA, whereas plasma concentrations of insulin, glucose, non-esterified fatty acids, and beta-hydroxybutyrate were not associated with C-LA (Patton et al., 2007). Westwood et al. (2000) reported higher concentrations of growth hormone in cows with high genetic merit for milk yield, whereas Gutierrez et al. (2006) found higher concentrations of growth hormone and beta-hydroxybutyrate, and lower concentrations of insulin, glucose, and IGF-1, in cows with high genetic merit for milk yield. Hayhurst et al. (2007) reported that the amounts of free fatty acids and glucose in male calves were moderately heritable and could be used to enhance selection for improved female fertility. Calves that mobilized large amounts of free fatty acids tended to have female offspring with reduced fertility. Thus, selection for milk yield could lead to lower levels of IGF-1, more body tissue mobilized and hence higher level of free fatty acids leading to reduced fertility such that the interval from calving to C-LA is increasing.

It should be emphasized that further research is necessary to investigate which factors other than negative EB affect the phenotypic difference in days to C-LA between cows selected for low or high milk production.

Milk yield, EB, and reproductive performance in Norwegian Red

The Norwegian Red is characterized as a moderate yielding breed with an average 305-days milk yield of 6,921 kg in 2008 (TINE Rådgiving, 2009). The genetic potential for milk yield in the breed has probably not been fully exploited because of constraints on milk production due to quota regulations and political framework present in Norway (Refsdal, 2007; Steine et. al., 2008). The lack of association between milk yield and the likelihood of pregnancy (**Paper I**) could be because of moderate milk yield combined with selection for fertility, health and functional traits in the breeding program.

The cows selected for high milk yield (**Paper II**) had a longer interval from calving to C-LA than cows selected for low milk yield, whereas the interval to C-LA was intermediary in cows selected for both yield and fertility. The present thesis also show that less selection pressure for milk yield and more weight on fertility in a breeding program does not necessarily reduce milk yield substantially, despite the fact that an increase in C-LA per unit of predicted transmitting ability for milk yield is expected in high producing cows (Royal et al., 2002). There was a strong association between C-LA and EB in the models with and without selection lines included. The interval to C-LA was reduced for the HMP and HI cows after adjustment for phenotypic differences in EB. This is in agreement with the unfavorable genetic correlations between milk yield and fertility reported in previous studies both at a phenotypic level (Gutierrez et al., 2006; Patton et al, 2007), and a genetic level (Veerkamp et al., 2001; Royal et al., 2002; Pryce et al., 2004), because the degree of negative EB stems from the milk yield in early lactation and hence influences the interval to C-LA.

The organic cows (**Paper IV**) were older, milked less, had lower SCC, and were fed less concentrate than conventional cows during the whole lactation period. The CFAI interval was shorter in conventionally managed cows, whereas there were no differences in calving interval, days open, or CLAI between the management systems. Reksen et al., (1999b) found lower milk yield and impaired reproductive performance, due to limited energy intake, in Norwegian organic cows. At that time, the energy requirement provided by concentrate for most organic herds was 20% because a maximum of 20% of the feed could be non organic in origin and the production of organic grain was limited. Currently, 60% of the energy fed on a daily basis in organic farming should be roughage (Mattilsynet, 2007). The average annual forage: concentrate ratio was 75:25 in organic herds and 63:37 in conventional herds. Reksen et al. (2001) reported that Norwegian Red cows were able to maintain ovarian activity by decreasing milk yield when the forage to concentrate ratio was 75:25 or lower. Milk yield was not associated with any of the fertility parameters investigated in the study. This could be because organic cows decreased milk yield according to their energy intake and compensated for the energy deficit post partum by reducing milk yield and minimizing the deleterious effects of negative energy balance on reproductive performance (**Paper IV**). Hence, the suggestions presented by Reksen et al. (1999b) that the level of concentrate fed should be increased to ensure optimal feeding of the cows in accordance with their individual needs is supported. The ability to reduce production in the current lactation without compromising reproductive performance means that the breed is adaptable to extensive production systems like organic farming.

Associations between C-LA and PCL

In **Paper II,** it was concluded that the increase in the interval from calving to C-LA by selection for high milk yield can be reduced by inclusion of fertility traits in the breeding program. Selection for shorter intervals to C-LA, which has a moderate heritability, may be a feasible approach to improving fertility (Darwash et al., 1997b). However, despite obtaining a shorter interval to C-LA by the inclusion of fertility as non return rate in the breeding program, inclusion of C-LA as the only fertility trait may be unwise because of the increased risk of a PCL rather than continuous cyclical activity after early C-LA (Opsomer et al., 2000; Royal et al., 2002; Petersson et al., 2007).

The interval from calving to C-LA was associated with the occurrence of PCL (**Paper III**) in the present study. Heritability for early C-LA is reported to be 0.16 to 0.21 (Darwash et al., 1997b; Royal et al., 2002; Petersson et al., 2007). Breeding for increased fertility is based on traditional fertility traits such as non return rate, calving interval, CFAI, and number of inseminations per conception, which have heritabilities that range between 0.02 and 0.04 (Pryce et al., 1997; Wall et al., 2003; Jamrozik et al., 2005). The interval to C-LA was approximately 5 d shorter in cows that subsequently experienced a PCL, which demonstrates the importance of elucidating the potential negative side effects of breeding for early C-LA.

Cows and management factors related to PCL

The occurrence of PCL (**Paper III**) was generally low in the Norwegian Red compared with studies performed with other dairy breeds (Opsomer et al., 1998; Royal et al., 2000; Shrestha et al., 2004; Samarütel et al., 2008). Whether this is related to the inclusion of female fertility in the breeding program since 1972 is unknown (Andersen-Ranberg et al., 2005; Steine et al., 2008).

No associations could be found between the occurrences of PCL and milk yield, concentrate allocation, parity, year of trial, or disease before C-LA or DOV **(Paper III).** Windig et al. (2008) could not find an association between prolonged luteal activity and milk production. Whereas Pollott and Coffey (2008) reported a higher than expected number of PCL type II in cows fed high levels of concentrate, while genetic line did not affect the occurrence of PCL type I or type II. Opsomer et al. (2000) reported parity to be a risk factor for prolonged luteal phases, and the most important risks for delayed luteolysis were early C-LA, occurrence of metritis, retained placenta, calving problems, and abnormal vaginal discharge. Petersson et al. (2006) also reported endometritis to be a risk factor for PCL. The low occurrence of PCL (**Paper III**) may partly be explained by the very low number of puerperal problems in the study population. The incidence rates per 100 cow-years in 2005 were 1.2, 3.1, 0.2, and 0.9 for dystocia, retained placenta, abortion, and metritis/endometritis, vaginitis and salphangitis, respectively (Østerås et al., 2007). The incidence rates per 100 dairy cow years treated for reproductive disorders were 1.71, 0.59, 1.12, 1.01, and 0.20 for anestrus, estrus synchronization, cystic ovaries, silent estrus, and silent repeated breeding, respectively (Østerås et al., 2007).

Occurrence of PCL and pregnancy rate

A previous episode of PCL (**Paper III**) was not associated with the likelihood of pregnancy to first AI in cows that were inseminated while having low progesterone levels. The latter contrasts with both Royal et al. (2000) who reported PCL to be associated with a decrease in pregnancy to first AI, and Shrestha et al. (2004) who reported lower pregnancy rate within 100 DIM. Cows in our study that experienced a PCL were more likely to be inseminated during the luteal phase, which may explain the results obtained in previous studies of associations between pregnancy rate and PCL by Royal et al. (2000) and Shrestha et al. (2004).

In the field study in **Paper I**, AI during the luteal phase was performed in 4.9% of the cows, which supports Andresen and Onstad (1979) and Grimard et al. (2006) who reported 4.4% and 5.0% AI in the luteal phase, respectively. Cairoli et al. (2006) found that 10.2% of the cows showing spontaneous estrus were inseminated at high progesterone concentrations. In **Paper III,** first AI was performed during the luteal phase in 12.4% of the lactations. Thus, the likelihood of AI during the luteal phase was higher in the population studied for **Paper III** than in the general population. This may also, at least partly, explain why the occurrence of a PCL was associated with the likelihood of AI during the luteal phase.

Selection traits for fertility included in breeding programs

Petersson et al. (2007) suggested using C-LA instead of CFAI in the selection index because there is only a delay of 4 to 5 d between C-LA and first ovulation post partum, and both factors are determined by individual physiology whereas CFAI interval is affected by management factors. As reported in **Paper III**, cows experiencing a PCL had shorter interval to C-LA. So far the trait for fertility selection in Norwegian Red has been non return rate. From 2009, CFAI will also be included in the selection index (Geno, 2009d). The CFAI interval has increased in Norwegian Red which could be due to longer voluntary waiting periods, but also may be the result of changes in estrus detection routines (Refsdal, 2007). The

CFAI in **Paper I** was longer than reported by Royal et al. (2000), but shorter than reported by Norman et al. (2009) in US Holsteins of 92 d in 1996, decreasing to 85 d in 2007.

C-LA and other endocrine fertility traits, e.g. length of first luteal phase or occurrence of PCL, in the selection index may have higher heritabilities than traditional fertility traits, but selection for these traits would exclude ordinary dairy herds from the breeding program. The breeding work in the Norwegian Red has so far been based on data reported to NDHRS from ordinary herds. Data for the recording of endocrine fertility traits have been based on progesterone measurement which would be extremely difficult to collect from ordinary herds. Hence, the sustainability of the breed may be reduced, because herds selected for inclusion in the breeding program could be biased and not representative of the population as a whole. Furthermore, the risk of inbreeding may increase because effective breeding population size would be reduced.

The use of non return rate as an indication of pregnancy overestimates pregnancy incidence. In **Paper I** the difference between 60 d non return rate and pregnancy incidence was 6.9% for virgin heifers and 11.3% for > 2nd lactation cows. Hence, the non return rate was subjected to more bias in older cows than in heifers. It is important to be aware of this difference when future breeding indices are based on non return rate of both heifers and older cows. A likely explanation of this bias is that older cows are more likely to be culled if non pregnant after first AI. However, culling of older cows (> 2nd parity) before 60 d after first AI was not an important reason for lower pregnancy incidence in **Paper I**. Only seven animals were culled prior to 60 d after first AI, and four of these animals were older cows.

The proportion of pregnant animals may vary with number of days from AI to pregnancy examination; this could be due both to the accuracy of pregnancy examination and to fetal mortality. In Norway, the calculation of non return rate after first AI includes all AI performed in heifers and cows. Reksen et al. (1999a) reported that approximately 50% of Norwegian Red cows were pregnancy diagnosed, and that cows presented for pregnancy diagnosis were less likely to be pregnant than herd mates. Thus, if pregnancy diagnosis is used as a fertility measure in the calculation of the breeding index, care must be taken to avoid selection bias (Dohoo et al., 2003). In the future, pregnancy incidence in Norwegian Red cows may be approximated from the parity specific differences between non return rate and pregnancy incidence (**Paper I**).

The occurrence of DOV

Delayed ovulation type I was present in 14.7% of the profiles in **Paper III**, which is lower than in some previous studies of Holstein cows and Swedish Red and Whites (Opsomer et al., 1998; Petersson et al., 2006; Samarütel et al., 2008) and higher than reported by Shresta et al. (2004) and Royal et al. (2000). The incidence of 2.8% for DOV type II agrees with previous studies in Holstein cows (Opsomer et al., 1998; Shrestha et al., 2004; Samarütel et al., 2008), but the incidence is lower than reported by Royal et al. (2000) and Petersson et al. (2006).

Season of AI

A previous study showed good reproductive efficiency in spring calving organic cows whereas calving during the fall resulted in impaired reproductive performance (Reksen et al., 1999b). In **Paper IV**, there was no difference between season of AI and the two types of farming. First AI was performed during the summer season (April-September) in 58.4% of conventional and 56.3% of the organic farms, which contrasts with the findings of Reksen et al. (1999b) where 52% of the organic cows and 36% of the conventional cows were bred during summer. Season was not associated with interval to first AI or last AI in the models. In **Paper I,** season for AI was almost significantly associated with pregnancy rate in cows. If more animals had been included in the study, we would probably have predicted a significant association between season and pregnancy rate. An earlier study found lower calving rate for cows inseminated during December to March, compared with cows inseminated during the rest of the year (Haugan et al., 2005).

Threshold values for progesterone level

Different threshold values for progesterone have been used both to indicate onset of estrus and to assess onset of luteal activity. Darwash et al. (1997a), Royal et al. (2000), and Petersson et al. (2007) defined C-LA as the occurrence of two or more consecutive milk progesterone concentrations ≥ 3ng/mL in whole milk. Shresta et al. (2004) defined C-LA as two consecutive skimmed milk samples ≥ 1ng/mL, Samarütel et al. (2008) defined C-LA as the first day with progesterone concentration ≥ 5ng/mL in pooled quarter milk samples, and Opsomer et al. (1998; 2000) defined C-LA as first rise in milk progesterone ≥ 15 ng/mL in milk fat. Opsomer et al. (1998; 2000) and Shresta et al. (2004) collected milk twice weekly, whereas Darwash et al. (1997a), Royal et al. (2000), Petersson et al. (2007) and Samartüel et al. (2008) collected milk thrice weekly. In **Paper III**, milk for progesterone analysis was collected twice weekly in 25.2% (127/502) of the profiles, and thrice weekly in the remaining lactations.

The procedure for analyses of progesterone content in whole milk, described by Waldmann et al. (1993; 1999), was used during a period of 14 years (**Papers I, II, and III**). The milk samples were obtained in a representative sample during the milking in the experiments conducted at the Norwegian University of Life Sciences **(Papers II and III)** and by the farmer or veterinarian/AI technicians in the field study conducted in **Paper I**. The progesterone concentration can vary with fat content in the milk and the concentration is highest in residual milk because of higher fat level. Hence, in **Paper I** the progesterone content in milk could increase artificially if residual milk was collected from the cow, which could explain why the lowest progesterone level in pregnant cows was 7 ng/mL.

Pregnancy diagnosis by rectal palpation and PAG

Pregnancy associated glycoprotein concentrations equal to 2.5 ng/mL (six weeks after AI) gave the greatest sensitivity (94.3%) and specificity (94.6%) in the assessment of pregnancy (**Paper I**). Sampling for PAG and rectal palpation for pregnancy were both conducted at the same time, and should express pregnancy or non pregnancy independently of each other. The use of rectal palpation for pregnancy diagnosis is valuable, provided that the veterinarians possess the necessary diagnostic skills.

CONCLUSIONS

1) A pregnancy incidence of 63.7% and a calving rate of 57.2% must be regarded as representing a high reproductive efficiency in Norwegian Red cows. For further estimation of pregnancy incidence from non return rate it is important to be aware of differences in non return rate and pregnancy incidence between heifers and older cows. This difference should also be considered when the non return rate in cows is used for the calculation of breeding indices.

2) Lactation number did not affect pregnancy incidence when heifers were excluded from the analysis, but heifers had a higher pregnancy incidence than cows. The interval to first AI was not associated with pregnancy incidence in the current study, possibly due to a relatively long voluntary waiting period. Milk yield was also not associated with the pregnancy incidence.

3) A PAG value of 2.5 ng/mL in plasma six weeks after AI optimized the sensitivity (94.3%) and specificity (94.6%) in the assessment of pregnancy.

4) The cows selected for low milk yield (LMP) had a shorter interval to C-LA and had lower milk yield than the cows selected for high milk yield (HMP) and cows selected for high milk yield and fertility (HI). The interval to C-LA for the HMP and HI cows decreased after adjustment for EB. The EB, which itself differed between selection lines, did not explain the whole difference in the interval to C-LA between the selection lines. Selection for milk yield negatively affects C-LA over and above the effects caused by changes in EB. The increase in days to C-LA caused by selection for high milk yield can be reduced if selection for milk yield is combined with fertility in the breeding program.

5) The occurrence of DOV and PCL in Norwegian Red cows was generally low compared with most other dairy populations.

6) Cows experiencing a PCL had shorter interval to C-LA. The occurrence of PCL did not affect the pregnancy to first AI when AI was performed at low progesterone concentrations, but cows with a PCL were more likely to be inseminated during the luteal phase. The likelihood of AI during the luteal phase was higher in the study

population than in the general population of Norwegian Red. This may, at least partly, explain why the occurrence of persistent corpus luteum was associated with the likelihood of AI during the luteal phase.

7) The interval to first AI was shorter in conventionally, as opposed to organically managed, cows, whereas there were no differences in the interval to last AI and calving interval between the two management systems. The cows were older in organic farming. Conventional cows yielded more, had higher SCC, and received more concentrates than organic cows. Higher level of concentrate fed to organic cows in recent years is probably an important factor for the higher reproductive efficiency than ten years ago.

The studies showed that the reproductive performance in Norwegian Red cows is relatively high. Inclusion of fertility in the breeding program is probably an important reason for such high fertility. The Norwegian Red is a sustainable breed that adapts well to both intensive and more extensive production systems like organic production.

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Pregnancy Incidence in Norwegian Red Cows Using Nonreturn to Estrus, Rectal Palpation, Pregnancy-Associated Glycoproteins, and Progesterone

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ABSTRACT

The objectives of the study were to estimate pregnancy incidence and calving rate after first artificial insemination (AI) in Norwegian Red cows undergoing spontaneous estrus, to assess the relationship between pregnancy and management factors at herd or cow level, to evaluate differences between 60-d nonreturn rate (NRR60d) and pregnancy incidence, and to compare the accuracy of pregnancy diagnosis by rectal palpation and plasma pregnancy-associated glycoproteins (PAG) analysis supported by progesterone measurements. In total, 829 animals (n = 229 heifers, 234 first-lactation, 173 second-lactation, and 193 >second-lactation cows) were included. Milk samples for progesterone analysis were collected both at AI and 3 wk later. Cows with progesterone concentrations <3 ng/mL at AI were considered in estrus or having nonactive ovaries, whereas cows with progesterone concentrations >7 ng/mL 3 wk later were considered pregnant. Blood sampling for PAG analysis and pregnancy diagnosis by rectal palpation were conducted 57.6 ± 0.92 d after AI. Pregnancy-associated glycoprotein concentrations equal to 2.5 ng/mL gave the greatest sensitivity (94.3%) and specificity (94.6%) in the assessment of pregnancy. The number of days from calving to first AI was 85.3 ± 1.71. Overall NRR60d after first AI was 72.5%. The corresponding values for heifers, first-lactation, second-lactation, and >second-lactation cows were 76.9, 67.1, 69.9, and 76.2%. Overall pregnancy incidence after first AI was 63.7%. The corresponding values for heifers, first-lactation, second-lactation, and >second-lactation cows were 70.0, 58.2, 61.6, and 64.9%. Overall calving rate to first AI was 57.2%. The corresponding values for heifers, first-lactation, second-lactation, and >second-lactation cows were 64.9, 54.3, 54.7, and 53.9%. The overall difference between NRR60d and pregnancy incidence was 8.8%, whereas the parity-specific differences were 6.9, 8.9, 8.3, and 11.3% for heifers, first-lactation, second-lactation, and >second-lactation cows, respectively. Eight animals with PAG <2.5ng/mL and classified as pregnant by rectal palpation calved, whereas 5 animals with PAG ≥2.5 ng/mL and classified as nonpregnant by rectal palpation also calved. The study showed that Norwegian Red cows have relatively high reproductive performance. Breeding for fertility traits over 35 yr is probably an important reason for such high fertility.

Key words: pregnancy incidence, dairy cow, reproductive performance

INTRODUCTION

Since the 1970s, milk yield per cow has increased because of intense genetic selection, improved management, and better nutrition (Lucy, 2001). Yet fertility in dairy cows has declined in recent decades, resulting in economic losses in the dairy industry. There is ongoing discussion about reasons for infertility in dairy cows, including factors such as increased milk production associated with negative energy balance, larger herd size, and greater inbreeding percentages. To improve fertility in dairy cows, several estrous cycle management systems, involving use of reproductive hormones and timed AI, were developed (Thatcher et al., 2006). There are a few published studies from recent years in which cows were inseminated at spontaneously occurring estrus (Andersson et al., 2006; Cairoli et al., 2006) compared with induced estrus and timed AI (Lopez-Gatius et al., 2004; Howard et al., 2006). In the Norwegian dairy industry, pharmacological methods to control estrous cycles are not routinely used, and pharmaceutical treatment of reproductive disorders is only applied after clinical examination and diagnosis (Østerås et al., 2007).

Despite modern estrous cycle management systems, fertility is still declining and the importance of the inclusion of fertility-related traits in the genetic selection

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indices used for the dairy industry has been stressed (Philipsson and Lindhé, 2003; Chagas et al., 2007). Contrary to breeding management in most countries, female fertility has been included in the total merit index for the Norwegian Red breed since 1972. The relative weight on fertility has been 8 to 15% over the entire period (Andersen-Ranberg et al., 2005). Reproductive performance in the breed increased throughout the last decade despite a moderate increase in milk yield (Østerås et al., 2007; Refsdal, 2007). Therefore, an assessment of the pregnancy incidence in the Norwegian Red may be used to indicate the potential of improvement in breeds that have experienced impaired reproductive performance during the same period.

Nonreturn rate has been regarded as a reasonably accurate indicator of reproductive performance. Moreover, the nonreturn rate to first AI will be biased because of variation in breeding management, culling practices, season, parity, season of semen collection, and type of bull used (Haugan et al., 2005; Refsdal, 2007). A good estimate of the pregnancy incidence in Norwegian Red is not available because only 50% of the cows in Norway are pregnancy diagnosed by rectal palpation (Reksen et al., 1999). From an international perspective, there is increasing interest in importing Norwegian Red semen for improvement of reproductive efficiency. Thus, more accurate information about the pregnancy incidence and calving rate of the breed is required.

Pregnancy diagnosis by rectal palpation is the most common way to assess pregnancy. However, pregnancy-associated glycoproteins (**PAG**) can be used for pregnancy diagnosis. Pregnancy-associated glycoproteins constitute a large family of glycoproteins specifically expressed in the outer epithelial cell layer (trophectoderm or chorion) of the placenta in ungulate species (Xie et al., 1997). Although their function remains unknown, PAG can be detected in the maternal blood from around wk 3 of pregnancy (Zoli et al., 1992). Plasma PAG concentrations were useful for pregnancy diagnosis 28 d after AI, provided that the time interval between calving and AI is at least 60 d (Haugejorden et al., 2006).

The objectives were 1) to estimate the pregnancy incidence and calving rate to first AI after spontaneous estrus in Norwegian Red cows; 2) to assess the relationship between pregnancy and management factors at herd or cow level; 3) to evaluate differences between nonreturn rate at 60 d (**NRR60d**) and pregnancy incidence; and 4) to compare the accuracy of pregnancy detection by rectal palpation with PAG analysis supported by progesterone measurements.

MATERIALS AND METHODS

The study began in October 2004, and ended in October 2005. Veterinary practices were selected for inclusion on the basis of being located within a short geographical distance from the Norwegian School of Veterinary Science, and the Norwegian AI and breeding association, Geno (Hamar, Norway). The ambulatory clinic at the Norwegian School of Veterinary Science (Akershus County) performed 104 AI. There were 19 AI technicians and veterinarians (AI personnel) in Oppland and Hedmark counties who participated in the study with a range from 1 to 187 AI (median = 20). The AI were performed in 308 herds with AI ranging from 1 to 20 (median = 2). Among 596 cows with designated housing systems, 116 cows were managed in 41 freestall herds, whereas 480 cows were managed in 219 tiestall herds. The unit of the study was cows or heifers presented for first AI. Treatment with reproductive hormones for reproductive disorders during the current lactation or at all for heifers was an exclusion criterion. In total, 851 animals were enrolled but 22 animals were excluded because of previous AI (n = 17), treatment for reproductive disorders (n = 4), or belonging to a breed other than Norwegian Red (n = 1); thus, the total number of animals included in the analyses was 829. For 7 animals (2 heifers and 5 cows) data for nonreturn rate and calving were available, but data for pregnancy diagnosis were missing. For 2 cows data for calving or culling were missing.

Milk samples for progesterone analyses were obtained by the farmer or AI personnel at AI and 3 wk later. A tablet of Broad Spectrum Microtab (D&F Control Systems Inc., Dublin, CA) was added as a preservative, and the milk samples were stored frozen until assayed for progesterone content. Progesterone concentrations in milk were measured by an enzyme immunoassay (Waldmann, 1993), modified by using the second-antibody coating technique. The specificity of the antibody was described (Waldmann, 1999). The interassay coefficients of variation for milk progesterone concentrations of 1.48 and 19.66 ng/mL were 9.2 and 5.3%, respectively. The intraassay coefficient of variation was <10%.

Blood samples for PAG were collected at the time of rectal examination for pregnancy performed by veterinarians preferably 6 wk after AI, if the animal did not return to estrus. A heterologous double-antibody RIA, modified from the method described previously by Zoli et al. (1992), was used to determine PAG concentrations in the plasma. Bovine PAG (bPAG) was used as standard and tracer, anti-caprine $PAG_{708,\ 709}$ as first antibody (Garbayo et al., 1998), and sheep anti-rabbit IgG as the second antibody for precipitation. The anti-caprine $PAG_{708,\ 709}$ cross-reacts with the bovine plasma. Because plasma dilutions did not completely parallel the standard curve, the assay must be regarded as semiquantitative. The standard curve ranged from 0.2 to 25 ng of bPAG equivalents/mL. To minimize nonspecific interferences, zero plasma was added to all standard tubes. Samples and standards were incubated with antibody overnight at room temperature, and labeled bPAG (33,333 dpm) was added the following day and incubated for a further 4 h at room temperature. Sheep anti-rabbit IgG was added, and free and bound fractions were separated by centrifugation (3,400 × g for 15 min). The precipitates were counted in a Wizard Gamma Counter (Wallac, Turku, Finland). Interassay coefficients of variation were 11.6% (2.59 ng of bPAG equivalents/mL) and 5.0% (10.27 ng of bPAG equivalents/mL), respectively. Assay sensitivity was 0.80 ng of bPAG equivalents/mL.

Data on previous calving date, date of AI, date of new AI, date for next calving, use of double AI (new AI within 0 to 3 d), parity, the milk yield measure within 43 d after AI, heifers' age at AI, AI personnel, herd size, culling date, and reasons for culling were obtained from the National Dairy Herd Recording System (**NDHRS**) files (Tine BA, Ås, Norway).

Determination of Pregnancy Incidence

The most reliable standard for pregnant animals was considered to be animals calving after first AI (n = 473 of 827 animals). Among animals that calved, the criteria used to determine whether the pregnancy was because of the reported AI were either a gestation period of <295 d without a recording on new AI, or return to estrus 18 to 24 d after first AI. For animals with a gestation period >290 d, the identity of the sire and the bull used at AI was controlled using the NDHRS files.

For cows with uncertain pregnancy status that did not calve within the defined gestation period, rectal palpation and progesterone measurements were considered in addition to PAG analysis. By including these criteria to determine pregnancy, the numbers of pregnant animals increased by 51; thus, the total number of pregnant animals was 524.

Analysis of Milk Progesterone. A progesterone concentration of 7 ng/mL was chosen as the threshold value for the indication of pregnancy 3 wk after first AI. This threshold value was based on the lowest milk progesterone values observed in pregnant animals of the current study. Milk progesterone concentrations 3 wk after AI in 4 pregnant cows that calved 280, 282, 278, and 280 d after first AI were 7.1, 8.0, 9.4, and 9.8 ng/mL, respectively. A threshold value of milk progesterone ≥3 ng/mL (Royal et al., 2000; Petersson et al., 2007) indicated luteal activity. Thus, to be compatible with pregnancy, milk progesterone concentrations at AI needed to be <3 ng/mL, and >7 ng/mL at the second milk sample obtained 3 wk later. Progesterone concentration ≥3 ng/mL in the first milk sample was indicative of AI in diestrus.

Analysis of PAG. The PAG value for determination of pregnancy was interpolated to an estimated PAG value at d 42 for all cows. The natural logarithm of PAG was used for the estimation, and regressed against days from AI to blood sampling. The coefficient β = 0.01978 was used to obtain the changes in PAG per day. The adjusted 42-d PAG value for the pregnant animals that delivered a calf (n = 473) was used to create a sensitivity curve for PAG. Animals whose blood was analyzed for PAG concentrations but were determined nonpregnant (n = 37) were used to create a specificity curve for PAG. Animals used to create a specificity curve had progesterone concentrations <3 ng/ml at AI and ≤7 ng/mL 3 wk after AI or PAG value <0.5 ng/mL or both in addition to being evaluated nonpregnant by rectal palpation.

Rectal Palpation. Local veterinarians were instructed to perform pregnancy examinations approximately 6 wk after AI by rectal palpation simultaneously with blood sampling for animals believed pregnant because of nonreturn to estrus or rebreeding.

Pregnancy Diagnosis by PAG and Rectal Palpation

Only cows that calved after first AI (n = 473) were used to determine the accuracy of pregnancy diagnosis by rectal palpation and by PAG analysis. Fourteen of these animals had no measurements for PAG, and 2 had no records of pregnancy examination; all 16 animals were excluded from the evaluation of the accuracy of pregnancy diagnosis by rectal palpation and PAG analysis.

Statistical Analyses

The calculation of NRR60d, pregnancy incidence, and calving rate was performed separately for all AI and for single AI only. The relationships between the outcome pregnant or nonpregnant and the predictor variables were tested both univariately using ordinary logistic regression in SPSS (SPSS Inc., 2004), and in multivariable analysis using a general estimating equation (**GEE**) approach with the GENMOD procedure in SAS (Stokes et al., 1995).

The following variables were included when the likelihood of pregnancy (1 or 0) was evaluated in the univariate logistic regression model: milk yield at the DIM closest to AI (milk yield), heifers' age at AI, days from calving to first AI (**CFI**), lactation number (heifer, 1, 2, >2), season (Jan to March, April to June, July to Sep, and Oct to Dec), double AI, housing (tie stall, free stall), personnel performing AI, and herd size. Personnel performing <30 AI were categorized into one group, whereas personnel performing ≥30 AI were included as single levels in the model. Herd size was categorized into 3 equally sized groups: 5.7 to 15.3, 15.4 to 21.3, and 21.4 to 134.5 cow-years.

The univariate analyses were performed both simultaneously for all parities and separately for cows and heifers. Animals calving during January, February, and March; animals with a single AI during estrus; herd size from 5.7 to 15.3 cow-years; personnel performing <30 AI; and heifers were the designated comparison groups and were assigned the zero value of an odds ratio = 1.

When the association between likelihood of pregnancy and milk yield was assessed, CFI was included in the model to adjust for changes in milk yield by DIM. Variables in the univariate analyses with a $P \leq 0.20$ (overall Wald statistics) were included in the multivariable model and the backward selection procedure was applied. The variable with the greatest P-value was omitted and only predictor variables with a $P \leq 0.20$ from the type 3 score statistics in the GEE analysis remained in the model. The following predictor variables remained in the model when the likelihood of pregnancy in all animals was assessed: lactation number, double AI (1 or 0), and AI personnel. None of the predictor variables remained in the heifer model. The following predictor variables remained in the model when the likelihood of pregnancy in cows was assessed: season, double AI (1 or 0), and CFI. Cows were nested within herd, which was accounted for by using a compound symmetry (exchangeable) correlation structure (Stokes et al., 1995). Confounding was assessed by comparing crude and adjusted parameter estimates. If the estimates varied >10%, confounding was regarded as present (Dohoo et al., 2003).

RESULTS

The distribution of AI (n = 829) by parity for heifers, first-lactation, second-lactation, and >second-lactation cows is in Table 1. The overall average CFI interval was 85.3 d (SD ± 41.9). The corresponding values were 84.4 ± 38.1, 86.8 ± 52.0, and 84.9 ± 35.8 d for first-lactation, second-lactation, and >second-lactation cows, respectively. The proportion of double AI was evenly distributed by parity: heifers 10.5%, first lactation 11.1%, second lactation 9.8%, and >second lactation 10.9%. The number of days from first AI to blood sampling for PAG analysis and rectal palpation was 57.6 ± 22.2 d (n = 584). Low progesterone concentration (<3 ng/mL) was present in 95.1% (520/547) of the cows at first AI, whereas progesterone concentration ≥3 ng/mL was detected in 4.9% (27/547) of the cows.

Nonreturn Rate, Pregnancy Incidence, and Calving Rate

Parity-specific NRR60d, pregnancy incidence, and calving rate, including double AI, are in Table 1.The difference between NRR60d and pregnancy incidence was 8.8%. The parity-specific differences between NRR60d and pregnancy incidence were 6.9, 8.9, 8.3, and 11.3% for heifers, first-lactation, second-lactation, and >second-lactation cows, respectively. The pregnancy incidence was 10.3% greater in heifers compared

Table 1. Overall and parity-specific 60-d nonreturn rate, pregnancy incidence at 6 wk, and calving rate in Norwegian Red cows after first AI[1]

	Nonreturn rate		Pregnancy incidence		Calving rate	
Group	n/n	%	n/n	%	n/n	%
All	601/829	72.5	524/822[2]	63.7	473/827[2]	57.2
Heifers	176/229	76.9	159/227	70.0	148/228	64.9
Cows	425/600	70.8	365/595	61.3	325/599	54.3
1st lactation	157/234	67.1	135/232	58.2	127/234	54.3
2nd lactation	121/173	69.9	106/172	61.6	94/172	54.7
>2nd lactation	147/193	76.2	124/191	64.9	104/193	53.9
Single AI only						
All	532/741	71.8	462/734[2]	62.9	416/739[2]	56.3
Heifers	155/205	75.6	143/203	70.4	133/204	65.2
Cows	377/536	70.3	319/531	60.1	283/535	52.9
1st lactation	139/208	66.8	118/206	57.3	111/208	53.4
2nd lactation	106/156	67.9	92/155	59.4	81/155	52.3
>2nd lactation	132/172	76.7	109/170	64.1	91/172	52.9

[1]Results are reported separately for all animals, including those that received double AI (new AI within 0 to 3 d, n = 829), and for animals that received only 1 AI (n = 741).

[2]The different numbers of observations for nonreturn rate, pregnancy incidence, and calving rate are because of missing data for pregnancy diagnosis at 6 wk for 7 animals, and missing data on calving date or culling date for 2 animals.

Table 2. Univariate analysis for all parity groups, cow only, and heifers only, and *P*-value for the predictor variables: lactation number, days from calving to first AI (CFI), double AI (dAI), season, person performing AI (pAI), herd size, housing, heifers' age at AI, and milk yield in Norwegian Red cows

Item	All	Cows	Heifers
Parity	0.04	0.37	—
CFI	—	0.17	—
Season	0.15	0.07	0.88
dAI	0.18	0.07	0.64
pAI	0.12	0.14	0.85
Herd size	0.81	0.43	—
Housing	—	0.44	—
Heifers' age at AI	—	—	0.73
Milk yield	—	0.95	—

with cows. The corresponding fertility measures after a single AI are in Table 1.

Univariate Analysis in Cows and Heifers

Univariate models for the all parity groups, only cows, and only heifers and the predictor variables are presented in Table 2. Parity was the only significant predictor when both cows and heifers were included in the analysis.

Milk Yield

The milk recordings were obtained on 15 ± 9.9 d after AI. Milk yield at the milk recording day, distributed by parity, was 22.6 ± 4.5, 26.4 ± 5.4, and 28.6 ± 5.7 kg for first-lactation, second-lactation, and >second-lactation cows, respectively. There was no relationship between likelihood of pregnancy to first AI and the predictor variables milk yield and CFI.

Multivariable Model

For all animals, the overall type III statistics were not significant for lactation number ($P = 0.13$), double AI ($P = 0.13$), and person performing AI ($P = 0.20$). Estimates for each level of the predictors are presented in Table 3.

For cows, the overall type III statistic was not significant for CFI ($P = 0.13$), double AI ($P = 0.08$), or season ($P = 0.19$). Estimates for each concentration of the predictors are in Table 4. Confounding between the covariates was not apparent in the multivariable analyses.

Sensitivity and Specificity for PAG

The curves for sensitivity and specificity for PAG are in Figure 1. A PAG value of 2.5 ng/mL optimized the sensitivity (94.3%) and specificity (94.6%) in the assessment of pregnancy.

Abortion and Early Partus

The proportion of pregnant animals that aborted was 1.0% (5/524). The 5 animals aborted 62, 149, 243, 252, and 259 d after first AI. The proportion of animals that calved 260 to 269 d of gestation was 3.2% (17/524).

Pregnant Animals Not Calving Within the Defined Gestation Period (<295 d)

Fifty-one animals (9.7%, 51/524) diagnosed pregnant 6 wk after first AI by progesterone, PAG, and rectal

Table 3. General estimating equation model for assessing the pregnancy incidence in heifers and cows of Norwegian Red from the following predictors: lactation number, double AI (dAI), and person performing AI

Item	β	SE	df	Odds ratio	*P*-value
Intercept	1.065	0.233	—	2.90	<0.01
Heifers[1]	—	—	—	—	—
1st lactation	–0.530	0.223	1	0.59	0.02
2nd lactation	–0.404	0.224	1	0.67	0.07
>2nd lactation	–0.238	0.225	1	1.25	0.29
dAI, no[1]	—	—	—	—	—
dAI, yes	0.445	0.288	1	1.56	0.12
<30 AI[1,2]	—	—	—	—	—
A[3]	–0.614	0.261	1	0.541	0.02
B	–0.303	0.414	1	0.739	0.47
C	–0.388	0.336	1	0.678	0.25
D	0.252	0.406	1	1.287	0.54
E	–0.338	0.306	1	0.713	0.27
F	–0.548	0.372	1	0.578	0.14
G	–0.027	0.348	1	0.973	0.94
H	0.421	0.443	1	1.523	0.32
I	–0.369	0.283	1	0.691	0.20

[1]Categorical variables assigned as baselines.

[2]Person performing <30 AI categorized to 1 group.

[3]A to I = letters identifying different persons performing ≥30 AI.

Table 4. General estimating equation model for assessing the pregnancy incidence in Norwegian Red cows from the following predictors: days from calving to first AI (CFI), double AI (dAI), and season

Item	β	SE	df	Odds ratio	*P*-value
Intercept	–0.185	0.274	—	0.83	0.50
CFI	0.003	0.002	1	1.00	0.17
dAI, no[1]	—	—	—	—	—
dAI, yes	0.537	0.317	1	1.71	0.09
Season, Jan–March[1]	—	—	—	—	—
Season, Oct–Dec	0.383	0.240	1	1.47	0.11
Season, Apr–June	0.320	0.270	1	1.38	0.23
Season, July–Sep	0.534	0.241	1	1.71	0.03

[1]Categorical variables assigned as baselines.

palpation did not deliver a calf after the current AI. Eleven were presented for a new AI, 12 animals were sold as milking cows, and 2 cows died. Culling reasons for the 12 animals were low fertility (n = 3) and other reasons (n = 9). Four animals aborted or calved 62 to 252 d of gestation, whereas 2 animals calved 296 and 297 d of gestation. Five animals were still alive 295 d after AI without a calving or a new recorded AI. No information was available regarding the last 3 animals.

Low Fertility as a Culling Reason

In the present study, 3.6% (30/829) of the animals were culled because of low fertility ≤290 d after first AI. Nine animals were not presented for a new AI, and 3 were retrospectively diagnosed as pregnant. Twenty-one animals were presented for a new AI.

Pregnancy Diagnosis by PAG and Rectal Palpation

Among 457 animals that calved after first AI and that had complete records regarding both PAG measurements and rectal pregnancy diagnosis, 444 had a PAG value >2.5 ng/mL and were classified pregnant by veterinarians. Eight animals with PAG ≤2.5 ng/mL that were classified as pregnant by veterinarians calved between 270 and 287 d after first AI, whereas no animals that calved with PAG ≤2.5 ng/mL were classified as nonpregnant by veterinarians. Five animals classified

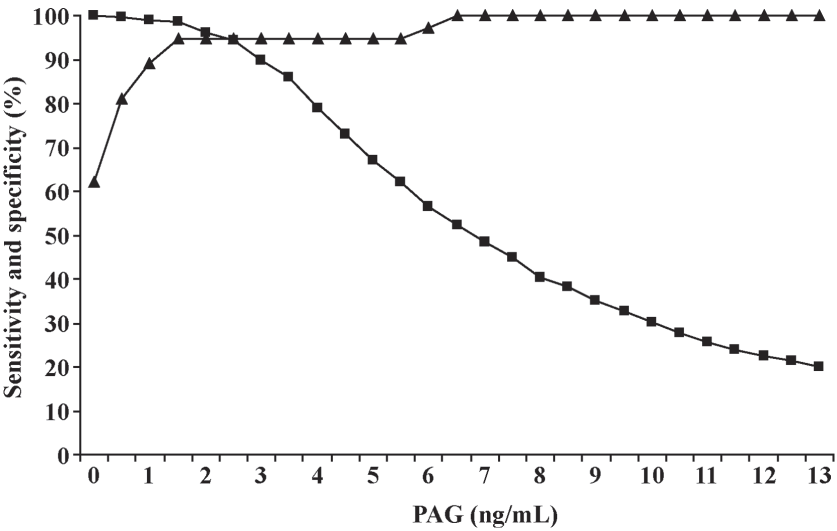

Figure 1. Sensitivity (n = 473; ■) and specificity (n = 37; ▲) for the determination of pregnancy by measurement of pregnancy-associated glycoproteins (PAG) in Norwegian Red cows.

as nonpregnant by veterinarians had a PAG value >2.5 ng/mL and calved after first AI.

DISCUSSION

A nonreturn rate of 71.8% at 60 d, a pregnancy incidence of 62.9% at 6 wk, and a calving rate of 56.3% after a single AI (Table 1) must be considered as indicators of high reproductive efficiency in Norwegian Red cows compared with previous and comparable studies in dairy cattle (Andersson et al., 2006; Cairoli et al., 2006), and superior compared with AI after induced estrus or timed AI (Lopez-Gatius et al., 2004; Howard et al., 2006). When cows subjected to double AI were excluded, the average NRR60d in Norwegian Red cows for 2005 was 72.7% (Refsdal, 2007), and the percentage of return rates within 0 to 3 d post-AI (double AI) was 12%. These findings are well supported with those obtained in the present study.

Different threshold values for progesterone have been used both to indicate onset of estrus and to assess onset of luteal activity. Roelofs et al. (2006) reported milk progesterone concentrations <2 ng/mL before ovulation. Milk progesterone concentrations ≥3 ng/mL were used as threshold value for luteal activity (Royal et al., 2000; Petersson et al., 2007). Accordingly, milk progesterone concentration <3 ng/mL was used to determine that a cow was not in the luteal phase of the estrous cycle at AI in the present study. Thus, milk progesterone was only used to determine pregnancy in addition to pregnancy diagnosis by PAG or manual rectal palpation.

The observed increase in NRR60d from 68.1% in 1985 to 72.7% in 2005 for Norwegian Red cows (Refsdal, 2007) may be biased because of decreased ability of farmers to identify and rebreed nonpregnant animals, which corresponds well with an increase in herd size during the period (Østerås et al., 2007). It may be because of increased emphasis on fertility and health in the breeding strategy. Female fertility has been included in the total merit index since 1972 with relative weight on fertility between 8 and 15% (Andersen-Ranberg et al., 2005). When the fertility index was calculated, the nonreturn rate at 56 d in heifers was weighted by 67%, whereas first-lactation cows were weighted by 33% (Geno Breeding and AI Association, 2007). In other Nordic countries, nonreturn rate after 56 d in heifers and cows, CFI, days from first AI to last AI, and days open are commonly used for female fertility traits evaluation of bulls (Interbull, 2007).

Overall, NRR60d overestimated the pregnancy incidence by approximately 9%. In heifers, the difference between NRR60d and pregnancy incidence was 6.9%, whereas the same estimate for >second-lactation cows was 11.3%. This demonstrates that the nonreturn rate was subjected to more bias in older cows than among heifers. But culling of older cows before 60 d after AI was not an important reason for the lower pregnancy incidence; only 7 animals in the study group were culled before 60 d after first AI, and of these 7 animals, 4 were >second-lactation cows. The likely explanation of this bias is that the farmer presents an older cow for AI and when the cow returns to estrus the farmer decides to cull her at the end of lactation rather than performing a new AI. Consequently, NRR60d overestimates the pregnancy incidence.

The pregnancy incidence may vary with number of days from AI to pregnancy examination; this could be due both to the accuracy of pregnancy examination and to fetal mortality. In Norway, calculation of nonreturn rate after first AI includes all AI performed in heifers and cows. Reksen et al. (1999) reported that approximately 50% of Norwegian Red cows were pregnancy diagnosed, and that cows presented for pregnancy diagnosis were less likely pregnant than herd mates. Thus, if pregnancy diagnosis is used as a fertility measure in the calculation of the breeding index, care must be taken to avoid selection bias (Dohoo et al., 2003). In the future, pregnancy incidence in Norwegian Red cows may be approximated from the parity-specific differences between nonreturn rate and pregnancy incidence.

Lactation number was a significant predictor of the likelihood of pregnancy when all parity categories were included in the analyses, but when heifers were excluded from the analysis, lactation number did not affect the pregnancy incidence. It is known that reproductive performance in heifers is better than in cows, because of milk production and the risk of a prolonged period of negative energy balance leading to delayed ovarian activity (Butler and Smith, 1989).

The interval from calving to first AI, which was 84.4, 86.6, and 84.9 d for first-lactation, second-lactation, and >second-lactation cows, respectively, was not associated with pregnancy incidence. The explanation for this could be a relatively long voluntary waiting period before first AI. The CFI interval in the present study was longer than that reported by Royal et al. (2000) in which the CFI interval increased from 74.0 d in the 1970s to 77.6 d in the 1990s. The average CFI interval in Norwegian Red cows increased from 79 d in 1990 to 86 d in 2005 (Refsdal, 2007).

In addition to an increased weight on fertility in selection, the lack of association between milk yield and days from calving to AI on the likelihood of pregnancy could be because of the moderate milk yield in the Norwegian Red cows, which was 6,605 kg/cow-year in 2005 (Østerås et al., 2007). Yet the genetic potential for high milk yield in Norwegian Red is not fully exploited because of the political framework present in Norway, which affects price mechanisms and feeding regimens such that milk yields remain moderate (Refsdal, 2007).

In the present study, AI in the luteal phase was performed in 4.9% of the cows, which supports Andresen and Onstad (1979) and Grimard et al. (2006) who reported 4.4 and 5.0% AI in the luteal phase, respectively. Cairoli et al. (2006) found that 10.2% of the cows showing spontaneous estrus were inseminated at high progesterone concentrations.

Sampling for PAG and rectal palpation for pregnancy was conducted at the same time, and both should express pregnancy or nonpregnancy independently of each other. Haugejorden et al. (2006) recommended that for cows inseminated n days before 60 d postpartum, pregnancy diagnosis by PAG be carried out at 28 + ($n \times 0.5$) d of pregnancy. Using this formula, in the current study, PAG analysis was conducted in 6 animals before the recommended day of gestation. But in these animals, PAG values were >7 ng/mL, and additionally they were confirmed as pregnant by rectal palpation.

CONCLUSIONS

In conclusion, Norwegian Red cows have a relatively high reproductive performance, and the study population was probably representative of the population of Norwegian Red cows. Breeding for fertility traits over the last 35 yr is likely an important factor contributing to such high fertility. For further estimation of the pregnancy incidence from nonreturn rate, it is important to be aware of differences in nonreturn rate and pregnancy incidence between younger and older cows. The study showed that the use of rectal palpation for pregnancy diagnosis is valuable, provided that veterinarians possess the necessary diagnostic skills.

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Commencement of luteal activity in three different selection lines for milk yield and fertility in Norwegian Red cows

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ABSTRACT

Relationships among commencement of luteal activity (C-LA), milk yield, and energy balance (EB) were investigated in 3 selection lines of Norwegian Red cows at the Norwegian University of Life Sciences from 1994 through 2001. The cows were selected for low genetic merit for milk yield (LMP), high genetic merit for milk yield (HMP), and a combination of high indices for milk yield and fertility (HI). Breeding values for fertility were based on 56-d nonreturn rate. The material included 268 lactations from 147 cows. Milk samples for progesterone analysis were drawn 3 times weekly from 1994 through 1998, and 2 times weekly from 1999 to 2001. Commencement of luteal activity was defined as the first 2 consecutive measurements of progesterone concentration >3 ng/mL not earlier than 10 d after calving. Selection line was significantly related to C-LA, so that the least squares mean days from calving to C-LA were 22.5, 30.4, and 27.2 d for LMP, HMP, and HI cows, respectively. The HMP cows produced more milk than the LMP cows. The average milk yield in the sixth week of lactation was 24.0, 27.1, and 25.3 kg for LMP, HMP, and HI cows, respectively. The interval to C-LA decreased for the HMP and HI cows after phenotypic adjustment for EB in the model. Least squares means for the interval to C-LA were 23.2, 29.7, and 25.6 d for the LMP, HMP, and HI cows, respectively, in a model that included parity, selection lines, and EB as covariates. Cumulated EB during the first 4 wk of lactation, which itself differed between selection lines, did not fully account for differences in interval to C-LA between selection lines. Thus, the results of the present investigation indicate that selection for milk yield negatively affects C-LA over and above the effects caused by concurrent changes in EB. The increase in days to C-LA caused by selection for high yields can be reduced if selection for milk yield is combined with fertility in the breeding program.
Key words: luteal activity, genetic merit, fertility, dairy cow

INTRODUCTION

Since the 1970s, milk yield per cow has increased rapidly because of intense genetic selection, improved management, and better nutrition. However, fertility in dairy cows has declined in recent decades. Potential factors such as increased milk production that is associated with negative energy balance (**EB**), larger herd size, and higher inbreeding percentages have been suggested as reasons for infertility in dairy cows (Lucy, 2001).

In recent years, the effects of genetic merit of milk yield on fertility have been of considerable interest. Unfavorable genetic correlations exist between milk yield and reproductive performance (Veerkamp et al., 2001; Royal et al., 2002; Pryce et al., 2004). Several studies on reproductive efficiency have been performed. Gutierrez et al. (2006) reported that commencement of luteal activity (**C-LA**) occurred later in cows with high EBV for milk production than in cows with low EBV for milk production. This is in accordance with MacKey et al. (2007) who reported that reproductive performance was negatively associated with both genetic merit for milk yield and actual level of milk production. The level of milk production had the greatest negative effect on reproductive performance, suggesting that poor fertility is caused by an inability to meet the nutritional requirements for an individual cow. Patton et al. (2007) found no associations between milk yield and resumption of ovarian activity, but reported a more negative EB to be associated with later C-LA and noted that DMI was the primary component of EB affecting reproduction. Reksen et al. (2001) found that cows with late resumption of ovarian activity produced more milk than cows with early and middle responses, suggest-

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Table 1. Distribution of number of lactations by parity and selection lines: low milk production (LMP), high milk production (HMP), and high index for milk yield and fertility (HI)

	Selection line		
Parity	LMP	HMP	HI
First parity	26	32	59
Second parity	21	31	50
>Second parity	26	18	5

ing that moderately yielding cows may compensate for energy deficits by decreasing milk yield, which on a genetic level could be explained by an increase in C-LA per unit of PTA for milk yield in high-producing cows (Royal et al., 2002). Thus, there are marked indications of unfavorable phenotypic and genotypic associations between milk yield and C-LA. Although the phenotypic correlation between C-LA and EB has been recognized (Reksen et al., 2001; Patton et al., 2007), less is known about the relationship between C-LA and selection for milk yield when phenotypic differences in EB are accounted for.

Fertility traits can be divided into traditional fertility traits and endocrine fertility traits (Royal et al., 2000). Traditional fertility traits such as calving interval, days to first service, 56-d nonreturn rate, and number of inseminations per conception have very low heritabilities (Pryce et al., 1997; Wall et al., 2003; Jamrozik et al., 2005), whereas endocrine fertility traits, which include interval to C-LA, length of first luteal phase, and occurrence of persistent corpus luteum, all have moderate heritability (Darwash et al., 1997; Royal et al., 2002; Petersson et al., 2007). In contrast to the majority of countries, in Norway, female fertility has been included in the total merit index of the Norwegian Red since 1972 (Andersen-Ranberg et al., 2005). The relative emphasis on fertility has been 8 to 15% throughout this period. Historically, the trait used in selection was the 56-d nonreturn rate of virgin heifers. Since 2002 a combination of 56-d nonreturn rate in heifers and first-lactation cows has been used (Andersen-Ranberg et al., 2005).

The unfavorable correlated response to selection for milk yield on C-LA has been proposed to be caused by a strong association between the length of the anovulatory period postpartum and EB (Butler, 2003). The proportion of the interval between calving and C-LA that is due to differences in EB within and between selection lines can be determined for by including EB in the statistical model. Thus, an estimate of the correlated response between selection for milk yield and C-LA, which is accountable to factors other than EB, may be obtained by adjusting for EB.

In the current study, days to C-LA was compared between 3 selection lines for milk yield and fertility: low milk yield (**LMP**), high milk yield (**HMP**), and high index for both milk yield and fertility (**HI**). The objectives of the study were 1) to quantify the differences in C-LA, milk yield, and EB between the selection lines; and 2) to assess how much of the differences in C-LA between the selections lines could be accounted for by the phenotypic differences in EB.

MATERIALS AND METHODS

The material included 3 selection lines of Norwegian Red cows (LMP, HMP, and HI), managed in a feeding trial designed to assess the effects of different levels of concentrate on reproduction, milk yield, and EB at the Norwegian University of Life Science from 1994 to 2001. The cows were followed from calving until pregnancy was verified, and the material included 268 lactations from 147 Norwegian Red cows (Table 1).

Selection Lines

The selection lines for the LMP and HMP cows were established between 1978 and 1989 by the use of low- and high-ranked sires for 305-d milk yield, respectively (Heringstad et al., 2003). Throughout the study period, cows in the LMP selection line were bred to a group of 11 sires that were progeny tested in 1978 and 1979 with EBV of 92.0 (SD ± 3.7) for milk yield and 102.0 (SD ± 10.6) for fertility from their first progeny test. To avoid inbreeding, none of the bulls were used for AI in their own progeny lines. The EBV for the maternal grandsires were 92.2 (SD ± 3.1) for milk yield and 101.7 (SD ± 7.0) for fertility from their first progeny test. The HMP cows were sired by the 3 to 4 highest ranking proven sires (total 22 sires) for milk production from the most recent group of progeny-tested Norwegian Red sires each year. The EBV for the sires were 111.7 (SD ± 4.1) for milk yield and 102.4 (SD ± 8.3) for fertility. The sires were only used for 1 yr to avoid inbreeding. The EBV for the maternal grandsires were 110.8 (SD ± 4.9) for milk yield and 103.5 (SD ± 8.4) for fertility. The HI cows were sired by the progeny-tested, top-ranked Norwegian Red bulls (elite bulls), before commencement of the study and until the end of study in 2001. The EBV for the sires were 109.0 (SD ± 5.6) for milk yield and 102.9 (SD ± 6.9) for fertility. The EBV for the maternal grandsires were 108.1 (SD ± 5.6) for milk yield and 101.3 (SD ± 6.3) for fertility. The cows comprising the selection line for high milk production and fertility reflected the genetic trend for the population of Norwegian Red throughout the study period. Fertility has been included in the total merit index as 56-d nonreturn rate since 1972. The relative emphasis on fertility has been between 8 to 15% during the entire period (Andersen-Ranberg et al., 2005). The following traits (and relative weights) were included in the breeding program in 1998: milk yield (21%), mastitis resistance (21%), udder conformation (11%), fertility (14%), growth rate (12%), conformation (6%), temperament (4%), diseases other than mastitis (3%), stillbirth (4%), and dystocia (4%; Geno Breeding and AI Association, 1998).

Estimated breeding values for the cows in the current lactation were obtained from the Norwegian Dairy Herd Record System and were estimated from the following formula: EBV = (I − 100) × 0.8 + 1/2 SBV + 1/4 MSBV + 1/8 SMMBV, where I = the milk index for the cow, SBV = EBV for the sire excluding milk traits, MSBV = EBV for the maternal grandsire excluding milk traits, and SMMBV = EBV for the sire of the maternal grandmother excluding milk traits. The EBV for the cows were −12.8, 3.6, and 6.2 for the LMP, HMP, and HI cows, respectively.

Management

The cows were milked twice daily. The breeding season started in early November and continued until February each year. The cows were on pasture from late May to early September. Only healthy untreated cows that calved during the indoor season were included in the study.

Milk Yield and Milk Progesterone

Milk weights were recorded on 3 consecutive days each week. The average weekly milk yield for the first 10 wk postpartum was calculated for each lactation. Milk progesterone concentrations were measured every Monday, Wednesday, and Friday from 1994 throughout 1998, and every Monday and Friday from 1999 to 2001, by an enzyme immunoassay (Waldmann, 1993), modified by using the second antibody coating technique. The specificity of the antibody has been described previously (Waldmann, 1999). The interassay coefficients of variation for milk progesterone concentrations of 1.48 and 19.66 ng/mL were 9.21 and 5.32%, respectively. The intraassay coefficient of variation was <10%. Milk progesterone concentrations were used to determine the interval from calving to C-LA. The interval from calving to C-LA was defined as the first 2 consecutive measurements of progesterone concentration >3 ng/mL more than 10 d after calving.

Concentrate Allocation

The cows were assigned group wise to different concentrate rations such that the groups were similar in terms of BW, age, body condition, and selection line for milk production. The first-parity cows received either 5 or 9 kg/d of concentrate during peak lactation (until 90 DIM), whereas the second-parity cows received either 6.5 or 11 kg/d during peak lactation from 1994 to 1998. From 1999 to 2001, the cows were divided into 4 groups balanced for calving date and parity and which received 4, 8, 12, or 16 kg/d of concentrate during peak lactation.

Energy Requirements

The DM of the feed was determined weekly, and ash, CP, crude fat, crude fiber, and NDF content were calculated monthly according to standard procedures (AOAC, 1995). Protein degradability of roughage was measured in a composite feed sample collected over a 3-mo period. Protein degradability of concentrate was analyzed in a composite sample collected throughout the entire indoor feeding period. Digestibility of DM was assessed in trials conducted for each of the 2 main feeds (grass silage and concentrate; Madsen et al., 1995). Dry matter intake was calculated as DM offered minus DM refused, on 3 consecutive days in a week.

Energy content in dry matter and energy requirements were calculated according to the Norwegian feeding value per unit of milk (**FUM**) system (Ekern, 1991), in which 1 FUM is equal to 6.9 MJ of NE_L. Energy intake was calculated as FUM_{intake} = DMI × food FUM concentration. Energy requirements are dependent upon maintenance, growth, and milk production according to the following formulae (Van Der Honing and Alderman, 1988):

$$\text{Energy requirements} = FUM_{maintenance} + FUM_{milk} + FUM_{growth}$$

$$FUM_{maintenance} = 0.0424 \times BW^{0.75},$$

where BW = body weight;

$$FUM_{milk} = 0.44 \times ECM + 0.0007293 \times ECM^2,$$

where ECM = daily ECM yield [kg] = kg milk × [0.25 + (0.122 × fat %) + 0.077 × protein %)]; and

$$FUM_{growth} \text{ (first-parity cows)} = 0.45.$$

Table 2. Cumulative average energy balance for the first 4 wk (CAEB wk 1–4), and weekly point estimates of average energy balance (EB) and milk yield (MY) from wk 2 to 10 postpartum between cows selected for low milk yield (LMP), high milk yield (HMP), and high genetic merit for milk yield and fertility (HI) as assessed by mixed linear models[1]

		LMP		HMP		HI	
Item	n	LSM	SE	LSM	SE	LSM	SE
CAEB wk 1–4	268	−9.77[a]	2.04	−13.97[ab]	1.93	−15.71[b]	1.61
EB wk 2	239	−11.54[a]	2.43	−17.02[ab]	2.35	−21.16[b]	2.07
EB wk 4	263	−4.89[a]	2.25	−8.47[a]	2.11	−11.49[a]	1.80
EB wk 6	258	−4.64[a]	2.21	−6.02[a]	2.11	−8.25[a]	1.79
EB wk 8	253	−3.02[a]	2.28	−6.33[a]	2.10	−6.25[a]	1.81
EB wk 10	252	−2.46[a]	2.04	−3.45[a]	1.87	−3.82[a]	1.60
MY wk 2	239	22.27[a]	0.72	24.48[a]	0.69	23.01[a]	0.60
MY wk 4	263	23.74[a]	0.66	27.03[b]	0.63	24.96[a]	0.54
MY wk 6	258	23.96[a]	0.68	27.14[b]	0.65	25.31[ab]	0.56
MY wk 8	253	23.85[a]	0.68	26.68[b]	0.62	24.90[ab]	0.53
MY wk 10	252	23.29[a]	0.66	26.07[b]	0.60	25.09[ab]	0.52

[a,b]Different superscripts indicate significant differences between selection lines by Bonferroni adjustment for multiple comparisons, $P < 0.05$.
[1]Least squares means.

Energy balance in FUM was estimated as the difference between energy intake and energy requirements for maintenance, growth in first-lactation cows, and milk production.

Statistical Analysis

All calculations were performed using SAS, version 9.1 (SAS Institute Inc., Cary, NC). Mixed linear models were performed (Littell et al., 1996) to assess differences in EB and milk yield between selection lines (LMP, HMP, HI) for each of the 12 postpartum weeks. The models adjusted for multiple lactations within the same cow by the use of a compound symmetry correlation structure.

Mixed linear models were also performed to assess relationships between the natural logarithm of days to C-LA (**lnC-LA**) and explanatory variables. The outcome variable, days to C-LA, was transformed by natural logarithm to obtain approximate normality of residuals. The first model included selection line and parity (1, 2, and >2) as fixed effects. A second model included parity and the cumulative average EB for the first 4 wk postpartum (**CAEB 1–4**) as fixed effects. Cumulative average EB for the first 4 wk postpartum was used to avoid random fluctuations that may occur if point estimates were used. A third model included parity, selection line, and CAEB 1–4 as fixed effects. The interaction between selection line and CAEB 1–4 was tested in this model, but omitted as it was not significant. Covariance between multiple lactations within the same cow was included as a compound symmetry correlation structure. Level of concentrate allocation was included in the models as a random effect. A single index notation of these models can be expressed as: $\text{lnC-LA}_{ijk} = \beta_0 + \beta_1 \ldots \beta_n + Z_{\gamma ij} + U_{\gamma k} + e$, where i corresponds to ith lactation number; j to observation at jth individual; β_0 = intercept; $\beta_1 \ldots \beta_n$ = fixed effects; $Z_{\gamma ij}$ represents the repeated effects for the ith lactation period within jth individual; $U_{\gamma k}$ = random effect of concentrate allocation; and e = residual error term. Multiple comparison adjustment for the pairwise difference in least squares means was performed using the Bonferroni option in SAS. Statistical significance was considered at P-values <0.05.

RESULTS

Least squares (**LS**) means of the cumulative average weekly EB, CAEB1–4, and milk yield during the first 10 wk of lactation are presented by selection line in Table 2. There was no significant difference in weekly EB between the selection lines except for the second week, where the HI cows had lower EB than the LMP cows (Table 2). Milk yield differed between selection lines in that HMP cows yielded more than LMP cows, whereas no significant differences in milk yield were observed between the HI and LMP cows or the HI and HMP cows (Table 2).

In the model with parity and selection line as the fixed effects, lnC-LA was only associated with selection line ($P = 0.02$) as assessed by the F-test. The LS means for lnC-LA were 3.106 ± 0.078, 3.407 ± 0.073, and 3.287 ± 0.066 for the LMP, HMP, and HI cows, representing an interval from calving to C-LA of 22.3, 30.2, and 26.8 d for LMP, HMP, and HI cows, respectively.

In the model with parity and EB included as fixed effects, lnC-LA was associated with both parity ($P <$

Table 3. Associations between number of days to commencement of luteal activity (C-LA, natural logarithmic transformed) as assessed by a mixed linear model corrected for level of concentrate allocation as random effects variable and fixed effects variables; parity, selection lines for high (HMP), low (LMP) milk yield, and high genetic merit for milk yield and fertility (HI)

Item	Least squares means	SE	Days to C-LA
Parity			
First parity	3.398[a]	0.052	29.9
Second parity	3.199[b]	0.054	24.5
>Second parity	3.178[ab]	0.081	24.0
Selection line			
LMP	3.144[a]	0.074	23.2
HMP	3.390[b]	0.068	29.7
HI	3.241[ab]	0.063	25.6

[a,b]Different superscripts indicate significant differences between parities or selection lines by Bonferroni adjustment for multiple comparisons, $P < 0.05$.

0.01) and EB ($P < 0.01$). The LS means for lnC-LA were 3.402 ± 0.053, 3.205 ± 0.053, and 3.173 ± 0.081 for first parity, second parity, and >second parity, representing an interval from calving to C-LA of 30.0, 24.7, and 23.9 d for first parity, second parity, and >second parity, respectively. The estimate for EB in this model was −0.0098 ± 0.0019.

Associations between lnC-LA and the fixed effects selection line, parity, and EB in the third model are presented in Table 3. In this model, lnC-LA was associated with parity ($P < 0.01$), selection line ($P = 0.04$), and EB ($P < 0.01$). The estimate for EB in this model was −0.0094 ± 0.0020. The LS means for days from calving to C-LA were 23.2, 29.7, and 25.6 d for the LMP, HMP, and HI cows, respectively, and 29.9, 24.5, and 24.0 d for first parity, second parity, and >second parity, respectively, when EB was included in the model in addition to parity and selection line.

The difference in C-LA between the HMP and LMP cows was 7.9 d when parity and selection line were included in the first model, but the difference between the HMP and LMP cows decreased to 6.5 d when phenotypic differences in EB were accounted for in the model.

DISCUSSION

It has been emphasized that fertility should be included in breeding goals to reverse the trend of declining fertility in dairy cows, because unfavorable genetic correlations exist between milk yield and reproductive performance (Veerkamp et al., 2001; Royal et al., 2002; Pryce et al., 2004). Currently, female fertility is included in selection indices in several other countries (VanRaden, 2004; Miglior et al., 2005). In the present study, cows selected for high milk yield had a longer interval from calving to C-LA than cows selected for low milk yield, whereas the interval to C-LA was intermediary in cows selected for both yield and fertility. Thus, less selection pressure for milk yield and more weight on fertility in a breeding program does not necessarily reduce milk yield substantially, despite the fact that an increase in C-LA per unit of PTA for milk yield is expected in high-producing cows (Royal et al., 2002).

There was a strong association between C-LA and EB that was of similar magnitude in the models with and without selection line included as a fixed effect. The interval to C-LA was reduced for the HMP and HI cows after adjustment for phenotypic differences in EB between the selection lines. This is in agreement with the unfavorable genetic correlations between milk yield and fertility reported in previous studies on both a phenotypic level (Gutierrez et al., 2006; Patton et al., 2007) and a genetic level (Veerkamp et al., 2001; Royal et al., 2002; Pryce et al., 2004), because the degree of negative EB stems from the milk yield in early lactation and hence influences the interval to C-LA.

To our knowledge, the present study is the first to evaluate and compare C-LA in cows selected for low milk yield, high milk yield, or the combination of high indices for milk yield and fertility. By managing the cows in a single herd, phenotypic variation resulting from different management and feeding practices was minimized. Additionally, valuable information about the reproductive performance and milk production of Norwegian Red cows from the 3 different selection lines was obtained. The LMP cows may be used as a baseline, representing the phenotype between 1978 and 1979; HMP cows may be viewed as a phenotype representing reproductive performance and milk yield in Norwegian Red cows, in which selection has been more focused toward milk yield and less toward fertility and other functional traits; and the HI group can be considered as representative of current breeding objectives.

In previous experiments (Westwood et al., 2000; Gutierrez et al., 2006) and population studies (Royal et al., 2000) of differences in reproductive performance between cows with high and low genetic merit for milk yield, contemporary sires with breeding indices for high and low milk yield have been used. In the present study, sires of cows selected for low milk yield were dated back to progeny test results from 1978 and 1979, whereas the sires used for AI in cows selected for high milk yield were the 3 to 7 highest ranking proven sires for milk yield in the most recent group of progeny-tested Norwegian Red sires during each year of the study. Thus, cows expressing selection responses from 16 to 23 yr of breeding were housed and fed beside cows in which no selection responses could be expected since 1979, even though changes in gene frequency to some extent could occur by chance alone in small populations (Simm, 2000).

After adjustment for phenotypic differences in EB, there was still a difference in days to C-LA of 6.5 and 2.4 d for the HMP and HI cows, respectively, compared with the LMP cows. Thus, the reduction in reproductive efficiency following selection for high milk yield must be attributable to factors other than negative EB because of high milk production. It has been shown that cows with a high genetic merit for milk yield mobilize more body tissue in early lactation than cows with average merit for milk yield on a genetic level (Pryce et al., 2001) and on a phenotypic level (Westwood et al., 2000; Pryce et al., 2001). The heritability for BCS is high (0.38; Veerkamp et al., 2001), and there is a strong and negative genetic correlation between BCS and milk yield (Veerkamp et al., 2001; Wall et al., 2003), which may lead to greater negative EB and reduction in fertility in cows selected for high milk yield. Westwood et al. (2000) reported that mean BCS and pattern of BCS changes differed between high- and low-yielding cows because high-genetic-merit cows mobilized more body tissue in early lactation than cows with a lower production potential. Hayhurst et al. (2007) reported that the amounts of free fatty acids and glucose in male calves were moderately heritable and could be used to enhance selection for improved female fertility. Calves that mobilized large amounts of free fatty acids tended to have female offspring with reduced fertility. Thus, selection for milk yield could lead to more body tissue mobilized and hence a higher level of free fatty acids leading to reduced fertility such that the interval from calving to C-LA is increasing.

Previous phenotypic studies reported that cows with higher plasma concentrations of IGF-1 had an increased likelihood of a shorter interval to C-LA, whereas plasma concentrations of insulin, glucose, NEFA, and BHBA were not associated with C-LA (Patton et al., 2007). Westwood et al. (2000) reported higher concentrations of growth hormone in cows with high genetic merit for milk yield, whereas Gutierrez et al. (2006) found higher concentrations of growth hormone and BHBA, and lower concentrations of insulin, glucose, and IGF-1 in cows with high genetic merit for milk yield. Although assessments of hormone and metabolic profiles were not objectives of the current study, it should be emphasized that further research is necessary to investigate which factors other than negative EB affect the phenotypic difference in days to C-LA between cows selected for low or high milk production.

Selection for shorter intervals to C-LA, which has moderate heritability, may be a feasible approach to improving fertility (Darwash et al., 1997). However, despite obtaining a shorter interval to C-LA by inclusion of fertility as nonreturn rate in the breeding program, inclusion of C-LA only as a fertility trait in a breeding program should be considered with caution because of the increased risk of persistent corpus luteum rather than continuous cyclical luteal activity after early C-LA (Royal et al., 2002; Petersson et al., 2007). Thus, both traditional and endocrinological fertility traits should be included in a breeding program to improve cow fertility in a sustainable manner.

First-parity cows had longer intervals to C-LA compared with second-parity cows. This agrees with Petersson et al. (2006), but contrasts with the reports from Darwash et al. (1997) who reported that the interval increased with parity. Previous studies have reported the interval to C-LA to be 33.8 d (Petersson et al., 2006), 31.0 d (Patton et al., 2007), 29.5 d (Veerkamp et al., 2000), and 25.6 d (Darwash et al., 1997). These figures correspond with those obtained for the HMP cows of the present study.

CONCLUSIONS

Cows selected exclusively for high milk yield experienced a longer interval from calving to C-LA than cows with no selection response for milk yield. The interval to C-LA in cows selected for both milk production and fertility was intermediate between the high and low milk production lines. Energy balance was significantly related to C-LA, and correction for EB accounted for part of the difference in time to C-LA between the selection lines. The increase in the interval from calving to C-LA by selection for high milk yields can be reduced by inclusion of fertility traits in the breeding program.

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Characterization of progesterone profiles in fall-calving Norwegian Red cows

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ABSTRACT

Progesterone profiles in Norwegian Red cows were categorized, and associations between the occurrence of irregularities in the profiles and the commencement of luteal activity were investigated. The cows were managed in 3 feeding trials from 1994 to 2001 and from 2005 to 2008 at the Norwegian University of Life Sciences. The cows were followed from calving, and the milk samples collected represented 502 lactations from 302 cows. Milk samples for progesterone analysis were taken 3 times weekly from 1994 throughout 1998 and from 2005 to 2008 and 2 times weekly from 1999 to 2001. Commencement of luteal activity was defined as the first day of 2 consecutive measurements of progesterone concentration $\geq$3 ng/mL not earlier than 10 d after calving. Delayed ovulation type I was defined as consistently low progesterone concentration, <3 ng/mL for $\geq$50 d postpartum. Delayed ovulation type II was defined as prolonged interluteal interval with milk progesterone measurements <3 ng/mL for $\geq$12 d between 2 luteal phases. Persistent corpus luteum (PCL) type I was defined as delayed luteolysis with milk progesterone $\geq$3 ng/mL for $\geq$19 d during the first estrous cycle postpartum. Persistent corpus luteum type II was defined as delayed luteolysis with milk progesterone $\geq$3 ng/mL for $\geq$19 d during subsequent estrous cycles before first artificial insemination. Delayed ovulation type I was present in 14.7%, delayed ovulation type II in 2.8%, PCL type I in 6.7%, and PCL type II in 3.3% of the profiles. Commencement of luteal activity was related to milk yield, parity, PCL type I, and the summated occurrence of PCL type I and II. The least squares means for the interval to commencement of luteal activity were 24.2 d when PCL type I and II were present and 29.5 d when PCL type I and II were absent. The likelihood of pregnancy to first service was not affected in cows with a history of PCL when artificial insemination was carried out at progesterone concentrations <3 ng/mL (i.e., during estrus); however, cows that had experienced PCL were more likely to be inseminated during a luteal phase. The occurrence of delayed ovulation and PCL in Norwegian Red cows was less than that reported in most other dairy populations.
Key words: progesterone profile, fertility, pregnancy, dairy cow

INTRODUCTION

The fertility of dairy cows has declined while milk yield has increased in recent decades (Lucy, 2001). The inclusion of fertility indices in dairy cattle breeding has been prioritized to avoid a further decline of fertility due to the unfavorable genetic correlations between milk yield and reproductive performance (Veerkamp et al., 2001; Royal et al., 2002; Pryce et al., 2004). In contrast to the majority of countries, female fertility has been included in the total merit index in the Norwegian Red since 1972 (Andersen-Ranberg et al., 2005; Steine et al., 2008). The relative emphasis on fertility has been 8 to 15% throughout this period. The population-average pregnancy and calving percentage to first AI is estimated to be 61 and 54%, respectively, in this breed (Garmo et al., 2008).

Previous investigations of postpartum progesterone profiles in high-yielding Holstein cows reported the incidence of delayed first ovulation to be between 5.5 and 25.3%, cessation of ovarian activity between 3.3 and 12.9%, and persistent corpus luteum (**PCL**) between 7.3 and 35.2% (Opsomer et al., 2000; Royal et al., 2000; Shrestha et al., 2004; Petersson et al., 2006; Samarütel et al., 2008).

A previous study reported that cows with early commencement of luteal activity (**C-LA**) after calving have an increased probability for early AI, shorter interval to conception, and greater conception rate (Darwash et al., 1997a). However, it has also been reported that

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early C-LA is a risk factor for irregular luteal cyclicity in the form of PCL (Opsomer et al., 2000; Royal et al., 2002; Petersson et al., 2007) and that PCL is associated with a decrease in pregnancy to first AI (Royal et al., 2000; Shrestha et al., 2004). It has also been reported that delayed first ovulation and cessation of cyclical activity increase the calving interval (Petersson et al., 2006) and decrease the pregnancy to first AI (Royal et al., 2000).

Measurement of traditional fertility traits such as nonreturn rate and days to first AI (Garmo et al., 2008), as well as effect of selection for milk yield and fertility on the postpartum interval to C-LA, has been investigated previously in Norwegian Red cows (Garmo et al., 2009). Thus there has been no classification of progesterone patterns postpartum in the breed, and the relationship between the progesterone patterns, C-LA, and subsequent reproductive performance has not yet been investigated.

The objectives for the study were 1) to quantify the occurrence of typical and atypical progesterone profiles in Norwegian Red cows, 2) to assess the relationship between time to C-LA and the occurrence of PCL, and 3) to investigate cow- and management-specific factors related to the occurrence of PCL.

MATERIALS AND METHODS

The milk progesterone data set for the current study was derived from Norwegian Red cows managed in 3 feeding trials designed to assess the effects of different levels of concentrate and different quality of roughage on milk yield, energy balance, and reproduction at the Norwegian University of Life Sciences from 1994 to 2001 and from 2005 to 2008. Complete milk samples were obtained from 502 lactation periods. The distribution of lactations by parity was 180, 170, and 152 for first, second, and >second parity, respectively. The average 305-d milk yields for all lactations in the herd from 1998 to 2008 were 6,275, 7,144, and 7,733 kg for first, second, and >second lactation, respectively. The average milk fat and milk protein in the bulk milk for 2007 were 4.08 and 3.32%, respectively. The herd consisted of 134 dairy cows in 2007.

Management

The cows were milked twice daily and housed in either the freestall or the tiestall barn. There were 495 lactations with housing data: 344 lactations in the freestall barn and 151 in the tiestall barn. The breeding season started in November each year such that the voluntary waiting period varied between cows. The cows were on pasture from late May to early September each year.

Table 1. Frequency of reproductive and obstetrical conditions, mastitis, and other diseases before and after commencement of luteal activity (C-LA; n = 502)

Item	Before C-LA	After C-LA
Reproductive and obstetrical conditions[1]	25	27
Clinical mastitis	53	63
Other diseases[2]	18	27

[1]Dystocia, retained placenta, metritis, vaginitis, cystic ovaries, abortion.

[2]Laminitis, ketosis, indigestions, milk fever.

Only cows that calved during the fall indoor season were included in the study. Skilled teaching veterinarians from the Ambulatory Practice at the Norwegian School of Veterinary Science performed all AI in the herd during the entire study period. Pregnancy was verified by rectal palpation by veterinarians approximately 6 wk after AI. Cows that did not subsequently deliver a calf (n = 36) were regarded as pregnant to first AI if milk progesterone was <3 ng/mL at AI with a subsequent increase in progesterone content ≥3 ng/mL for at least 24 d after AI. The cows were not exposed to a synchronization program and did not receive any hormone treatment before C-LA. The frequencies of diagnosed reproductive disorders, clinical mastitis, and other diseases before C-LA are presented in Table 1. Estrus detection was performed daily by visual observation in the morning, afternoon, and late evening. In addition, estrus observations were occasionally carried out during other routine work in the barn. All observations were recorded on a designated 21-d calendar.

Feeding Protocol

The cows were assigned to different concentrate rations such that the groups were similar in terms of weight, age, and body condition at calving (Edmonson et al., 1989). There were 484 lactations with complete records on concentrate allocation. The distribution of concentrate allocation by parity during peak lactation is presented in Table 2. Cows were given ad libitum access to roughage. The distribution of the feedstuff in the herd in 2007 was 34% concentrate, 40% roughage, and 26% pasture.

Milk Yield and Milk Progesterone

The average weekly milk yield for the fourth week postpartum was calculated for 430 lactation periods. For an additional 54 lactations with missing records on weekly milk yield, records for monthly milk yield were obtained from the Norwegian Dairy Herd Record System. The milk yield record closest to d 30 of lactation

Table 2. Distribution of concentrate allocation (1 kg = 6.9 MJ) in peak lactation by parity from 1994 to 2008 (n = 484)

Concentrate (kg/d)	First parity	Second parity	>Second parity
≤6	51	58	39
7–9	96	26	72
≥10	24	79	39

was used. Together, these 484 observations expressed the milk yield during the fourth week of lactation.

Milk progesterone concentrations were measured in a representative sample of whole milk collected during milking every Monday, Wednesday, and Friday from 1994 throughout 1998 (n = 232) and from 2005 to 2008 (n = 143) and every Monday and Friday from 1999 to 2001 (n = 127) by an enzyme immunoassay (Waldmann, 1993) modified by using the second antibody coating technique. The specificity of the antibody has been described previously (Waldmann, 1999). The interassay coefficients of variation for milk progesterone concentrations of 1.48 and 19.66 ng/mL were 9.21 and 5.32%, respectively. The intraassay coefficient of variation was less than 10%.

Definitions of DOV, C-LA, PCL, Luteal Phase, and Interovulatory Interval

Delayed ovulation (**DOV**) type I was defined as consistently low progesterone concentration (<3 ng/mL) for ≥50 d postpartum. Delayed ovulation type II was defined as prolonged interluteal interval with milk progesterone measurements <3 ng/mL for ≥12 d between 2 luteal phases (Royal et al., 2000). There were 502 lactations with data for DOV type I or type II.

The interval from calving to C-LA was defined as the first day of the 2 consecutive measurements of milk progesterone concentration ≥3 ng/mL (Royal et al., 2000) more than 10 d after calving. Of the 502 lactations, adequate data were available to identify C-LA in 497 lactations. The remaining 5 cows were removed from the herd before C-LA, and these cows had progesterone measurements until 56, 67, 100, 124, and 130 DIM.

Persistent corpus luteum type I, PCL type II, the length of the first luteal phase, and the first interovulatory interval postpartum were measured according to the definitions by Royal et al. (2000). Persistent corpus luteum type I was defined as delayed luteolysis during the first estrous cycle postpartum with milk progesterone ≥3 ng/mL for ≥19 d, whereas PCL type II was defined as delayed luteolysis with milk progesterone ≥3 ng/mL for ≥19 d during subsequent estrous cycles before AI. The length of the first luteal phase was measured as the time from the first sample of milk progesterone ≥3 ng/mL to the last consecutive progesterone sample ≥3 ng/mL (Royal et al., 2000). Among the 497 lactations that were available for the determination of C-LA, individual progesterone samples were missing for 8 lactation periods, and in 9 lactation periods, AI was performed at first estrus. Therefore, the length of the first luteal phase and PCL could be determined for 480 lactations. The same limitation also applies to the assessment of interovulatory intervals. In addition, one cow was culled after the first luteal phase but before the start of a new cycle, such that there were 479 lactations available for the calculation of interovulatory intervals. The length of the interovulatory intervals was considered to be a measurement of the total estrous cycle length and was defined as the interval between the milk progesterone rise (≥3 ng/mL) in one estrous cycle to the rise of milk progesterone (≥3 ng/mL) in the next cycle (Royal et al., 2000). The lengths of the luteal phases and interovulatory intervals were calculated with and without profile abnormalities (DOV and PCL) included (Table 3).

Pregnancy to First AI Related to a Previous Episode of PCL

Of the 502 lactations included in the study, 476 were presented for AI after calving. Cows in which AI was performed in the luteal phase (n = 56) and one cow (n = 1) experiencing PCL after first AI were omitted. In addition, all profiles with abnormalities were excluded from the baseline control group, such that the occurrence of PCL type I or the summated occurrence of PCL type I and II was compared with only normal progesterone profiles (n = 316).

AI During the Luteal Phase Related to a Previous Episode of PCL

The definition of first AI during a luteal phase was based on the progesterone measurements taken 2 or 3 times weekly. The exact determination of whether a cow was presented for first AI during the luteal phase was not possible in 26 cows because data on the progesterone measurement within ±2 d to first AI were missing. These cows were omitted from the analysis. The progesterone measurement closest to AI was at the day for insemination for 37% of the cows, ±1 d for 54%, and ±2 d for 9% of the cows. In addition, the profiles were evaluated to confirm that these cows actually were inseminated during the luteal phase. One cow that experienced PCL after first AI was also omitted. All profiles with abnormalities were excluded from the baseline control group in the analysis, such that the occurrence of PCL type I, PCL type II, or the summated

Table 3. Number of lactations and means and 95% confidence intervals (CI) for the lengths of luteal phases and interovulatory intervals during the first 4 cycles postpartum in Norwegian Red cows presented with profile abnormalities (PCL[1] and DOV[2]) included and excluded

	Luteal phase			Interovulatory interval		
Cycle no.	n	Mean	95% CI	n	Mean	95% CI
PCL and DOV included						
1	480[3]	10.5[a]	9.7–11.3	479[3]	19.3[a]	18.4–20.2
2	376	12.5[b]	12.0–12.9	359	20.6[b]	20.3–21.0
3	261	12.4[b]	12.0–12.9	250	20.9[b]	20.4–21.3
4	153	12.5[b]	11.9–13.2	144	20.7[b]	20.1–21.3
PCL and DOV excluded						
1	372[3]	9.0[a]	8.5–9.4	371[3]	17.4[a]	16.9–18.0
2	300	12.3[b]	11.9–12.6	294	20.7[b]	20.3–21.1
3	210	11.9[b]	11.5–12.4	207	20.8[b]	20.3–21.2
4	124	12.2[b]	11.6–12.7	121	20.7[b]	20.1–21.4

[a,b]Different superscripts under luteal phase indicate significant differences between the lengths of the luteal phases based on the 95% CI. Different superscripts under interovulatory interval indicate significant differences between the interovulatory intervals based on the 95% CI.

[1]PCL = persistent corpus luteum; PCL type I = delayed luteolysis with milk progesterone concentration ≥3 ng/mL for ≥19 d during the first estrous cycle postpartum; PCL type II = delayed luteolysis during estrous cycles after the first cycle postpartum.

[2]DOV = delayed ovulation; DOV type I = prolonged anovulatory period with milk progesterone concentration <3 ng/mL for ≥50 d postpartum; DOV type II = prolonged interluteal interval with milk progesterone <3 ng/mL for ≥12 d between 2 luteal phases.

[3]One cow was culled after first luteal phase but before start of a new estrous cycle.

occurrence of PCL type I and II was compared with only normal progesterone profiles (n = 335).

Statistical Analysis

Associations Between C-LA and PCL, Milk Yield, Parity, Housing, Year, and Disease Before C-LA. Mixed linear models were performed using SPSS (SPSS Inc., 2006) to assess relationships between the natural logarithm of days to C-LA (**lnC-LA**) and the explanatory variables milk yield at the fourth week of lactation (**MY**), parity (first, second, >second), year of study (1994 to 1998, 1999 to 2001, and 2005 to 2007), housing (freestall or tiestall), disease treatment before C-LA (1/0), PCL type I (1/0), and the summated occurrence of PCL type I and II (1/0). The outcome variable days to C-LA was transformed by natural logarithm to obtain approximate normality of residuals. Explanatory variables associated with lnC-LA with $P < 0.20$ were included in an extended model. Level of concentrate was included in the model as a random effect. Covariance between multiple lactations within the same cow was accounted for by a compound symmetry correlation structure. Multiple comparison adjustment for the pairwise difference in least squares means (**LS-means**) was performed using the Bonferroni option in SPSS (SPSS Inc., 2006).

Associations Between PCL and MY, Concentrate Allocation, Parity, Year, Housing, Disease Before C-LA, and DOV. The associations between the outcome PCL type I (1/0), PCL type II, or the summated occurrence of PCL type I and II, and the explanatory variables were tested separately using a general estimating equation (**GEE**) approach in SPSS adjusted for multiple lactations within the same cow by the use of a compound symmetry correlation structure (SPSS Inc., 2006). The following variables were used when the likelihood of PCL in separate (univariate) models was evaluated: MY, concentrate allocation (≤6 kg, 7–9 kg, and ≥10 kg), parity (first, second, >second), year of study (1994 to 1998, 1999 to 2001, and 2005 to 2007), housing (freestall or tiestall), disease treatment before C-LA, and the summated occurrence of DOV type I and II (1/0). An extended model was not constructed because year of trial was the only explanatory variable with $P < 0.20$.

Likelihood of Pregnancy to First AI Related to a Previous Episode of PCL. The associations between the likelihood of pregnancy to first AI (1/0) and the explanatory variables PCL type I, PCL type II, or the summated occurrence of PCL type I and II were tested separately using the GEE approach in SPSS (SPSS Inc., 2006). The associations were adjusted for multiple lactations within the same cow by the use of a compound symmetry correlation structure. The interval from calving to first AI was originally included in all models but omitted from the analyses because it was not associated with the outcome variable, pregnancy to first AI.

Likelihood of AI During the Luteal Phase Related to a Previous Episode of PCL. The associations between the likelihood of performing the first AI during the luteal phase (progesterone concentration ≥3 ng/mL; 1/0) and the explanatory variables PCL type I, PCL type II, or the summated occurrence of PCL types I and II were tested separately using the GEE approach in SPSS (SPSS Inc., 2006). The associations were adjusted for multiple lactations within the same cow by the use of a compound symmetry correlation structure. Statistical significance was considered to be $P < 0.05$ for all analyses.

RESULTS

The overall average lnC-LA was 3.261 ± 0.025, representing an interval from calving to C-LA of 26.1 d. Average milk yields in the fourth week of lactation were 22.0, 27.2, and 30.1 kg for first, second, and >second parity, respectively.

Length of Luteal Phases and Interovulatory Intervals

Lengths of the first 4 luteal phases and interovulatory intervals with PCL and DOV included and excluded are presented in Table 3. Length of first luteal phase was shorter than the lengths of consecutive luteal phases. Length of first interovulatory interval, which is an estimate of estrous cycle length, was shorter in the first cycle compared with subsequent cycles.

Progesterone Profile Abnormalities

Delayed ovulation type I was present in 14.7% (74/502) of the lactations; n = 42, 22, and 10 in first, second, and >second parity, respectively. The length of DOV type I was 50 to 130 d, with a mean of 76.0 ± 2.4 d. Delayed ovulation type II was present in 2.8% (14/502) of the lactations; n = 5, 6, and 3 in first, second, and >second parity, respectively. The length of DOV type II was 12 to 101 d, with a mean of 30.9 ± 6.8 d. Five cows experienced both DOV type I and type II during the same lactation.

In total, PCL was present in 9.6% (46/480) of the lactations. Two cows experienced both types of PCL in the same lactation. Persistent corpus luteum type I was present in 6.7% (32/480) of lactations; n = 13, 9, and 10 for first, second, and >second parity, respectively. The length of PCL type I was 19 to 105 d, with a mean of 33.8 ± 3.2 d. Persistent corpus luteum type II was present in 3.3% (16/480) of lactations; n = 4, 4, and 8 for first, second, and >second parity, respectively. The length of PCL type II was 19 to 42 d, with a mean of 27.5 ± 2.2 d.

Three cows experienced both DOV type I and PCL type I during the same lactation, and one cow experienced both DOV type I and PCL type II during the same lactation.

Associations Between C-LA and MY, PCL, Parity, Year, Housing, and Disease Before C-LA

When the variable lnC-LA was tested against individual explanatory variables, P-values ≤0.20 (type III statistic) were calculated for MY ($P = 0.03$), parity ($P < 0.01$), housing ($P < 0.01$), the summated occurrence of PCL type I and II ($P = 0.04$), PCL type I ($P = 0.08$), and PCL type II ($P = 0.18$).

Associations between lnC-LA and the explanatory variables MY, parity, housing, and PCL (type I or the summated occurrence of PCL type I and II), when entered simultaneously in the extended mixed-model analysis, are presented in Table 4. In the type III statistics F-test, lnC-LA was associated with MY ($P < 0.01$), parity ($P < 0.01$), housing ($P = 0.01$), and PCL type I ($P = 0.01$) or the summated occurrence of PCL type I and II ($P = 0.02$). In the model with PCL type I included as explanatory variable, the LS-means for lnC-LA were 3.141 ± 0.111 when PCL type I was present and 3.382 ± 0.065 when PCL type I was absent, representing an interval from calving to C-LA of 23.1 and 29.4 d, respectively. The LS-means for lnC-LA were 3.468 ± 0.090, 3.179 ± 0.085, and 3.137 ± 0.085, representing an interval from calving to C-LA of 32.1, 24.0, and 23.0 d for first, second, and >second parity, respectively. The LS-means for lnC-LA were 3.194 ± 0.079 (freestall) and 3.329 ± 0.087 (tiestall), representing an interval from calving to C-LA of 24.4 and 27.9 d for freestall and tiestall, respectively.

In the model with the summated occurrence of PCL type I and II included as explanatory variable, the LS-means for lnC-LA were 3.187 ± 0.099 when PCL type I or II was present and 3.384 ± 0.065 when PCL type I or II was absent, representing an interval from calving to C-LA of 24.2 and 29.5 d, respectively. The LS-means for lnC-LA were 3.490 ± 0.087, 3.201 ± 0.080, and 3.165 ± 0.080 for first, second, and >second parity, representing an interval from calving to C-LA of 32.8, 24.6, and 23.7 d for first, second, and >second parity, respectively. The LS-means for lnC-LA were 3.216 ± 0.075 (freestall) and 3.355 ± 0.082 (tiestall), representing an interval from calving to C-LA of 24.9 and 28.6 d for freestall and tiestall, respectively.

Associations Between the Likelihood of PCL and: MY, Concentrate Allocation, Parity, Year, Housing, DOV, and Disease Before C-LA

The separate GEE analyses on the relationships between the summated occurrence of PCL type I and II and the explanatory variables MY, concentrate allocation, parity, year, housing, the occurrence of DOV type I or II, and disease treatment before C-LA are pre-

Table 4. Associations between number of days to commencement of luteal activity[1] (natural logarithmic transformed) and the explanatory variables

	PCL type I[2]			PCL type I and II[2]		
Item	β	SE	*P*-value	β	SE	*P*-value
First parity	—	—	—	—	—	—
Second parity	−0.290	0.060	<0.01	−0.289	0.060	<0.01
>Second parity	−0.331	0.072	<0.01	−0.325	0.072	<0.01
PCL: no[3]	—	—	—	—	—	—
PCL: yes	−0.241	0.093	0.01	−0.198	0.079	0.01
Tiestall[3]	—	—	—	—	—	—
Freestall	−0.136	0.05	0.01	−0.139	0.054	0.01
MY	0.029	0.005	<0.01	0.029	0.005	<0.01
Intercept	2.885	0.125	<0.01	2.893	0.125	<0.01

[1]Associations assessed in 2 separate multivariable mixed linear models corrected for level of concentrate allocation as random effect variable and adjusted for multiple lactations within the same cow by the use of a compound symmetry correlation matrix. Explanatory variables included in the models were parity, milk yield in the fourth week of lactation (MY), housing (freestall, tiestall), and persistent corpus luteum (PCL) type I or the summated occurrence of PCL type I and type II.

[2]PCL type I = delayed luteolysis with milk progesterone concentration ≥3 ng/mL for ≥19 d during the first estrous cycle postpartum; PCL type II = delayed luteolysis during estrous cycles after the first cycle postpartum.

[3]Categorical variables assigned as baselines.

sented in Table 5. There were no associations between the summated occurrence of PCL type I and II and the predictors when assessed separately in the GEE analysis.

Likelihood of Pregnancy to First AI Related to a Previous Episode of PCL

Of 476 lactations presented for AI after calving, 256 (53.8%) resulted in pregnancy after first AI. Number of observations and odds ratios for pregnancy to first AI in cows that experienced PCL type I or the summated occurrence of PCL type I and II are presented in Table 6. There were no associations between the likelihood of pregnancy to first AI and a previous episode of PCL type I or the summated occurrence of PCL type I and II compared with cows with normal progesterone profiles.

Likelihood of AI During the Luteal Phase Related to a Previous Episode of PCL

Overall, 12.4% (56/450) of the AI were performed during the luteal phase. Pregnancy to first AI was recorded to be 60.7% (239/394) in the 394 first AI performed when milk progesterone concentrations were known to be low. Associations between first AI during the luteal phase and the occurrence of PCL type I, PCL type II, or the summated occurrence of PCL types I and II are presented in Table 7. The likelihood of performing the first AI during the luteal phase was 6.8 times higher in cows with PCL type II and 3.2 times higher for the summated occurrence of PCL type I and II compared with cows with normal progesterone profiles.

DISCUSSION

The feeding regimens and overall herd management employed in the current study reflect those commonly employed by Norwegian farmers. Thus, the results presented should give reliable information about luteal function in Norwegian Red cows. However, clinical trials differ from field environments, and this limitation should be taken into account when the findings of the study are interpreted.

The definitions of DOV and PCL and sample frequency in the current study are the same as used by Royal et al. (2000) and Petersson et al. (2007). In the studies conducted by Opsomer et al. (2000) and Shrestha et al. (2004), the definitions of DOV and PCL are slightly different but comparable with the definitions of the current study.

The occurrence of PCL was generally low in Norwegian Red compared with other dairy breeds studied (Opsomer et al., 1998; Royal et al., 2000; Shrestha et al., 2004; Samarütel et al., 2008). Whether this is related to the inclusion of female fertility in the breeding program since 1972 remains unknown (Andersen-Ranberg et al., 2005; Steine et al., 2008). However, population studies of reproductive performance in the breed have shown encouraging results (Garmo et al., 2008). The interval to C-LA and the effect of energy balance, parity, and genetic merit for milk yield and fertility in Norwegian Red cows has been described previously (Garmo et al.,

Table 5. Likelihood of persistent corpus luteum (PCL)[1] type I and II as assessed by general estimating equations adjusted for multiple lactations within the same cow by the use of a compound symmetry correlation matrix[2]

Item	n = PCL/overall	β	SE	OR	*P*-value
MY	43/465	−0.003	0.026	0.997	0.92
Concentrate	46/464	—	—	—	0.27
≤6 kg[3]	143	—	—	1.0	—
7–9 kg	185	−0.106	0.383	0.899	—
≥10 kg	136	0.452	0.385	1.571	—
Parity	46/480	—	—	—	0.63
First[3]	167	—	—	1.0	—
Second	164	−0.188	0.363	0.829	—
>Second	149	0.184	0.360	1.203	—
Year	46/480	—	—	—	0.10
1994–1998	223	—	—	1.0	—
1999–2001	120	0.410	0.444	1.507	—
2005–2007	137	0.803	0.377	2.231	—
Housing	45/473	—	—	—	0.60
Tiestall[3]	141	—	—	1.0	—
Freestall	332	0.194	0.370	1.214	—
Disease before C-LA	46/480				0.30
No[3]	401	—	—	1.0	—
Yes	79	0.381	0.369	1.463	—
DOV type I and II[4]	46/480	—	—	—	0.31
Not present[3]	414	—	—	1.0	—
Present	66	−0.559	0.552	0.572	—

[1]PCL type I = delayed luteolysis with milk progesterone concentration ≥3 ng/mL for ≥19 d during the first estrous cycle postpartum; PCL type II = delayed luteolysis during estrous cycles after the first cycle postpartum.

[2]The associations were assessed in separate models for each of the fixed-effect variables: milk yield in the fourth week of lactation (MY), concentrate allocation (≤6, 7–9, and ≥10 kg), parity (first, second, and >second), year of study, the sum of delayed ovulation (DOV) type I and II, disease before commencement of luteal activity (C-LA), and housing (freestall, tiestall).

[3]Categorical variables assigned as baselines.

[4]DOV type I = prolonged anovulatory period with milk progesterone concentration <3 ng/mL for ≥50 d postpartum; DOV type II = prolonged interluteal interval with milk progesterone <3 ng/mL for ≥12 d between 2 luteal phases.

2009). Petersson et al. (2006) reported a slightly less frequent occurrence of PCL, compared with the current study, in the Swedish Red and Whites and Swedish Holstein, in which fertility also has been included in the breeding program since the 1970s. Petersson et al. (2006) also reported that Swedish Holstein had a greater risk for atypical progesterone profiles compared with the Swedish Red and Whites.

The interval from calving to C-LA was associated with the occurrence of PCL in the current study. Heritability for early C-LA is reported to be 0.16 to 0.21 (Darwash et al., 1997b; Royal et al., 2002; Petersson et al., 2007), whereas traditional fertility traits such as nonreturn rate, calving interval, interval to first AI, and number of inseminations per conception, which are the basis in breeding for increased fertility, have

Table 6. Likelihood of pregnancy to first AI assessed by general estimating equations adjusted for multiple lactations within the same cow by the use of a compound symmetry correlation matrix[1]

Item	n	β	SE	Odds ratio (OR)	95% Confidence interval for OR	*P*-value
PCL type I[2]						
Present	20	−0.725	0.467	0.484	0.194–1.210	0.12
Normal profiles	316	—	—	1.0	—	—
PCL type I and II[2]						
Present	29	−0.456	0.396	0.634	0.292–1.377	0.25
Normal profiles	316	—	—	1.0	—	—

[1]The analyses were performed in separate analyses for persistent corpus luteum (PCL) type I or the summated occurrence of PCL type I and II. The baseline control group consisted exclusively of lactations in which normal luteal activity had been recorded.

[2]PCL type I = delayed luteolysis with milk progesterone concentration ≥3 ng/mL for ≥19 d during the first estrous cycle postpartum; PCL type II = delayed luteolysis during estrous cycles after the first cycle postpartum.

Table 7. Likelihood of performing first AI during the luteal phase (progesterone ≥3 ng/mL) assessed by general estimating equation adjusted for multiple lactations within the same cow by the use of a compound symmetry correlation matrix[1]

Item	n	β	SE	Odds ratio (OR)	95% Confidence interval for OR	*P*-value
PCL type I[2]						
Present	25	0.915	0.512	2.498	0.916–6.811	0.07
Normal profiles	335	—	—	1.0	—	—
PCL type II[2]						
Present	11	1.921	0.627	6.826	1.996–23.349	<0.01
Normal profiles	335	—	—	1.0	—	—
PCL type I and II[2]						
Present	38	1.193	0.378	3.295	1.570–6.916	<0.01
Normal profiles	335	—	—	1.0	—	—

[1]The analyses were performed in separate analyses for persistent corpus luteum (PCL) type I, PCL type II, or the summated occurrence of PCL type I and II. The baseline control group consisted exclusively of lactations in which normal luteal activity had been recorded.

[2]PCL type I = delayed luteolysis with milk progesterone concentration ≥3 ng/mL for ≥19 d during the first estrous cycle postpartum; PCL type II = delayed luteolysis during estrous cycles after the first cycle postpartum.

heritabilities from 0.018 to 0.040 (Pryce et al., 1997; Wall et al., 2003; Jamrozik et al., 2005). Selection for shorter intervals to C-LA, which has a moderate heritability, may be a feasible approach to improving fertility (Darwash et al., 1997b). However, inclusion of C-LA only as a fertility trait in a breeding program should be considered with caution because of the increased risk of PCL rather than continuous cyclical luteal activity after early C-LA (Opsomer et al., 2000; Royal et al., 2002; Petersson et al., 2007). In the current study, the interval to C-LA was approximately 5 d shorter in cows that subsequently experienced PCL, which demonstrates the importance of elucidating the potential negative side effects of breeding for early C-LA.

A previous episode of PCL was not associated with the likelihood of pregnancy to first insemination in cows that were inseminated while having low progesterone levels. The latter is in contrast with both Royal et al. (2000), who reported PCL to be associated with a decrease in pregnancy to first AI, and Shrestha et al. (2004), who reported a lower pregnancy rate within 100 DIM. However, cows experiencing PCL were more likely to be inseminated during the luteal phase, which might explain the results obtained in previous studies of associations between pregnancy rate and PCL by Royal et al. (2000) and Shrestha et al. (2004). To our knowledge, the relationship between the increased likelihood of insemination during the luteal phase and the occurrence of PCL has not been reported before. The occurrence of PCL type II was highly significantly associated, whereas PCL type I showed a tendency to be associated with the likelihood of AI during the luteal phase. Cows that experienced PCL type II could have expressed signs of estrus during a previous cycle and, hence, presented for insemination at the expected time for the following estrus. Another reason could be altered behavior in these animals because of ovarian pathology (Vanholder et al., 2006). First AI was performed during the luteal phase in 12.4% of the lactations, which is greater than the population estimate of 4.4% (Andresen and Onstad, 1979) and 4.9% (Garmo et al., 2008) in Norwegian Red cows.

There were no associations between the occurrence of PCL and milk yield, concentrate allocation, parity, year of trial, disease before C-LA, or DOV. Windig et al. (2008) could not find an association between prolonged luteal activity and milk production. Pollott and Coffey (2008) reported a greater-than-expected number of PCL type II in cows fed high levels of concentrate, and genetic line did not affect the occurrence of PCL type I or type II. Parity was not associated with the occurrence of PCL in the current study, which contrasts with Opsomer et al. (2000), who reported parity to be a risk factor for prolonged luteal phases. Opsomer et al. (2000) reported that the most important risks for delayed luteolysis were early C-LA, occurrence of metritis, retained placenta, calving problems, and abnormal vaginal discharge. Petersson et al. (2006) also reported endometritis to be a risk factor for PCL. Thus, prolonged luteal phases could be the result of uterine problems rather than ovarian pathology. It has been suggested that ovulation before complete uterine involution might increase the risk of developing pyometra (Opsomer et al., 2000). In the current study, the low occurrence of PCL could partly be explained by the very low number of puerperal problems in the cows included. This corresponds well with the population of Norwegian Red cows in which the incidence rates per 100 cow-years in 2005 were 1.2, 3.1, 0.2, and 0.9 for dystocia; retained placenta; abortion; and metritis, vaginitis or salphangitis, respectively (Østerås et al., 2007).

Delayed ovulation type I was present in 14.7% of the profiles, which was less than in some previous studies of Holstein cows in which DOV type I was reported to be 25.3% (Samarütel et al., 2008) and 20.5% (Opsomer et al., 1998), whereas an estimate of 15.6% was found in Swedish Holstein and Swedish Red and Whites (Petersson et al., 2006). However, Shrestha et al. (2004) reported the proportion of DOV type I to be 13.2%, whereas Royal et al. (2000) reported 10.9 and 12.9% from 1975 to 1982 and from 1995 to 1998, respectively. In the current study, DOV type II occurred in only 2.8% of the profiles. The incidence agrees with previous studies in Holstein cows that reported the proportion of DOV type II to be 4.2 (Samarütel et al., 2008), 3.3 (Shrestha et al., 2004), and 3.0% (Opsomer et al., 1998) but is less than the DOV type II estimates of 12.9 and 6.6% reported by Royal et al. (2000) and Petersson et al. (2006), respectively.

Length of first luteal phases (10.5 d) agreed with Royal et al. (2000), who reported the length of first luteal phase to be 10.8 d in British Friesian from 1975 to 1982. The same study reported the length of first luteal phase to be 14.6 d from 1995 to 1998 after there had been a large breed substitution of British Friesian with North American Holstein and a significant increase in milk yield in the population.

Length of first interovulatory interval, a measure of the estrous cycle length, was 17.4 and 19.3 d when profiles with abnormalities were excluded and included, respectively. First interovulatory interval was shorter in the first cycle compared with consecutive cycles (20.6 to 20.7 d) in cows with normal profiles. Royal et al. (2000) reported increased length of first interovulatory interval from 18.9 d (1975 to 1982) to 22.5 d (1995 to 1998) when both normal and abnormal profiles were included. Fonseca et al. (1983) reported the length of the first estrous cycle to be 17.1 d and the length of the second estrous cycle to be 21.4 d in Holstein cows in North Carolina when only normal cows were included. Thus, the current length of the estrous cycles, with or without abnormalities, in Norwegian Red cows corresponds well with estrous cycle length observed in the Holstein breed 25 years ago. For future estimates of estrous cycle length, it is feasible to investigate cycle length with profile abnormalities included and excluded. This would provide a more reliable assessment of whether the increased cycle length is due to PCL and DOV type II or other causes.

CONCLUSIONS

The occurrence of DOV and PCL in Norwegian Red cows in the current study is less frequent than that reported in most other dairy populations. Cows experiencing PCL had shorter interval to C-LA. The occurrence of PCL did not affect pregnancy to first AI when AI was performed at low progesterone concentrations, but cows with PCL were more likely to experience AI during a luteal phase.

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IV

Reproductive Performance, Udder Health, and Antibiotic Resistance in Mastitis Bacteria isolated from Norwegian Red cows in Conventional and Organic Farming

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ABSTRACT

Background: During the last 35 years, the Norwegian Red breeding program has paid attention to functional breeding objectives, including health and fertility traits. Hence, the breed may be expected to perform well with regards to fertility and udder health in both conventional and organic farming systems. The objectives of this study were to investigate whether there were differences between Norwegian Red cows in conventional and organic farming with respect to reproductive performance, udder health, and antibiotic resistance in udder pathogens.

Methods: Twenty-five conventional and 24 organic herds from south-east and middle Norway participated in the study. Herds were selected such that geographical location, herd size, and barn types were similar across the cohorts. All organic herds were certified as organic between 1997 and 2003. All herds were members of the Norwegian Dairy Herd Recording System. The herds were visited once during the study. The relationship between the outcomes and explanatory variables were assessed using mixed linear models.

Results: There were less >2nd parity cows in conventional farming. The conventional cows had higher milk yields and received more concentrates than organic cows. Although after adjustment for milk yield and parity, somatic cell count was lower in organic cows than conventional cows. There were a higher proportion of dry quarters at the herd visit in organic herds. The interval from calving to first AI was shorter in conventional cows (LS-means: 75.0 vs 77.7 d), whereas there were no differences in the interval to last AI or calving interval. There was no difference between conventional and organic cows in quarter samples positive for mastitis bacteria from the herd visit. Milk yield and parity were associated with the likelihood of at least one quarter positive for mastitis bacteria. There was few *S. aureus* isolates resistance to penicillin in both management systems. Penicillin resistance against Coagulase negative staphylococci isolated from subclinically infected quarters was 48.5% in conventional herds and 46.5% in organic herds.

Conclusion: There were no large differences between reproductive performance and udder health between conventional and organic farming. Norwegian Red is a sustainable breed that adapts well to both intensive and more extensive production systems like organic production.

Background

Organic agriculture aims to be a holistic production management system which promotes and enhances ecosystem health, including biological cycles and soil biological activity. The primary goal for organic agriculture is to optimize the health and productivity of inter-dependent communities of soil life, plants, animals and people [1]. Organic farms are supposed to be self-sufficient for animal feed, and the use of chemical fertilizers or herbicides are prohibited. In recent years, there has been increased attention to organic farming. According to recent statistics, 3.9% of the cultivated land, 2.1% of total milk production, and 2.6% (6800 cows) of the dairy cows are managed organically [2]. The legislation governing Norwegian organic farming is based on principles derived from International Federation of Organic Agriculture Movements [2]. At least 50% of feed on an organic farm should be produced on the farm itself and roughage should constitute 60% of energy fed in dry matter intake. The proportion of roughage can be reduced to 50 % the first three months of lactation.

Use of synthetic veterinary products prophylactically is prohibited. Withdrawal times for prescribed products are twice as long as corresponding time periods for conventional farming. During one year a maximum of three treatments periods with drugs are allowed for each individual. Natural mating is preferred over artificial insemination (**AI**). Embryo transfer and estrus synchronization programs are prohibited. On the individual level, hormones such as gonadotropin releasing hormone and prostaglandins are only allowed for treatment of ovarian cysts or luteolysis [3].

Compared with conventional cows a lower milk yield, and impaired reproductive performance has been reported in organic cows [4] The differences were due to a limited energy intake and increased winter breeding in the organic cows. In Sweden it has been reported that cows in organic herds tend to produce less milk than cows in conventional herds. [5]. Whereas the calving interval, and the intervals from calving to first and last AI, were shorter for organic cows compared to conventional cows. Nauta et al. reported an extended calving interval in cows managed on organic farms that converted to an organic management system between 1990 and 2003 [6].

Differing results have been reported for udder health when organic and conventional dairy herds were compared. A lower mastitis treatment rate has been found in organic herds, which might be due to lower milk yield in these herds [7-9]. However, Hardeng and Edge did not find any significant difference in individual cow milk somatic cell count (**SCC**) between organic and conventional herds [8], and Valle et al. found no difference in bulk milk SCC [9].

The lower treatment rate and, thus, reduced use of antibiotics, may reduce antibacterial selection pressure. A few studies have been carried out comparing the occurrence of antibiotic resistant udder pathogens in organic and conventional farming [10-12]. Roesch et al. reported no difference in antibiotic resistance [12], Tikofsky et al. (2003) found good susceptibility to the most commonly used antibiotics [10], and Sato et al. (2004) reported small differences between conventional and organic farming [11].

The FAO has characterized the Norwegian Red as a sustainable breed [13]. From the 1970s the breeding program has included health, fertility and functional traits, along with the production traits of milk yield and growth. Currently, 10 traits are included in the total merit index. The following traits (and relative weights) were included in the breeding program in 2008; milk yield (24%), mastitis resistance (22%), milk fever, ketosis, and retained placenta (2%), fertility (15%), udder (15%), leg confirmation (6%), growth rate (9%), temperament (4%), calving ease (1%), and still birth (1%) [14].

The relationship between selection for milk yield and reproductive performance and time to onset of luteal activity post partum has been investigated previously [15]. Cows selected exclusively for high milk yield had a longer interval to the commencement of luteal activity after calving than cows bred according to other breeding objectives. A prolonged period of ovarian quiescence was found to be reduced if selection for milk yield was combined with fertility in the breeding program. Rozzi et al. reported that organic farmers emphasized functional traits rather than production traits in Holstein cows [16]. For Norwegian Red cows, it is reported that it is possible to obtain genetic improvement for clinical mastitis and milk yield simultaneously if the traits are given sufficient weight in selection [17] and that selection against clinical mastitis is favorable correlated with selection responses for ketosis and retained placenta [18]. Since the breeding program for the Norwegian Red has paid attention to fertility, health and functional traits for a long period, this breed may perform well with regards to fertility and udder health in both conventional and organic farming systems.

The objectives of this study were to investigate whether there were differences between Norwegian Red cows managed conventionally and organically with respect to reproductive performance, udder health, and resistance against penicillin in udder pathogens.

Methods

Sampling of herds

The conventional and organic herds present in the selected cohort were registered in the Norwegian Dairy Herd Recording System (**NDHRS**). The organic farms converted at least four years before the start of the study. The organic herds were certified as organic between 1997 and 2003. All organic herds in south east (n = 26) and middle Norway (n = 21), excluding herds with less than nine cow-years (one cow-year = 365 d for a cow in the herd during one year), received an invitation letter for participation in the study. Farmers were requested to reply within one week to be included in the study. Those that did not were contacted by telephone one week after the deadline. Thirty of the 47 farms contacted agreed to participate in the study. Four of the thirty were excluded because they contained breeds other than the Norwegian Red. A further herd was excluded as it was farmer cooperatively and so had an elevated number of herdsmen. Another herd decided to not participate at the start of the study.

Thirty eight owners of conventional herds located in the same geographical areas as organic herds were asked to participate. Herds were selected to the organic farms according to herd size (± five cow-years) and type of housing. Eleven farmers were not interested (mostly due to the extra labor), one farmer planned cooperative farming, and one farmer planned conversion to organic farming.

In total, 24 organic and 25 conventional herds were enrolled in the study. One organic herd ended milk production during the study period. The distribution of geographical area, herd size, and barn type is presented in Table 1. The veterinarians practicing on the selected farms were informed about the study before it started, and were asked to collect quarter milk samples and complete a standard form when treating cases of clinical mastitis in these herds. Bacteriological examination of milk samples were performed free of charge for the farmer.

Herd visit

Each herd was visited once by the same researcher (first author) between February and June in 2006. Quarter milk samples for bacteriological examination were collected aseptically from 523 conventional and 487 organic lactating cows. Milk was collected aseptically from all quarters of cows in lactation on the respective day of the herd visit. The California mastitis test (**CMT**) was performed when the milk was sampled. The milk samples were refrigerated immediately after sampling and subsequently frozen until they underwent laboratory examination.

Milk samples obtained from cases of clinical mastitis

The farmers were requested to collect quarter milk samples from all cows affected by clinical mastitis or showing signs that might indicate the presence of clinical mastitis between February 2006 and August 2007. Samples were to be collected regardless of whether a veterinary surgeon was contacted. A written description of the signs of clinical mastitis (i.e., visible changes in milk such as clots, yellow like, blood like or water like milk, changes in quarters such as soreness/ache or pain by palpation), was given to the farmers. Standard packages for milk collection and transportation of quarter milk were distributed. The farmers received information about routines required for aseptic milk sampling and interpretation of CMT. Information about the condition of clinical cases was recorded on a standard form, which included the rectal temperature, appetite (recorded as normal, slightly decreased, markedly decreased, anorexia) and clinical signs of acute or chronic inflammation at quarter level, teat injury, and visual abnormality of secretion. CMT was recorded on a scale from 1 to 5 [19]. The farmers were instructed to keep the milk samples cold or frozen until submission by mail to the National Veterinary Institute.

Laboratory Methods

Bacteriological examinations of quarter milk samples were performed at the Mastitis Laboratory of the National Veterinary Institute, Oslo, Norway. Secretions were brought to room temperature, assessed visually and characterized by appearance. After being mechanically shaken, secretions (0.01 ml) were plated on Bacto Blood Agar Base No 2 (Difco Laboratories, Detroit, MI, USA) containing 5% washed bovine erythrocytes and incubated for 48 h in a 5% CO_2 atmosphere at 37 °C. Cultures were read at 24 and 48 h. If growth was not detected after 24 h incubation, the original sample was incubated for 4 h at 37 °C and 0.05 ml was plated on blood agar and incubated for 24 h under aerobic (5% CO_2 atmosphere) and anaerobic conditions.

Bacteria were identified according to the recommendations of the International Dairy Federation [20]. Species were identified tentatively by gross colony morphology and Gram staining; further confirmatory tests were used as necessary. Suspected staphylococcal colonies were tested using the tube coagulase test (Becton Dickinson Microbiology Systems, MA, USA). Staphylococci were differentiated from streptococci with a catalase test and *Streptococcus dysgalactiae* subspecies *dysgalactiae, Streptococcus uberis* and *Streptococcus agalactiae* were differentiated by the CAMP reaction and their ability to hydrolyze aesculin and inulin. *Escherichia coli* was identified by lactose and indole fermentation tests; other bacteria within the family *Enterobacteriaceae* were identified to species or genus level by the

API 20 E® identification system (bioMérieux, Marcy l'Etoile, France). Staphylococcal isolates were tested for ß-lactamase activity by the cloverleaf method [21] using *Staphylococcus aureus* ATCC 25923 as the indicator strain.

Individual and Herd data

Individual animal data for test-day milk yield, test-day SCC, and test-day concentrate allocation (**CA**), calving date, the intervals from calving to first and last AI, and use of natural mating or AI were obtained for all cows enrolled in NDHRS from 2005 throughout 2007 (n = 3209 lactations, 2093 cows). The calving interval was defined as time between two successive calvings ≤ 550 d, and days open were calculated as calving interval minus 281 d (expected gestation period). The milk yield recording closest to 28 DIM (range 15 to 45 d, n = 845) was used as an estimate for milk yield in the fourth week of lactation. Occurrence of clinical mastitis from February 2006 to August 2007 that was treated and reported was obtained from the NDHRS files. A new clinical mastitis treatment within 9 d after the initial treatment was not considered to be a new case. Cases which had been treated for retained placenta and reproductive disorders from 2005 throughout 2007 were obtained from the NDHRS files. The average annual forage: concentrate ratio for the herds and culling rate (number of culled cows per 100 cow-years) of cows in 2006 and 2007 was also obtained from the NDHRS files.

Statistical analyses

The unit of the study was lactation periods. Statistical significance was considered at $P \leq 0.05$ for all analyses.

Chi-square tests. Pearson Chi-square tests were performed to assess the univariate relationships between management systems and the following variables; parity distributions, proportion of dry quarters, AI versus natural mating, season for first AI, proportion of milk samples negative for pathogens, and proportion of milk samples from clinical mastitis negative for pathogens.

Associations between reproductive performance and management system, milk yield, season, parity, and AI versus natural mating. Mixed linear models [22] were performed using SPSS 15.0 to separately assess the relationships between the three outcome variables; interval from calving to first AI, interval from calving to last AI, and calving interval ≤ 550 d and the explanatory variables; management system, parity, milk yield in the fourth week of lactation, barn type, and season for first AI. In addition, calving interval was also assessed with AI versus natural mating. The interval from calving to first AI (**lnCFAI**) and the interval from calving to last AI (**lnCLAI**) were transformed by natural logarithm to approximate normality of the residuals. Explanatory variables with $P \leq 0.20$ in the separate analyses were included in the final models together with the interactions terms; management system by parity, parity by season, management system by season, barn type by season, barn type by management system, barn type by parity. The backward elimination procedure was applied for explanatory variables with $P > 0.05$ in the extended model. The covariance between multiple measurements of lnCFAI, lnCLAI and calving interval was correlated, and the models were adjusted for multiple lactations within the same cow by the use of a compound symmetry correlation structure for repeated effects. Multiple comparison adjustment for the pair wise difference in least square means (**LS-means**) was performed using the Bonferroni option in SPSS.

Lactations curves for test-day milk yield, somatic cell count and concentrate allocation.

For construction of the lactation curves for the continuous variables; test-day milk yield, test-day SCC and test-day CA multiple records on milk yield, SCC and CA were used for each lactation. Test-day milk yield and CA was recorded monthly whereas SCC was recorded bimonthly. Only recordings within 305 DIM were included in the analysis. Number of lactations used to construct the lactation curves was 3088, 3096, and 3035 for test-day milk yield, CA, and SCC, respectively. The outcome variable, SCC was transformed by natural logarithm (**lnSCC**) to obtain approximate normality of residuals. Mixed linear models for repeated outcomes were run using SPSS to assess the relationships between the outcome variables (milk yield, lnSCC, and CA) and the explanatory variables. Subjects were lactation within cows and week in milk were entered as repeated effect. Covariance between multiple measurements of milk yield, lnSCC, and CA within lactations were correlated and accounted for by the use of first order autoregressive correlation structure [23].

The lactation curves were expressed through inclusion of weeks in milk (**WIM**) and the natural logarithm of WIM (**lnWIM**) as described by [24-26]. The explanatory variables WIM and lnWIM were entered simultaneously in all models with test-day milk yield, lnSCC or CA as outcome variables. The explanatory variables; management system (conventional or organic) and parity (1st, 2nd, and > 2nd) were first assessed separately for each of the three outcome variables (milk yield, lnSCC, and CA). Secondly, interaction terms considered as biological important such as WIM by management, WIM by parity, lnWIM by management, and lnWIM by parity were included in the respective models. Thirdly, also test-day milk yield was included as an explanatory variable in a separate model expressing the lactation curve for lnSCC. The final model for each of the three outcomes was constructed such that the explanatory variables and their interactions terms with $P \leq 0.20$ when tested separately, were included in an extended model. The backward elimination procedure was applied for the explanatory variables and interaction terms with $P > 0.05$ in the extended model.

Associations between cows with milk samples positive for mastitis bacteria and management system, milk yield, parity, and WIM. Intra-mammary infection was considered to be present when an udder pathogen was isolated from at least one quarter. Associations between the presence of intra-mammary infection (= 1) or not (= 0) at the herd visit and the explanatory variables; management, milk yield in the fourth wk of lactation, WIM, and parity were tested separately using generalized linear models with logit as link function in SPSS. Explanatory variables with $P \leq 0.20$ when assessed separately and the interaction terms; parity by WIM, parity by milk yield, and WIM by milk yield were included in an extended model. The backward elimination procedure was applied for explanatory variables with P > 0.05 in the extended model.

Results

Herd characteristics

The average herd size in 2006 was 23.8 (SD ± 11.8) and 23.0 (SD ± 11.5) cow-year for the conventional and organic herds, respectively. Average milk yield per cow-year in 2006 was 7188 (SD ± 805) and 6155 kg (SD ± 963) for conventional and organic herds, respectively. The distribution of parities for conventional and organic farming from 2005 to 2007 is presented in Table 1. There were more 1st parity and less > 2nd parity cows in conventional compared to organic farming ($P < 0.01$). The culling rate for 2007 was 46.9/ 100 cow-year (95% CI: 35.0, 58.9) in conventional farming and 31.2/ 100 cow-year (95% CI: 25.3, 37.1) in organic farming. The average annual forage: concentrate ratio for conventional herds was 63:37 (95% CI: 35.1, 39.5) and 75:25 (95% CI: 22.6, 28.3) for organic herds.

There were 47 cases of retained placenta recorded in the NDHRS files from conventional herds, whereas there were 19 cases recorded from organic herds from 2005 throughout 2007. There were 148 cases of treatments for reproductive disorders (silent heat, heat synchronization, metritis, endometritis, vaginitits, cystic ovaries, and repeated breeding) in conventional herds during the same period, whereas the corresponding figures were 15 in organic herds.

Descriptive reproductive parameters

There were 1548 and 1403 lactations with records on AI or natural mating as first service for the conventional and organic herds, respectively. Natural mating was preferred as first service in 2.8% (43/1548) of the conventional observations and in 13.8% (199/1403) of the organic observations ($P < 0.01$). Natural mating was registered in six organic and six conventional farms. There were two organic farms using mostly natural mating as first service with 100% and 73.5% matings registered. For the conventional farms, natural mating as first service was used in 5.4% to 33.3% of the cows.

There were no differences between conventional and organic cows in the overall calving interval or in the calving interval when only cows presented for first AI were included (Table 2). When only cows presented for natural mating as first service were included, the organic cows had a shorter calving interval than conventional cows (355.9 versus 386.1 d). There were no differences between days open, days to first AI, days to last AI between conventional and organic cows (Table 2).

There was no difference between season for AI in conventional and organic farming. First AI was performed during the summer season (April- September) in 58.4% (875/1498) and 56.3% (675/1198) of the lactations in the conventional and organic farms, respectively.

Associations between interval to first AI and management, milk yield, parity barn type, and season

When lnCFAI was assessed in separate models for each of the explanatory variables separately, P-values ≤ 0.20 were observed for management ($P = 0.01$), parity ($P = 0.09$), barn type ($P \leq 0.01$), and season ($P = 0.16$). After the backwards selection procedure had been applied, only management system and barn type remained in the model. None of the interaction terms were associated with the lnCFAI. The results from the extended model are presented in Table 3.

The LS-means for the interval to first AI were 75.0 d and 77.7 d for conventional and organic cows, respectively. The LS-means for interval to first AI were 73.8 d and 78.9 d for free stall and tie stall cows, respectively.

Associations between interval to last AI and management, milk yield, parity, and season

When lnCLAI was assessed in separate models for each of the explanatory variables, P-values ≤ 0.20 were observed only for parity ($P < 0.01$) and barn type ($P < 0.01$). After the backwards selection procedure had been applied, both parity and barn type remained in the model. None of the interaction terms were associated with lnCLAI. The results from the extended model can be obtained in Table 3. The LS-means for the interval to last AI were 94.6, 88.7, and 91.8 d for 1st, 2nd, and > 2nd parity, respectively. The LS-means for the interval to last AI were 87.3 and 96.3 d for free stall and tie stall cows, respectively.

Associations between calving interval and management, season, breeding management, and parity

When calving interval was assessed in the separate models for each of the explanatory variables, *P*-values ≤ 0.20 were observed for breeding management ($P < 0.01$), parity ($P = 0.09$), barn type ($P < 0.01$), and season ($P = 0.20$). After the backwards selection procedure was applied only barn type remained to be associated with the calving interval. None of the interaction terms were associated with calving interval. The results from the extended model can be obtained in Table 3. The LS-means for calving interval were 372.0 d and 382.3 d for free stall and tie stall cows, respectively.

Test-day milk yield

All explanatory variables; WIM, lnWIM, management, parity, and interaction terms; WIM by management system, WIM by parity, lnWIM by management system, and lnWIM by parity were significantly associated ($P < 0.01$) with milk yield at the test day in the type III statistics. Lactation curves for 1st and > 2nd parity cows in conventional and organic farming are presented in Figure 1. The average 8 WIM yield for conventional cows were 23.8, 29.3, and 32.3 kg for 1st, 2nd, and > 2nd lactation, respectively. The corresponding figures for organic cows were 20.7, 26.2, and 29.3 kg.

Test-day concentrate allocation

All explanatory variables; WIM, lnWIM, management, parity, and interaction terms; WIM by management system, WIM by parity, lnWIM by management system, and lnWIM by parity were associated ($P < 0.01$) with concentrate allocation in the type III statistics. Concentrate allocation curves for 1st and >2nd parity cows in conventional and organic farming are presented in Figure 2. The average concentrate allocation 8 WIM for conventional cows were 50.7, 60.2, and 63.6 MJ for 1st, 2nd, and >2nd lactation, respectively. The corresponding figures for organic cows were 31.9, 41.4, and 44.7 MJ.

Test-day SCC

All explanatory variables; WIM, lnWIM, management, parity, and test-day milk yield, but none of the interactions were associated ($P < 0.01$) with test-day lnSCC in the type III statistics.

Curves for back-transformed SCC for 1st and >2nd parity cows in conventional and organic farming are presented in Figure 3. The average SCC 8 WIM for conventional cows were 79 400, 104 800, and 151 400 for 1st, 2nd, and >2nd lactation, respectively. The corresponding figures for organic cows were 64 900, 85 700, and 123 700.

Herd milk samples

The percentage of dry quarters was 2.2% (43/1948) and 1.2% (26/2092) ($P = 0.02$) in organic and conventional management, respectively. Number of quarter milk samples positive for mastitis bacteria is presented in Table 4. There was no difference in the percentage of quarter samples positive for mastitis bacteria between conventional and organic farming. Independent of management, there were more quarter milk samples positive for mastitis bacteria in cows > 2nd parity than in 1st and 2nd parity cows ($P < 0.01$).

Associations between cows with milk samples positive for mastitis bacteria and management system, milk yield, WIM, and parity

When the likelihood of a positive diagnosis for mastitis bacteria (subclinically infected cows) was assessed in separate models for each of the explanatory variables, P-values ≤ 0.20 were observed for milk yield in the 4th wk of lactation ($P < 0.01$), WIM ($P = 0.03$) and parity ($P < 0.01$).Multivariate associations between cows with milk samples positive for mastitis bacteria and the explanatory variables are presented in Table 5. In the type III statistics F-test, both parity ($P = 0.01$), milk yield ($P = 0.03$), and WIM ($P = 0.05$) were associated with the likelihood of at least one quarter positive for mastitis bacteria. Management system was not associated with bacteriological diagnosis when assessed separately, and hence not included in the model.

Samples of cases from clinical mastitis

In the study period, a total of 332 cases with clinical mastitis (229 in conventional and 103 in organic herds) were recorded in 271 cows in 24 conventional and 19 organic herds in the NDHRS-files. With the exception of three cases, all were treated by a veterinarian. In total, there were collected 238 (144 conventional and 94 organic) samples of quarters with clinical mastitis from 177 cows (107 conventional and 70 organic). Fifty-three quarter samples were registered in the same cow at the same date, such that the number of cases of clinical mastitis at the cow level was 185 (112 conventional and 73 organic) from 172 cows (104 conventional and 68 organic). Quarter milk was collected from 48.9% (112/229) of the cases of clinical mastitis cases in conventional herds and from 70.9% (73/103) of the cases in organic herds. Twenty-one conventional herds and 19 organic herds submitted samples from at least 1 cow with clinical mastitis.

The distribution of bacteria isolated from milk samples from quarters with clinical signs of mastitis is presented in Table 6. There was a higher proportion (30.9% versus 18.1%) of clinical mastitis cases where mastitis bacteria were not isolated on organic compared to conventional farms ($P = 0.02$).

Antibiotic resistance in milk samples from the herd visit and samples from clinical mastitis

Of the staphylococci isolated from milk samples of subclinically infected quarters collected during the routine visits to the conventional herds, 81 of the 167 isolates (48.5%) of coagulase-negative staphylococci (CNS) and 6 of the 68 isolates (8.8%) of *S. aureus* were resistant to penicillin. The corresponding findings in the organic herds were for CNS 93 penicillin-resistant isolates out of 200 (46.5%) and for *S. aureus* nine penicillin-resistant isolates out of 64 (14.0%). Of the isolates from quarters with clinical mastitis, resistance to penicillin was not found among the 59 *S. aureus* from conventional and organic herds. Whereas one out of one of the CNS isolates from the organic herds, and three out of five of the CNS isolates from conventional herds were penicillin-resistant.

Discussion

Previously, there have been separate Norwegian field studies comparing reproductive performance [4] udder health [8], and herd health and management [9] in conventional and organic dairying. The present study agrees with previous findings of higher parity distribution and lower milk yield in organic cows [4; 8]. However, the present study does not support the reported findings of impaired reproductive performance in organic herds [4] or the statement of no differences in SCC [8] between the management systems.

Roesch et al. reported that lower milk yields in organic farms were due to individual animal and farm level factors; such as udder health, breed, nutrition and management [27]. In this study conventional herds were selected to the organic herds on the basis of geographical location, housing system and herd size. Consequently, these potential confounders were unlikely to have influenced the findings of the present study. Since the cows included in the study were all Norwegian Reds, breed is not a potential confounder either. All organic herds included in the study converted form 1997 to 2003. During this period the government provided an economic incentive for conversion to organic farming. It may have been this, rather than the idealism of farming in a sustainable manner, that stimulated conversion, as described in Switzerland [28]. The governmental payment could have affected management decisions more than the holistic view of organic production in the present study, which could be interpolated to other Norwegian organic farmers converting to organic production after 1997.

The organic cows were fed less concentrate than conventional cows. Reksen et al. reported that the energy provided by concentrate for most organic herds was 20%, because a maximum of 20% of the feed could be non-organic in origin and production of organic grain was limited [4]. The percentage of non-organic feed allowed to use on organic farms has gradually been reduced. Since January 2008 no feed of non-organic origin has been allowed. Currently, 60% of the energy fed on daily basis should be roughage [3]. Hence, today it is possible to feed a higher amount of concentrate than the limit of 20% ten years ago. In 2007, 39.4% of the feed fed to Norwegian cows per cow-year was concentrate [29]. Valle et al. reported that the percentage concentrate fed was 39.3% and 27.4% for conventional and organic herds, respectively [9]. In the present study, the average annual forage: concentrate ratio was 63:37 in conventional herds and 75:25 in organic herds. Previously, it has been described that Norwegian Red cows are able to maintain ovarian activity by decreasing milk yield when the forage to concentrate ratio was 75:25 or lower [30]. Fall et al. reported that organic cows did not mobilize more body tissue than conventional cows, and that the organic cows adjusted the milk production according to feed intake [31]. It could be that the organic cows decreased milk yield according to feed intake and hence met the negative energy balance post partum with lower milk yield rather than impaired reproductive performance since milk yield was not associated with any of the fertility measurement investigated in the present study. Hence, the suggestions presented by Reksen et al. are met and the breed seems to adapt well in extensive production systems [30].

Natural mating as first service was more commonly used in organic than conventional farming, which agrees with the findings reported by Reksen et al. [4]. The interval to first AI was shorter for conventional cows, whereas there was no difference in calving interval, days open, and interval to last AI between the management systems. Reksen et al. [4] and Valle et al. [9] reported no difference in these reproduction measures except for a reduction in the number of days open for organic cows [4], whereas Löf et al. [5] reported shorter calving intervals and shorter interval to first and last AI in organic cows. Nauta et al. [6] found extended calving interval in cows managed on farms that converted between 1990 and 2003. Cows in free stall herds had shorter interval to first AI, last AI and calving interval in the present study. Valde et al. [32] reported higher fertility status index in free stall herds compared to tie stall herds. When cows only presented for natural mating were investigated, organic cows had shorter calving interval and fewer days open than conventional cows which could be explained by more complete systems with natural breeding as first service compared to conventional farming. There were no difference between season for AI and the two types of farming which contrasts with the findings of Reksen et al. where 52% of the organic cows and 36% of the conventional cows were bred during the summer [4].

Although adjusted for parity and milk yield, SCC was lower in organic cows during the whole lactation period compared to conventional cows which contrasts with the findings that no difference in SCC existed between the management systems [8; 33-34]. A Danish and a Dutch study reported lower milk yield in long-standing organic farms compared to later conversion and conventional farms, but the studies were conflicting regarding to SCC [6; 35]. A Swedish study found lower milk yield and lower proportion of cows with high SCC in organic farming [36], whereas Valle et al. [9] found no differences in bulk milk SCC and higher culling rate in conventional herds. In the present study, the culling rate was slightly higher in the conventional herds which should give the opportunity to cull cows with high SCC and hence potential to improve the udder health.

The percentage of dry quarters at the herd visits was higher in organic compared to conventional farming which could have given lower measurements of SCC in organic herds. Valle et al. reported a lower proportion of produced milk delivered to the dairy factory from organic herds [9]. Hamilton et al. reported that organic farmers were more unlikely to use veterinary treatment in cases of clinical mastitis [37]. In the present study, four organic farmers always preferred veterinary treatment in cases of clinical mastitis, whereas 12 conventional farmers always called the veterinarian. In a previous study, it was reported that conventional farmers on average called the veterinarian in 4.7 out of 10 mild mastitis cases, whereas the corresponding figure was 2.0 for organic farmers [9]. In 2007, 16 of the 24 organic farms had reported data about (veterinary) treatment in cases of mastitis to the NDHRS-files, whereas 22 of 25 conventional farms had reported cases of mastitis. Milk was sampled in 48.9% of the clinical mastitis cases treated in conventional herds whereas the corresponding figure was 70.9% in organic herds. The milk samples from cases with clinical mastitis were sampled from cows in 21 conventional herds and 19 organic herds. The number of samples submitted from farms varied considerably, especially for the conventional farmers (1 to 23). This variation could be due to differing levels of motivation to improve the udder health in each herd. Since the milk samples were analyzed for free, this could have been an important factor for motivation to submit milk samples for at least the conventional farmers.

The proportion of milk samples positive for mastitis bacteria at the herd visit were not associated with management. However, parity, stage of lactation, and milk yield were. There was no difference between organic and conventional herds with respect to bacteriologically positive milk samples at the herd visit. Coagulase-negative staphylococci were the bacteria most frequently isolated from quarters in both conventional (8.1%) and organic (10.5%) cows. This differs considerably from the reported frequency of 3.3% in a nationwide random assignment survey [38]. The percentage of milk samples positive for *Streptococcus dysgalactiae* at the herd visits was similar in the two management systems, and corresponds well with the 1.2% of cows previously reported to be infected national [38]. The proportion of *S. aureus* was similar in organic and conventional herds (3.3 and 3.4%), but lower than previous reports of 8.2% [38]. For *Streptococcus uberis*, the percentage (0.6%) of organic quarters infected corresponds with the report of Østerås et al. [38]. However the infection rate in conventional herds was twice as high (1.2%) as the earlier report. The deviation of the proportion of mastitis bacteria isolated in the present study compared to the study conducted by Østerås et al. [38] could be due to the design of the studies. In the present study, all cows in lactation in the herd were sampled, whereas the other study sampled every fifth cow from every 50th herd during each quarter of the year [38]. Hence environmental factors in the specific herd in the present study would have larger impact on the proportion of infected quarters compared to the other study.

In the clinical mastitis cases, there were a higher proportion of bacteriological negative milk samples from organic farms (30.9% versus 18.1%). The reason for this remains unknown, but it could be possible that some of this quarters originally were infected by *E.coli* which was not present at the time of milk sampling. Vaarst and Enevoldsen reported that bacteriological negative mastitis showed strong similarities with clinical coliform mastitis, and that 20% of the cases were bacteriological negative in a study of manifestation of clinical mastitis in Danish organic herds [39]. In another Norwegian study, 12.5% of the cases of clinical mastitis were bacteriological negative [40]. *Staphylococcus aureus* was the bacteria most frequently isolated from quarter samples with signs of clinical mastitis in both conventional (27.8%) and organic herds (20.2%), which is lower than previous reported in heifers (pre partum to 14 d post partum) of 44.3% [41]. Whist et al. reported *S. aureus* to be isolated in 47.4% of the cases with clinical mastitis in problem herds [40]. In the present study, *E. coli* was the second most frequent reason for clinical mastitis in both conventional (23.6%) and organic (19.1%) cows. The proportions in this study are higher than previous reported of 6.4% [41] and 10.7% [40]. The frequency of *Streptococcus dysgalactiae* in organic farming (17.0%) was close to previous studies with 18.2% [41] and 22.5% [40]. In conventional farming, the percentage of *Streptococcus dysgalactiae* was only 11.1%. The reason for such low proportion of *Streptococcus dysgalactiae* in conventional farming could be due to higher motivation to improve udder health and more use of dry cow therapy initiated by veterinarian than organic herds. The use of dry cow therapy was not investigated in the present study.

Cases selected for antibiotic treatment are assessed by the veterinarian before treatment. Benzyl penicillin and dihydrostreptomycin are the most common antibiotics used for intramammary treatment [40]. Penicillin resistance against *S. aureus* was not found in the cases of clinical mastitis. At the herd visit, penicillin resistance against *S. aureus* was found in 8.8% of the subclinically infected quarters in conventional farming and in 14.1% of subclinically infected quarters in organic farming. Østerås et al. reported 11.4% resistance against penicillin G for *S. aureus* in subclinically infected quarters [38]. Other studies investigating antibiotic resistance between the two management systems reported no difference in penicillin resistance between the two farming types [12; 42]. Tikofsky et al. found that *S. aureus* in US organic farms were more susceptible to antibiotics than in conventional farms, but also that *S. aureus* isolates in general showed good susceptibility [10]. Since the choice of antibiotics and cases for treatment are very different in US and Norway, comparisons between the countries are difficult. A study that investigated antimicrobial susceptibility of *S. aureus* in bulk tank milk in organic and conventional farms in Denmark and US, reported small differences between organic and conventional farms in each country, but large differences between the respective agricultural systems [11].

Penicillin resistance against CNS isolated from subclinically infected quarters was 48.5% in conventional and 46.5% in organic herds. Østerås et al. reported that 36.1% of the CNS isolates from subclinically infected quarters were penicillin resistant and that the resistance was highest during late indoor season (April-May) [38]. This could explain the higher percentage of penicillin resistance CNS in the present study, because most of the herd visits took place in the spring and early summer (late March to late May) in both conventional and organic herds.

Conclusion

The interval to first AI was shorter in conventional cows, whereas there were no differences in the interval to last AI and calving interval between the two management systems. The cows were older in organic farming. Conventional cows yielded more, had higher SCC, and received more concentrates than organic cows. Higher level of concentrate fed to organic cows in recent years is an important factor for higher reproductive efficiency than ten years ago. The Norwegian Red is a sustainable breed that adapts well to both intensive and more extensive systems like organic production.

Competing interests

The authors declare that they have no competing interests.

Author's contributions

RTG contributed to design, planning and administration of the study, performed the herd visits, collection, analysis, and interpretation of data, main author of the manuscript. SW contributed to study concept and design, analysis and interpretation of data, critical revision of the manuscript for important intellectual content. SS contributed in the udder health part and analysis of milk samples, reviewing the manuscript with special emphasis on the udder health part. BIFH contributed in the selection of organic herds, ensured correct information about organic farming and regulations, and production of the manuscript. OØ contributed in the planning of data needed for epidemiological analysis, collection of data from the Norwegian Dairy Herd Recording System, input on epidemiological and statistical approach in the manuscript. OR contributed in design, planning, analysis and interpretation of data, critical revision and production of the manuscript.

All authors read and approved the final manuscript.

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Figure 1. Lactation curves for milk yield. Predicted test-day milk yield (kg/d) by WIM for 1st (organic▲, conventional△) and > 2nd (organic●, conventional○) parity cows in conventional and organic farming.

Figure 2. Lactation curves for concentrate allocation. Concentrate allocation [MJ/d] by WIM for 1st (organic▲, conventional△) and > 2nd (organic●, conventional○) parity cows in conventional and organic farming.

Figure 3. Lactation curves for SCC. Test-day somatic cell count (SCC) by WIM for 1st (organic▲, conventional△) and > 2nd (organic●, conventional○) parity cows in conventional and organic farming.

Table 1. Distribution of herds in organic and conventional farming. Distribution of the organic (n = 24) and the conventional (n = 25) dairy herds according to geographical area, herd size, barn type, and parities from 2005 to 2007.

		Organic herds		Conventional herds	
		n	%	n	%
Geographical area	South-east	17	70.8	18	72.0
	Middle	7	29.1	7	28.0
Herd size	≤ 10 cow-years	1	4.2	1	4.0
	11-20 cow-years	12	50.0	11	44.0
	21-30 cow-years	8	33.3	10	40.0
	>31 cow-years	3	12.5	3	12.0
Barn type	Tie stall	10	41.7	9	36.0
	Free- tall	14	58.3	16	64.0
Parity 2005 to 2007	1st	580	37.9	706	42.0
	2nd	381	24.9	453	27.0
	> 2nd	568	37.1	521	31.0

Table 2. Reproduction parameters. Calving interval (overall, AI only, natural mating only), interval from calving to first AI (CFAI), interval from calving to last AI (CLAI), and number of AI per observations in conventional and organic farming.

	Conventional			Organic		
	n	mean	95 % CI	n	mean	95 % CI
Calving interval						
Overall	1112	376.3	373.5, 379.0	1102	375.6	372.7, 378.5
Only AI	1074 [1]	375.8	373.0, 378.5	922 [1]	379.5	376.4, 382.6
Only natural mating	37 [1]	386.1[a]	372.4, 399.7	177 [1]	355.0 [b]	347.7, 362.4
Days open						
Overall	1112	95.3	92.5, 98.0	1102	94.6	91.7, 97.5
Only AI	1074 [1]	94.8	92.0, 97.5	922 [1]	98.5	95.4, 101.6
Only natural mating	37 [1]	105.1 [a]	91.4,118.7	177 [1]	74.0 [b]	66.7, 81.4
CFAI	1505	79.5	77.8, 81.2	1204	82.0	80.2, 83.8
CLAI	1505	98.8	96.4, 101.2	1204	99.0	96.5, 101.5

[1] For one conventional and three organic cows that calved data was missing for breeding management and days open.

[a,b] Different subscripts indicates significant differences between conventional and organic farming by Bonferroni adjustment for multiple comparisons, $P < 0.05$

Table 3. Models for assessment of reproductive performance. Associations between outcomes for reproductive performance; natural logarithm of the interval to first AI (lnCFAI), natural logarithm of the interval to last AI (lnCLAI), and calving interval and the explanatory variables; management, season, parity, and breeding management as assessed by mixed linear models adjusted for multiple lactations within cow by the use of compound symmetry correlation structure.

		β	SE	95% CI	*P*	Ls-means
lnCFAI (n = 2709)						
Management	Organic [1]	-	-	-	-	4.353 [a]
	conventional	-0.037	0.016	-0.068, -0.005	< 0.01	4.317 [b]
Barn type	Tie stall	-	-	-	-	4.368 [a]
	Free stall	-0.066	0.017	-0.099, -0.032	< 0.01	4.302 [b]
Intercept		4.386	0.016	4.354, 4.418	< 0.01	
lnCLAI (n = 2709)						
Parity	1st parity [1]	-	-	-	-	4.550 [a]
	2nd parity	- 0.064	0.019	-0.102, - 0.026	< 0.01	4.485 [b]
	> 2nd parity	- 0.029	0.020	-0.068, 0.009	0.14	4.520 [a,b]
Barn type	Tie stall	-	-	-	-	4.567 [a]
	Free stall	-0.098	0.019	-0.134, -0.061	< 0.01	4.469 [b]
Intercept		4.599	0.018	4.563, 4.634	< 0.01	
Calving interval (n = 2195)						
Barn type	Tie stall	-	-	-	-	382.3 [a]
	Free stall	-10.31	2.211	-14.649, -5.974	< 0.01	372.0 [b]
Intercept		382.32	1.891	378.61, 386.04	< 0.01	

Table 4. Mastitis bacteria isolated in milk samples. Milk samples at quarter level positive for mastitis bacteria in conventional and organic farming at the herd visit (n = 3971).

	Conventional		Organic	
Diagnosis	n	%	n	%
Bacteria negative	1746	84.5	1577	82.8
Staphylococcus aureus	68	3.3	64	3.4
CNS	167	8.1	200	10.5
Streptococcus dysgalactiaee	26	1.3	32	1.7
Streptococcusococcus uberis	25	1.2	11	0.6
Other *Streptococcus* spp.	4	0.2	5	0.3
Escherichia coli	0	-	3	0.2
Enterococcus ssp.	29	1.4	9	0.5
Others [1]	1	0.04	4	0.2

[1] *Corynebacterium, Proteus, Archanobachter pyogenes*

Table 5. Factors associated with bacteriological positive milk samples. Associations between cows with milk samples positive for mastitis bacteria and explanatory variables; milk yield in the fourth week of lactation (MY), parity and weeks in milk (WIM) at sampling date at the herd visit in conventional and organic farming (n = 845).

		β	SE	OR	95% CI	*P*
WIM		0.011	0.006	1.011	1.000, 1.022	0.05
MY		0.026	0.012	1.027	1.003, 1.051	0.03
Parity	1st parity [1]	-	-	-	-	-
	2nd parity	0.040	0.190	1.041	0.718, 1.511	0.83
	> 2nd parity	0.537	0.197	1.710	1.164, 2.514	< 0.01
Intercept		- 1.480	0.327	0.228	0.120, 0.432	< 0.01

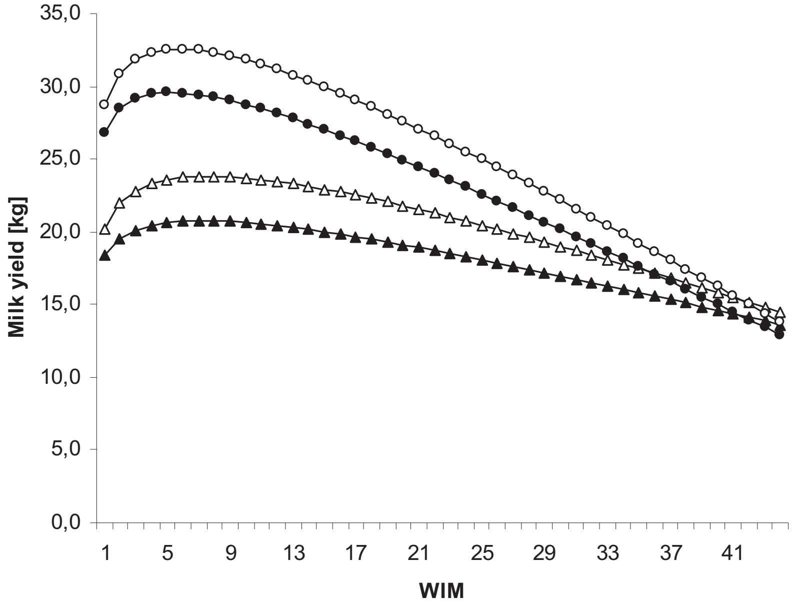
[1] Categorical variable assigned as baselines

Table 6. Bacteria isolated from cases of clinical mastitis. Distribution of bacteria isolated from milk samples from quarters with sign of clinical mastitis (changes in milk secret or symptoms from quarter) in conventional and organic farming (n = 238, from 177 lactations, 173 animals).

	Conventional		Organic	
Diagnosis	n	%	n	%
No bacteria	26	18.1	29	30.9
Mixed bacteria	2	1.4	1	1.1
Staphylococcus aureus	40	27.8	19	20.2
CNS	5	3.5	1	1.1
Streptococcus dysgalactiaee	16	11.1	16	17.0
Streptococcus uberis	8	5.6	2	2.1
Other *Streptococcus* spp.	3	2.1	2	2.1
Escherichia coli	34	23.6	18	19.1
Enterococcus spp.	1	0.7	0	-
Others [1]	9	6.3	6	6.4

[1] *Proteus, Klebsiella, Archanobacter pyogenes, Pasturella, Serratia,* yeast, combinations of *S. aureus* and *Streptococcus dysgalactiae.*

Figure 1.



35,0
30,0
25,0
20,0
15,0
10,0
5,0
0,0
Milk yield [kg]
1
5
9
13
17
21
25
29
33
37
41
WIM

Figure 2.

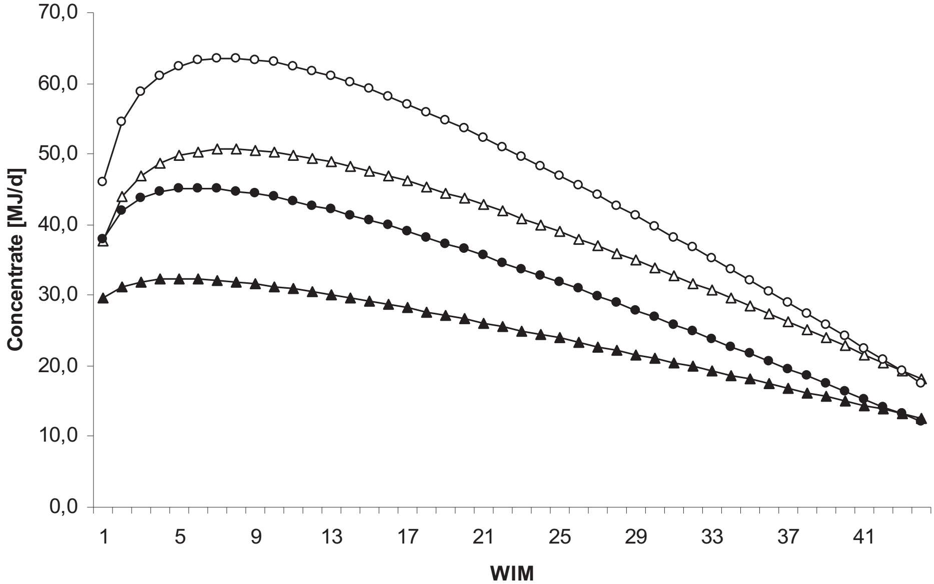

70,0
60,0
50,0
40,0
30,0
20,0
10,0
0,0
Concentrate [MJ/d]
1
5
9
13
17
21
25
29
33
37
41
WIM

Figure 3.