Milk Progesterone as a Tool to Improve Fertility in Dairy Cows

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Abstract

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Milk progesterone offers an opportunity to objectively study fertility in dairy cows, in contrast to traditional measures of dairy cow fertility, which in general are highly influenced by on-farm management decisions. The aim of this thesis was to study how milk progesterone could be used as a genetic and management tool to improve fertility in dairy cows. Progesterone-based measures were influenced by different systematic factors, *e.g.* breed, parity, season, housing and lameness, studied in a dataset from a Swedish experimental herd. The repeatabilities were higher for progesterone-based measures compared with traditional measures of fertility based on insemination data. If a cow had an atypical progesterone profile in one lactation, the risk of an atypical profile in the next lactation was increased. Genetic parameters for progesterone measures based on different milk sampling intervals were estimated in a British dataset. Heritability estimates were moderate, but decreased with increased sampling intervals. It was shown that progesterone analysis of monthly milk samples, resembling milk sampling as in the current Swedish milk recording system, could be used to increase the accuracy of genetic evaluation for an earlier start of cyclical ovarian activity after calving. Inclusion of monthly milk sampling for progesterone analysis in predictive models could also be used to identify cows with delayed ovarian cyclicity with a high accuracy already two months after calving. This enables an earlier treatment of ovarian dysfunction and therefore, probably, a shorter calving interval. In conclusion, this thesis shows that milk progesterone may be used for improved management and genetic evaluation of dairy cow fertility.

Keywords: dairy cow, fertility, genetic evaluation, luteal activity, management, progesterone profile

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Appendix

Papers I-IV

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

I. Petersson, K.-J., Strandberg, E., Gustafsson, H. & Berglund, B. 2006. Environmental effects on progesterone profile measures of dairy cow fertility. *Animal Reproduction Science 91,* 201-214.

II. Petersson, K.-J., Gustafsson, H., Strandberg, E. & Berglund B. 2006. Atypical progesterone profiles and fertility in Swedish dairy cows. *Journal of Dairy Science 89,* 2529-2538.

III. Petersson, K.-J., Berglund, B., Strandberg, E., Gustafsson, H., Flint, A.P.F., Woolliams, J.A. & Royal, M.D. 2007. Genetic analysis of postpartum measures of luteal activity in dairy cows. *Journal of Dairy Science 90,* 427-434.

IV. Petersson, K.-J., Strandberg, E., Gustafsson, H., Royal, M.D. & Berglund, B. 2007. Detection of delayed cyclicity in dairy cows based on progesterone content in milk sampled at different intervals. (Submitted).

Abbreviations

AI:	artificial insemination
CLA:	interval from calving to commencement of luteal activity (days)
CLA_m:	CLA, based on random monthly sampling (days)
lnCLA:	natural logarithm of CLA
CCI:	interval from calving to conception (days)
CFI:	interval from calving to first AI (days)
CFO:	interval from calving to first ovulation (days)
DOV1:	delayed ovulation, type I
DOV2:	delayed ovulation, type 2
FOE:	interval from calving to first ovulatory oestrus (days)
NINS:	number of inseminations per service period
PCL1:	persistent corpus luteum, type 1
PCL2:	persistent corpus luteum, type 2
PFI:	pregnancy at first AI
PLA:	percentage of samples the first 60 days after calving above the limit for luteal activity (%)
PLA_a:	PLA, based on actual sampling interval (%)
PLA_w:	PLA, based on weekly sampling (%)
PLA_f:	PLA, based on fortnightly sampling (%)
PLA_m:	PLA, based on random monthly sampling (%)

Introduction

Decreasing fertility

The fertility of dairy cows is decreasing in many countries and is probably most pronounced in the Holstein population. From the UK, Royal *et al.* (2000) reported a decline of 1% per year in pregnancy rate to first artificial insemination (AI) from 1975 to 1998. In a Spanish study, pregnancy rates to first AI decreased from 42% in 1991 to 33% in 2000 (López-Gatius, 2003). From the USA, an increase in number of AI per conception from 1.8 in 1970 to 3.0 in 1999 has been reported (Lucy, 2001). Moreover from the USA, Washburn *et al.* (2002) reported an increase in days open for Holstein cows from 124 days in 1976 to 168 days in 1999. In Sweden, the calving interval increased from 12.6 months in 1974/1975 to 13.3 months in 2005 for all cows in regular milk recording (Swedish Dairy Association, 2006). For Swedish Holstein cows, the pregnancy rate per AI decreased from 41% in 1998 to 38% in 2005. For Swedish Red cows the decrease was lower, from 43% to 41% (H. Gustafsson, Swedish Dairy Association, personal communication).

The ability of a cow to reproduce is fundamental for the milk production, the dairy cow, the dairy farmer and therefore for the whole dairy industry. The optimal calving interval is generally considered to be about 12 months from an economic point of view (Strandberg & Oltenacu, 1989; Sørensen & Østergaard, 2003). This means that the cow should conceive in about three months after the last calving. If not, this may lead to decreased production and later an increased risk of the cow being culled. Fertility problems is the most common reason for culling in Sweden; the cause of 24% of culls in 2005 (Swedish Dairy Association, 2006). The implication is that improving the fertility of dairy cows gives an opportunity to cull cows for other reasons, *e.g.* poor milk production. The increased calving interval is not the only influence of low fertility on the economy, increased number of inseminations and veterinary treatments also cause considerable costs in the dairy sector.

Progesterone and dairy cow fertility

Not only traditional fertility measures based mainly on AI data, such as conception rate as mentioned above, have deteriorated with increased milk production levels. Early reproductive functions also seem to have been affected by the intensive selection for higher milk production. From the UK, the decreased pregnancy rate from 1975 to 1998 was associated with increased proportion of atypical progesterone profiles (Royal *et al.*, 2000). Roche, Mackey & Diskin (2000) reported increased incidence of postpartum anoestrus, abnormal ovarian cycles and prolonged luteal phases when they compared a study of Irish Friesian cows from the 1980s (Fagan & Roche, 1986) with one of Dutch Holstein cows from the 1990s (Opsomer *et al.*, 1998).

Because of the high correlation between progesterone concentration in blood and milk (*e.g.* r = 0.88; Dobson & Fitzpatrick, 1976), progesterone analysis of milk samples can be used to study the postpartum ovarian activity in the dairy cow. Firstly, a period of low progesterone levels after calving occurs when the cow exhibits a period of anoestrus (Lamming & Bulman, 1976). This period is followed by an increased progesterone level, which is indicative of the first postpartum ovulation. The cavity of the ovulated follicle is gradually filled with progesterone-secreting luteal cells, which forms the corpus luteum. The corpus luteum then dominates the oestrous cycle during the luteal phase with high progesterone levels for about 14 days from about the fourth day after ovulation. After that, the corpus luteum is degenerated and a new ovulation can occur unless the cow becomes pregnant and the corpus luteum is maintained during the pregnancy (Peters & Ball, 1995).

Progesterone and the overall fertility of the dairy cow have been shown to be connected in many different ways. Low probability of embryo survival was shown to be associated with both low and excessive progesterone levels five to seven days after AI, which indicates an optimum in progesterone levels for embryo survival at this time after AI (Stronge *et al.*, 2005). Repeat breeding heifers tended to have higher basal progesterone concentrations at oestrus compared with virgin heifers (Båge *et al.*, 2002) and increased basal progesterone levels at AI have been associated with increased probability of repeat-breeding for cows and higher return rate at AI (Waldmann *et al.*, 2001). The ovarian activity after calving, measured with milk progesterone levels, also affects the overall fertility of the dairy cow. Early onset of oestrous cyclicity after calving has been shown to increase the probability of an early insemination after calving, shorten the interval from calving to conception, increase conception rate, and reduce number of services per conception (Darwash, Lamming & Woolliams, 1997b). Aberrations in the oestrous cyclicity after calving, studied with progesterone profiles, were also associated with longer interval from calving to first service and increased calving intervals (Royal *et al.*, 2000).

Genetics of fertility

Fertility is unfavourably genetically correlated to milk production traits (*e.g.* Janson & Andréasson, 1981; Roxström *et al.*, 2001; Wall *et al.*, 2003). Therefore it is important to include reproductive traits in a total merit index in breeding programmes for dairy cows (Philipsson, 1981). Owing to the general decline in fertility of dairy cows observed in several countries, the awareness has increased worldwide in recent years of the need to include fertility in dairy cattle breeding programmes (Jorjani, 2005).

Fertility has been included in the Swedish genetic evaluation scheme since the 1970s (Lindhé *et al.*, 1994). The genetic trends have shown a decrease in fertility of Swedish Holsteins whereas the genetic level has been relatively constant for the Swedish Red (Lindhé & Philipsson, 2001). It seems that inclusion of fertility in the breeding goal in Sweden has not been enough to withstand the effects of

importation of genetic material into the Swedish Holsteins from countries that have low, or no weighting on fertility in their breeding objective. This may partly be a result of the low heritabilities, usually below 5%, for traits included in the fertility index (Janson, 1980; Roxström *et al.*, 2001). These traits are highly influenced by management, which partly explains the low heritabilities. For instance, the interval from calving to first AI (CFI) is measured to obtain an indirect measure of interval from calving to first ovulation. However, CFI is affected by the farmer's decision of when to start the service period, which may vary between herds and between cows within herds (DeJarnette *et al.*, 2007). Traits other than CFI that are included in the present Nordic fertility index are number of inseminations per service period, interval from first to last insemination, interval from calving to last insemination, non-return rate and fertility treatments (http://www.nordicebv.info; 24-Mar-2007). As mentioned, CFI is an indirect measure of the cow's ability to resume ovarian cyclic activity after calving, whereas non-return rate and number of inseminations are indicators of the cow's ability to become pregnant. The interval from calving to last insemination is an example of a trait that combines the two abilities above (Jorjani, 2005).

To overcome the low heritability of female fertility, measures that more directly reflect the cow's own physiology should be examined. One example would be to use interval to first ovulation determined by the progesterone level in milk instead of CFI. Studies of the interval from calving to commencement of luteal activity (CLA; occurring about four to five days after first ovulation) have revealed heritabilities of 16 to 21%, which is considerably higher than for the present measures of fertility in dairy cows (Darwash, Lamming & Woolliams, 1997a; Veerkamp *et al.*, 2000; Royal, Flint & Woolliams, 2002). In these studies of CLA, progesterone samples were taken relatively frequently. Such frequent sampling would require in-line progesterone monitoring systems. A suggested alternative is to use milk samples from the routine milk recording for analysis of progesterone (van der Lende *et al.*, 2004). The disadvantage is that milk samples in the regular milk recording are taken relatively infrequently (once a month), and it needs to be determined how sampling intervals affect the genetic parameters of progesterone-based measures.

Aims of the thesis

The general aim of this thesis was to study how milk progesterone could be used both as a genetic and as a management tool to improve fertility in dairy cows.

More specifically the aims were:

- To study the interval from calving until regular ovarian cyclicity and the incidence of normal and various atypical progesterone profiles in Swedish dairy cattle.
- To investigate the influence of different systematic environmental factors and genetics on dairy cow fertility measures based on milk progesterone analysis.
- To investigate how milk progesterone measures based on different sampling intervals could be used
  - in breeding programmes for an earlier first ovulation after calving, and
  - as a diagnostic tool for disturbed ovarian function.

Overview of the investigations

Material

This thesis is mainly based on data from the experimental herd of the Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences (Paper I, II & IV). Paper III and part of Paper IV are based on a British material from University of Nottingham, UK and Roslin Institute, UK.

Animals

Swedish material

The data collected in the experimental herd of the Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences covered a period of 15 years from December 1987 to December 2002. The cows were in their 1st to 10th lactation and included almost 1100 lactations from about 500 cows, comprising more than 30,000 milk samples. The cows were of the two major breeds in Sweden, about 300 Swedish Red cows and about 200 Swedish Holstein cows. During the first years of the data collection, the experimental herd was kept at another location, Kungsängen research farm, but were moved to the present location at Jälla high school farm in 1992. At the former location all animals were tied, but at the present location the cows were either tied or in loose housing. From 1994 and onwards the cows were subject to a calving interval trial, in which they were inseminated for expected calving intervals of either 12 or 15 months. Since 1985, the Swedish Red cows in the herd were also subjected to a selection trial on high or low milk fat content but with the same energy content in the milk.

Milk sampling for progesterone analysis started in the second week after parturition. Milk was sampled twice weekly until ovarian cyclical activity was detected. Sampling was then reduced to once a week until first AI. As a consequence of the long study period for the Swedish data material, three different RIA kits were used for progesterone analysis in whole milk samples; Farmose, Spectra (Orion Diagnostica, Espoo, Finland) and Coat-A-Count (Diagnostic Products Corporation, Los Angeles, CA, USA). Progesterone observations from estimated day of ovulation were used to set limits for luteal activity for each of the three kits. Day of ovulation was estimated as the day after detection of oestrus (normal to very strong oestrous signs) or the day before onset of metoestrous bleeding. The limit for luteal activity was set using the progesterone concentration that 95% of the estimated days of ovulation fell below.

British material

The analysed data in Paper III were from a milk progesterone database from the University of Nottingham, UK and Roslin Institute, UK with additional information from two commercial databases from National Milk Records Plc, Chippenham, UK and Holstein United Kingdom, Ricksmanworth, UK. These data were collected between October 1996 and March 1999 and comprised 1212 lactations from 1080 British Holstein cows in eight herds. Milk samples for progesterone analysis were taken three times per week with start at day 2 to 8 after calving, until a maximum of 24 days after the first AI. Progesterone concentration was measured in whole milk samples with ELISA (Ridgeway Science Ltd, Alvington, Gloucestershire, UK).

Fertility measures

Progesterone concentrations were plotted against days postpartum to create individual lactation progesterone profiles. The progesterone profiles were used to derive various early, endocrine-based fertility measures and to categorise the profiles.

The first postpartum progesterone value above the limit for luteal activity, preceded by a low progesterone value, was used to define CLA, see example in Figure 1. Recordings of heat observations were used, together with the progesterone profiles, to decide the day of first ovulatory oestrus (FOE). All samples taken within the first 60 days postpartum were used to calculate the percentage of samples above the limit for luteal activity (PLA), see example in Figure 1. For the definition of PLA_a all samples in the databases were used for the calculation. For PLA based on weekly sampling (PLA_w), only the first sample in each week was included. For PLA based on one sample every fortnight (PLA_f), only the first sample in every two-week period was included. For PLA based on random, monthly sampling (PLA_m), a sample within the first four weeks of lactation was randomly chosen and was used for the calculation together with the sample taken about four weeks (28 to 37 days) later. For the calculation of CLA based on monthly random sampling (CLA_m) the same sampled days as the calculation of PLA_m was used. If the first sampled day had high progesterone level

this day was defined as CLA_m, whereas if the first sampled day had low progesterone level and the consecutive day about four weeks later had a high progesterone level, this day became CLA_m. If both days had low progesterone levels, CLA_m was set to 60 days.

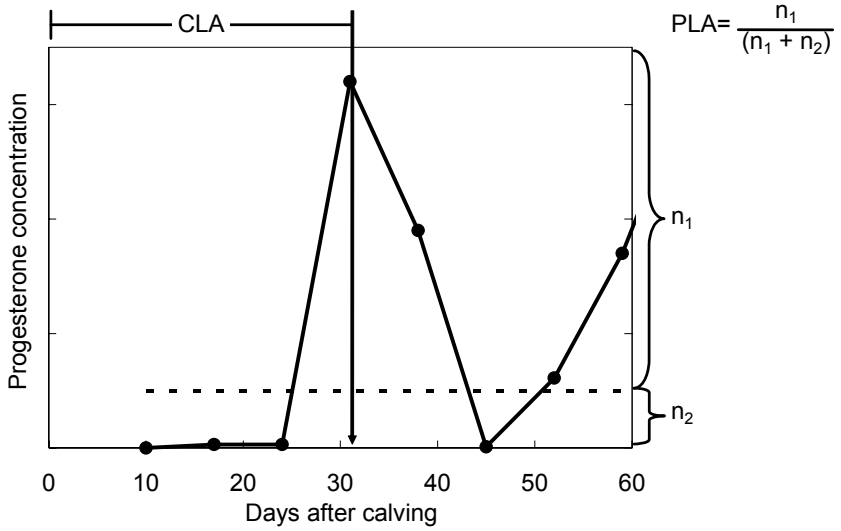

Fig. 1. Example of calculation of the interval from calving to commencement of luteal activity (CLA) and the percentage of samples the first 60 days after calving above the limit for luteal activity (PLA). n_1 = number of samples with luteal activity, n_2 = number of samples without luteal activity.

The progesterone profiles were categorized into (1) normal profile; (2) delayed cyclicity with low progesterone for at least 56 days after calving; (3) cessation of cyclicity, with a normal start but the cyclicity was interrupted with low progesterone for at least 14 days; and (4) prolonged luteal phase, with a normal start but with extended periods of high progesterone of at least 20 days. See Figure 2 for examples of the various types of profiles. In the British material, the definitions were somewhat different for the various aberrations in the progesterone profile and they were defined as (1) delayed ovulation type 1 (DOV1), with low progesterone levels for at least 45 days after calving; (2) delayed ovulation type 2 (DOV2), with low progesterone for at least 12 days between two luteal phases; (3) persistent corpus luteum type 1 (PCL1), with high progesterone levels for at least 19 days in the first postpartum oestrous cycle; and (4) persistent corpus luteum type 2 (PCL2), with high progesterone levels for at least 19 days in oestrous cycles after the first cycle. The aberration called DOV1 in the British material corresponds well to delayed cyclicity in the Swedish material DOV2 to cessation of cyclicity, and PCL1 and PCL2 together to prolonged luteal phase but for all of them somewhat different number of days were used in the definitions as shown above.

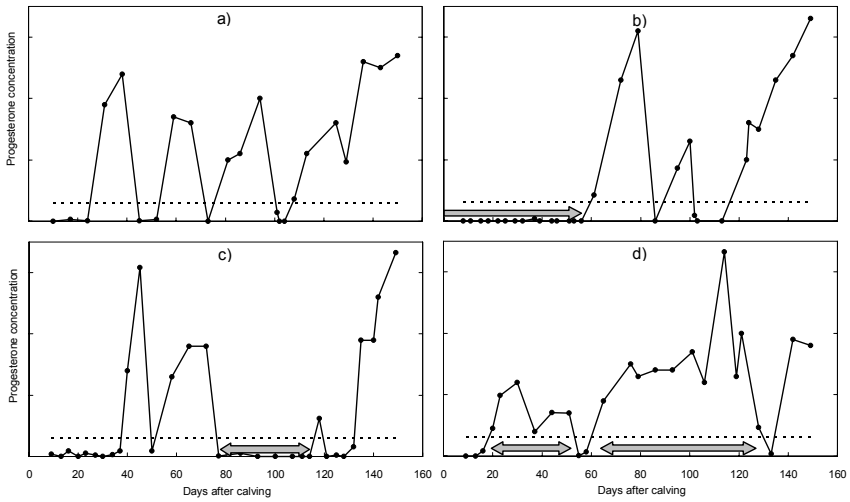

Fig. 2. Illustration of the various progesterone profiles for the Swedish material: a) normal profile, b) delayed cyclicity, c) cessation of cyclicity, and d) prolonged luteal phase. Solid lines represent the progesterone level, dotted lines represent the limit of progesterone for luteal activity and arrows indicate the special feature of each type of profile.

Records of calving dates and AI dates were used to calculate the more traditional fertility measures, such as pregnancy to first AI (PFI), number of AI per service period (NINS), interval from calving to first AI (CFI), and interval from calving to conception (CCI).

Methods

In Paper I, the influence of different fixed effects and the random effect of cow were analyzed with mixed linear models in procedure MIXED of SAS (SAS Institute Inc., 2002). For the analysis of normal and atypical progesterone profiles (Paper II), a multinomial model in procedure MULTILOG in SUDAAN (Research Triangle Institute, 2004) was used. The four different profiles have no natural order and therefore a generalized logit model was implemented to account for this nominal distribution. The three atypical profiles were also merged into a single category to allow a binomial analysis of the data. This model was analysed with the same procedure as the multinomial model but with a cumulative logit because of the ordinal response.

For the genetic analyses in Paper III, the REML option of the DMU package (Jensen & Madsen, 1994) was used to fit a mixed linear animal model to the data. Variance components and breeding values were obtained from a single-trait analysis but for correlations, a bivariate analysis was applied.

In Paper IV, procedure LOGISTIC in SAS (SAS Institute Inc., 2002) was used to fit logistic regression models to half of the Swedish dataset (test data). These models were then applied to the rest of the Swedish dataset and to the entire

British dataset for validation. To examine the ability of the different models to discriminate between delayed cyclicity and the other types of profiles we used non-parametric receiver operating characteristic (ROC) curves, which is a plot of sensitivity (true positive fraction) of a test versus 1-specificity (false positive fraction) calculated for several different decision thresholds or probability cut-offs. The diagnostic accuracy of a test can be obtained by calculating the area under the ROC curve (Dohoo, Martin & Stryhn, 2003). The non-parametric receiver operating characteristic curves were constructed using procedure LOGISTIC in the SAS package (SAS Institute Inc., 2002), and the area under the ROC curve was calculated using the "*%roc*" macro (SAS Institute Inc., 2006).

Main results

Early fertility measures (Paper I, II)

In the Swedish material, the average CLA was 33.8 days, the average FOE was 61.3 days and PLA had an average of 37.2%. Delayed cyclicity was the most common atypical profile, present in 15.6% of the included lactations. Profiles with cessation of cyclicity and prolonged luteal phase were present in 6.6% and 7.3%, respectively, of the lactations.

Prolonged CLA was mainly found in cows with a delayed cyclicity profile in the Swedish dataset or DOV1 in the British dataset (Table 1). The mean for PLA was significantly different for all various types of progesterone profiles in the Swedish dataset, with the highest mean for prolonged luteal phase profiles and lowest for delayed cyclicity profiles. In the British dataset, PLA differed significantly for all profiles, except for PCL2, which did not differ significantly compared with profiles with no atypical pattern or PCL1 profiles.

Table 1. *Mean ± SE for interval from calving to commencement of luteal activity (CLA) and percentage of samples the first 60 days after calving above the limit for luteal activity (PLA) per type of profile for the Swedish and the British dataset*[1]

Dataset	Profile	CLA (days)	PLA (%)
Swedish dataset	Normal profile	28.8 ± 0.4^{a}	42.1 ± 0.6^{a}
	Delayed cyclicity	76.3 ± 2.5^{b}	2.6 ± 0.4^{b}
	Cessation of cyclicity	29.4 ± 1.7^{ad}	26.6 ± 2.1^{c}
	Prolonged luteal phase	25.4 ± 1.5^{cd}	58.7 ± 2.0^{d}
British dataset	No atypical pattern	23.0 ± 0.3^{a}	51.7 ± 0.5^{a}
	Delayed ovulation, type 1	61.2 ± 1.5^{b}	13.5 ± 0.8^{b}
	Delayed ovulation, type 2	23.9 ± 1.7^{a}	39.8 ± 2.4^{c}
	Persistent corpus luteum, type 1	23.9 ± 0.8^{a}	60.2 ± 1.2^{d}
	Persistent corpus luteum, type 2	24.2 ± 1.3^{a}	$54.4 \pm 1.9^{a, d}$

[1]Different letter in subscript indicates difference ($P < 0.05$) between different profiles within type of fertility measure and within dataset.

A range of different systematic factors affected the interval measures and the probability of different types of progesterone profiles (Table 2). The Swedish Holstein cows were more likely to have an atypical progesterone profile compared with Swedish Red cows. This was mainly due to an increased proportion of profiles with prolonged luteal phase for the Swedish Holsteins. First parity cows had a higher incidence of delayed cyclicity profiles compared with older cows, which also resulted in a significantly longer CLA and FOE in the first lactation compared with later lactations. Delayed cyclicity was also more common for cows in tie stalls compared with cows in the loose housing system and consequently they also had longer CLA and FOE. Cows that calved during the winter season (November to April) were more likely to have both delayed cyclicity and cessation of cyclicity compared with cows that calved during the summer season (May to October). Both CLA and FOE were consequently longer during the winter season in contrast to the summer season. The calving interval trial affected both type of progesterone profile and FOE. For cows in their second or higher lactation with a planned longer calving interval (15 months) the risk of delayed cyclicity and cessation of cyclicity increased, and FOE was longer compared with cows in their second or higher lactation with a conventional calving interval (12 months). Incidence of mastitis increased both CLA and FOE, but did not affect type of progesterone profile. Cows with lameness had longer CLA and FOE and increased risk of delayed cyclicity compared with healthy cows. Generally, the effects of the different environmental factors on PLA followed the same trend as the effects for CLA.

Table 2. *LS-mean differences (Class A - Class B) for interval from calving to commencement of luteal activity (CLA), interval from calving to first ovulatory oestrus (FOE), percentage of samples the first 60 days after calving above the limit for luteal activity (PLA) and odds ratios (OR; Class A compared with Class B) for atypical versus normal progesterone profile for different systematic effects in the Swedish dataset*

Class A	Class B	CLA (days)	FOE (days)	PLA (%)	OR
Swedish Holstein	Swedish Red	-2.9	-1.5	2.9	1.50*
Parity 2	Parity 1	-18.1***	-19.7***	9.6***	0.48**
Parity ≥ 3	Parity 1	-14.8***	-19.3***	6.8**	0.66
Winter	Summer	10.5***	9.4***	-12.2***	2.55***
CI[a] 12	CI 15	-4.6	-11.0**	3.2	2.47**
Tie stall	Loose housing	7.5***	8.9**	-6.4***	2.29***
Mastitis	No mastitis	8.4***	12.3***	-6.9***	---
Lameness	No lameness	18.0***	18.9***	-9.8	1.83

[a]Second and later lactations in the calving interval trial of 12 or 15 months
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

To consider repeated measures within cow we entered cow as a random effect in the analyses of CLA and PLA. This effect was significant for both these measures and the repeatability estimates were 0.14 and 0.16 for CLA and PLA, respectively. In the analysis of progesterone profiles the risk of an atypical profile in the ongoing lactation was 2.2 times higher if the previous lactation had an atypical profile.

The various types of atypical profiles influenced the later, traditional, fertility measures. Taking all three types of atypical progesterone profiles together, they had 15 days longer CFI compared with normal profiles and 18 days longer CCI compared with normal profiles. Cows with an atypical progesterone profile also tended to have lower PFI compared with those with a normal profile, 47 and 52% respectively.

Milk sampling intervals for progesterone analysis (Paper III, IV)

The effect of changed milk sampling scheme for progesterone analysis for genetic analysis and management purposes was investigated with the PLA measure. For the genetic analysis (Paper III) the British progesterone database was used and for the management study (Paper IV) both the Swedish and the British materials were used. The different sampling regimes were all present samples, weekly sampling, fortnightly sampling and monthly sampling.

Heritabilities for the different PLA measures are presented in Table 3. The heritability estimate for PLA_a, using all samples, was 29.5%. The heritability estimates decreased with decreasing sampling frequency. An average heritability estimate for CLA_m was calculated and included in Table 3, in addition to the PLA measures that were presented in Paper III. The former had an average heritability of 9.2%. Both CLA_m and PLA_m in Table 3 are based on an average over 10 different random runs. The average heritability estimate for PLA_m over these 10 different runs became somewhat lower compared with the heritability estimate of 14% reported in Paper III.

Table 3. *Estimates of phenotypic variance (σ^2_P), genetic variance (σ^2_A), herd-year-season variance (σ^2_{HYS}, not included in phenotypic variance), heritability (h^2) and standard error of heritability (SE) for various measures in the British dataset*[1]

	σ^2_P	σ^2_A	σ^2_{HYS}	h^2 (%)	SE
PLA_a	364.9	107.7	19.5	29.5	5.8
PLA_w	398.1	98.4	14.8	24.7	5.7
PLA_f	433.7	87.4	28.0	20.1	5.5
PLA_m average of 10 runs	1112	125.7	26.9	11.3	5.0
CLA_m average of 10 runs	226.5	21.0	7.0	9.2	4.9

[1]PLA_a = Percentage of samples the first 60 days after calving above the limit for luteal activity, based on all samples. PLA_w = PLA, based on weekly sampling. PLA_f = PLA, based on fortnightly sampling. PLA_m = PLA, based on monthly sampling. CLA_m = Interval from calving to commencement of luteal activity based on monthly sampling.

The different PLA measures had high negative genetic correlations with DOV1 (-0.53 for monthly sampling, < -0.87 otherwise) and a moderate positive genetic correlation with PCL1 (> 0.65 if at least fortnightly sampling). The genetic correlation between all PLA measures and lnCLA was strong and negative. The average genetic correlation between PLA_m and lnCLA was -0.84, with an average standard error of 0.18. The average genetic correlation between CLA_m and lnCLA was 0.85, with an average standard error of 0.18. These averages were based on three genetic correlations between PLA_m and lnCLA and five genetic correlations between CLA_m and lnCLA. The bivariate analyses of the other of the 10 different datasets did not converge. The genetic correlation between PLA_m and lnCLA in Paper III was used in the selection index calculations and showed that milk sampling for progesterone analysis once per month could be used with high accuracy (0.80 with 50 daughters per bull) to predict breeding values for lnCLA.

The accuracy of using progesterone measures in different models for detection of delayed cyclicity was obtained using ROC curves and calculating the area under curve for half of Swedish dataset (validation data) and for the entire British dataset. The accuracy was highest (0.94 to 0.99), as expected, when CLA or PLA_a was included in the model without progesterone measures. The accuracy was also significantly higher when the CLA_m or PLA_m (0.85 to 0.88) was added to the model compared with the model with no progesterone measure (0.76 and 0.67). The overall percentage correct diagnosed lactations, for selected probability cut-offs, were 88 to 98% for CLA and PLA_a, 80 to 82% for CLA_m and PLA_m and 65 or 68% for the model with no addition of progesterone measures.

General discussion

Progesterone based fertility measures

Trends for Swedish dairy cows

CLA is an indicator of the interval from calving to first ovulation, which occurs on average four to five days before CLA (Darwash, Lamming & Woolliams, 1997a). The average CLA was 33.8 days (Paper I). This translated into the interval to first ovulation would indicate that the first ovulation occurred about two days later in this study compared with previous studies of Swedish cows (Larsson *et al.*, 1984; Berglund *et al.*, 1989). However, the overall mean of 61.3 days for FOE (Paper I), was considerably longer compared with earlier studies on Swedish dairy cows, where 47 days was reported (Larsson *et al.*, 1984; Berglund *et al.*, 1989). The comparison between the results in this thesis and earlier Swedish studies may indicate that oestrous signs, rather than the interval to first ovulation, have been affected by selection for increased production. From the USA, Washburn *et al.* (2002) reported that oestrus detection rates decreased from 51% to 42% for Holsteins between 1985 and 1999 and Wiltbank *et al.* (2006) reported a shorter duration of oestrus for cows with high milk production compared with low

producing cows. In Paper I, no changes in CLA and FOE were observed for the period of 15 years that were studied.

The proportion of atypical progesterone profiles was 30% (Paper II), which was lower than reported in recent studies from Belgium (49%; Opsomer *et al.*, 2000) and the UK (44%; Royal *et al.*, 2000) but higher than the 22% of atypical profiles reported in a study more than 25 years ago (Bulman & Wood, 1980). However, definitions of atypical profiles in these different studies differed slightly, *i.e.* the number of days used differed in the definitions, which make the results not directly comparable. The risk of delayed cyclicity (Paper II) was significantly increased in the most recent calving year groups (2000 to 2002) as compared with the two preceding periods (1994 to 1996 and 1997 to 1999).

The increased incidence of delayed cyclicity in the last calving year group may have been related to the general decrease in fertility for Swedish dairy cows. The overall fertility of the dairy cow is, however, influenced by a range of factors, including fertility in the early postpartum period as well as by oestrous signs, semen and oocyte quality, embryo survival *etc.* Atypical profiles was strongly associated with the late fertility measures; CFI was 15 days longer and CCI was 18 days longer in lactations with an atypical profile than in lactations with a normal profile (Paper II). An underlying factor for this association may be that an increased number of oestrous periods before the first service increases the probability of a successful first service (Whitmore, Tyler & Casida, 1974). With an early start of cyclicity after calving and regular ovarian activity the number of oestrous cycles may increase before the service period begins. Lamming & Darwash (1998) speculated that increased number of oestrous cycles before the first insemination positively affected the oviductal and uterine environment and therefore improved embryo survival.

Breed differences

The risk of atypical profiles was increased, explained mainly by prolonged luteal phase profiles, for the Swedish Holstein cows as compared with Swedish Red cows. This difference can be influenced by the genetic difference between the two breeds. The genetic trend in fertility for the Swedish Holstein breed has been negative, which is highly influenced by imported genetic material. By contrast, the genetic level has remained relatively constant in the Swedish Red breed (Lindhé & Philipsson, 2001). An increased proportion of profiles with prolonged luteal phase was observed by Royal *et al.* (2000), who compared data from 1975 to 1982 with those from more recent years (1995 to 1998) for British Holstein-Friesian cows. The higher incidence of prolonged luteal phase profiles that was observed for the Swedish Holstein cows compared with the Swedish Red cows in Paper II supports the findings by Royal *et al.* (2000) and may indicate a general increase of prolonged luteal phases in the worldwide Holstein population.

Other systematic effects

First parity cows had longer CLA and FOE than cows in their second and later parities. They also had a higher risk of delayed cyclicity profiles. In earlier Swedish studies, later onset of ovarian activity in first parity cows has also been reported, associated with negative energy balance (Berglund *et al.*, 1989). The relationships between fertility and metabolic traits were found to be different for first parity cows and older cows (Wathes *et al.*, 2007).

Taylor *et al.* (2003) reported that cows with delayed cyclicity had a long period of negative energy balance, the lowest body weight and the greatest losses in body condition score after calving. In Paper II, cows with a decrease of more than 50 kg in body weight during the first 8 weeks of lactation had an increased risk of delayed cyclicity profiles. The effect of these body weight changes may have affected the association between a longer dry period and increased incidence of delayed cyclicity profiles because cows with longer dry periods (≥ 68 days) were probably more obese and therefore may lose more body weight after calving compared with cows with a short dry period (≤ 46 days). Wathes *et al.* (2007) found that higher body condition score before calving for first parity cows was followed by lower body condition score after calving, which was associated with a longer CCI.

The effect of longer dry periods in the previous lactation on type of progesterone profile may explain the increased incidence of progesterone profiles with delayed cyclicity and cessation of cyclicity for cows that previously had had a lactation with a planned extended calving interval of 15 months. Longer dry periods for cows with a planned 15-month calving interval were shown by Rehn *et al.* (2000). Profiles were analysed until first AI, which is considerably later in cows with an extended calving interval. This increased study period may therefore in itself be a factor that might explain the higher probability of atypical profiles for cows with planned calving intervals of 15 months.

Increased energy corrected milk yield of 1 SD (about 420 kg) the first 60 days in milk were associated with increased CLA of 3.7 days and increased FOE of 6.3 days. Associations between higher milk yields and increased CFO and FOE were also found in earlier studies of Swedish dairy cattle (Berglund *et al.*, 1989). However, no significant phenotypic association between CLA and milk yield was observed by Royal, Flint & Woolliams (2002), but higher milk yield was genetically correlated with longer CLA. No significant association was detected between milk yield and type of progesterone profile in Paper IV.

Cows that calved during the winter season (November to April) had longer CLA and FOE, and an increased incidence of both delayed cyclicity and cessation of cyclicity compared with summer calving cows (May to October). Calving during the winter season increased the risk of delayed cyclicity (Opsomer *et al.*, 2000) and increased the interval from calving to first oestrus (Hansen and Hauser, 1983). In the later study, the effect of calving season was neither affected by diet nor management, so the effect was probably caused by photoperiod or temperature.

Tied cows had both longer CLA and FOE and increased proportion of delayed cyclicity compared with cows in loose housing. Claus *et al.* (1983) also found that intervals from calving to first ovulation were longer in tie stalls than in loose housing. Better fertility in the loose housing system could be explained by a better opportunity for exercise and social interaction between cows.

Cows that had been treated for mastitis or lameness had longer CLA and FOE (Paper I). In a meta-analysis of several published papers, Fourichon, Seegers & Mahler (2000) found no effect of mastitis on traditional fertility measures, whereas leg problems were associated with an average increase of 12 days to conception. The incidence of delayed cyclicity was higher in cows that had been treated for lameness (Paper II). This agrees with Garbarino *et al.* (2004), who showed an increased risk of delayed cyclicity in cows with lameness.

Repeatabilities

The Swedish data did not have a structure that enabled genetic analyses of the various fertility measures. However, each cow had generally several parities in the data, which made it possible to estimate the random effect of cow and the effect of atypical progesterone profiles in earlier parities. The repeatability for CLA (0.14) in the Swedish dataset was lower than that reported by Darwash, Lamming & Woolliams (1997a) (0.26-0.28), but the repeatabilities for both CLA and PLA were higher than the repeatabilities for PFI and NINS (Paper I). The repeatability for CLA corresponded well to the repeatability for interval to first ovulation reported by Berglund *et al.* (1989). The risk of an atypical progesterone profile was increased if the cow had displayed an atypical profile in the previous lactation (Paper II), which is indicative of a genetic influence on type of progesterone profile.

Progesterone analysis of milk sampled at different intervals

To examine how infrequent sampling of milk for progesterone analysis, *e.g.* as in the current Swedish milk recording with monthly milk sampling, could be used the measure PLA was introduced. This measure was calculated with milk sampling for progesterone analysis two to three times per week, weekly, fortnightly or monthly. However, the main purpose was to study the difference between using all samples and using only monthly sampling. Analysis of PLA was also complemented with CLA using all samples or monthly sampling. The advantage of PLA is that it is easy to automate and that it yields information on what happens after ovarian cyclicity has started. However, the interpretation of PLA is not as straightforward as it is for CLA.

The associations between CLA or PLA and the various types of progesterone profiles were studied in Paper II and III (Table 1). Some of these results were obvious and expected, such as the major delay in CLA for profiles with delayed cyclicity in the Swedish dataset and with DOV1 profiles in the British dataset. The

mean of PLA was different between all types of progesterone profiles in the Swedish dataset. This was also confirmed in Paper III, utilizing the British material, except for PCL2 profiles. The PLA measure was lowest for cows with a delayed cyclicity profile or a DOV1 profile and higher for cows with a prolonged luteal phase profile or a PCL1 profile compared with cows with normal or no atypical pattern in the progesterone profile.

Genetic aspects

The heritability estimates for PLA with different sampling intervals were moderate, ranging from 29.5% for the most frequent sampling to 14.0% with monthly sampling (Paper III). This was a surprisingly high heritability estimate for PLA_a (29.5%) compared with heritability estimates for CLA that previously has been reported (16% to 21%, Darwash, Lamming & Woolliams, 1997a; Veerkamp *et al.*, 2000; Royal, Flint & Woolliams, 2002). The further examination of PLA_m, with 10 new random selections of sampling days revealed a mean heritability estimate of 11.3% (Table 3). This was slightly lower than the heritability estimate of 14% reported in Paper III for PLA_m. For the 10 new datasets with PLA_m, CLA_m was also calculated. The average heritability estimate of 9.2% for CLA_m was lower, but close to the heritability for PLA_m.

High negative genetic correlations were found between DOV1 and the different PLA measures, and moderate to high positive genetic correlation between PCL1 and PLA. The genetic correlations with PCL1 raise concern with regard to fertility disorders because it has been shown that prolonged luteal phases are associated with pyometra (Etherington *et al.*, 1991). Thus, it appears that PLA has an intermediate optimum value: too high a value for PLA is unfavourable because it is associated with PCL1 but a low value could also be unfavourable because it is associated with DOV1. The genetic correlations between PLA_m, and DOV1 and PCL1 respectively indicated that monthly milk sampling for progesterone analysis could give an indication of DOV1 profiles because of the moderate correlation with this type of profiles, but this infrequent sampling regime cannot be used to detect PCL1 profiles.

In some breeding programmes for dairy cow fertility, *e.g.* the Nordic fertility index, CFI is used to measure the cows' ability to resume cyclical ovarian activity after calving (Jorjani, 2005), *i.e.* CFO. However, CLA is a more direct measure of CFO than is CFI. A fertility index with CLA would be improved with the addition of a measure that is more related to the cow's own physiology. With CLA as breeding goal trait in the selection index, PLA_m as index trait resulted in a much higher accuracy (0.80) compared with when CFI was the index trait (0.09), calculated with a daughter group size of 50 cows (Paper III). The new analysis of the 10 new PLA_m and CLA_m calculations revealed lower genetic correlations between PLA_m or CLA_m and lnCLA (on average an absolute value of 0.85) than reported between PLA_m and lnCLA in Paper III. However, the selection index calculation (Paper III) was tested also with lower genetic correlations (0.8 and 0.9) and the accuracy decreased but remained high (0.64-0.72). Because CLA_m and PLA_m are based on monthly sampling, it was concluded that milk sampling for

progesterone analysis in the regular milk recording could be used to increase the accuracy of a breeding programme towards an earlier start of cyclical ovarian activity after calving.

The PLA measure was mainly introduced to test how a measure based on progesterone analysis of milk samples was changed with altered sampling intervals. However, compared with CLA, the luteal activity after CLA also affected PLA (if CLA was shorter than 60 days). This could be seen as an advantage, because PLA could potentially detect also other problems than delayed cyclicity. However, with monthly sampling, this ability was poor. This resulted in that CLA based on monthly sampling, CLA_m was also examined. The heritability estimates for CLA_m was slightly lower than for PLA_m but because of the easier interpretation of CLA_m, this measure could have an advantage over PLA_m. Previously, an alternative measure to CLA_m and PLA_m has been studied, CLA50% introduced by van der Lende (2004). This measure is the lactation stage when 50% of the daughters of a sire had an active corpus luteum with 3 to 6 weeks intervals of milk sampling for progesterone analysis. The rationale for CLA50% is also infrequent sampling, like the PLA_m and CLA_m measures. However, PLA_m and CLA_m were calculated on the cow level in contrast to CLA50% which was calculated on the sire level. Obtaining the information on a cow level gives an opportunity to use this information not only for genetic evaluation but also for management purposes.

Management aspects

The possibility to use PLA_m or CLA_m for management purposes was investigated in Paper IV. Moreover PLA and CLA based on all samples, two to three samples per week, was included in the study. This frequent sampling is at present impractical and expensive to apply in commercial herds. A future in-line milk sampling system combined with progesterone analysis would allow more frequent milk sampling. Models with use of such data were described by Friggens & Chagunda (2005).

Progesterone measures based on milk sampling for progesterone analysis as in the regular milk recording (once a month) could be used to predict cows with delayed ovarian cyclicity (Paper IV). The similar outcome between the two different validation datasets, from two countries, strongly supports this conclusion. The results for the two validation datasets were surprisingly concordant even though some differences existed between the two materials, *e.g.* the definition of delayed cyclicity differed by 10 days. Inclusion of progesterone measures based on monthly milk sampling in the models gave a greater probability to predict delayed cyclicity compared with the basic model, which included only the herd and animal risk factors.

The predictive model in Paper IV may be used as it is, but probability cut-off values have to be set up by considering sensitivity and specificity demands. Setting up an optimal cut-off requires information about the incidence of delayed cyclicity in the population and consequences or costs for false-negatives and false-

positives (Greiner, Pfeiffer & Smith, 2000). With the selected probability cut-offs used in this study, 73 to 80% of all lactations in the British and Swedish validation datasets with delayed cyclicity were correctly identified (sensitivity) with PLA_m or CLA_m in the model. The results for the Swedish data showed an opportunity to correctly identify four out of five cows with delayed cyclicity, already at around 60 days after calving. This would allow a decrease in the time range from calving to start of treatment. Results from Swedish field data showed that the interval from calving to veterinary examination of anoestrous dairy cows averaged 98 days, which led to an average interval from calving to conception of 154 days for these cows (H. Gustafsson, Swedish Dairy Association, personal communication)

Ruiz, Oltenacu & Smith (1992) found in a cost-benefit evaluation of an on-farm progesterone test that it was profitable to monitor return to cyclicity but not to detect type of clinical condition. The approach in Paper IV was to use milk samples for progesterone analysis that could also be used to improve the genetic evaluation for fertility in dairy cows, as shown in Paper III. The cost for progesterone analysis of milk samples could therefore benefit from management returns as well as from improvements in genetic gains for fertility. The inclusion of progesterone analysis in the analysis of milk samples in the current Swedish milk recording system should therefore be considered. In Sweden, approximately 85% of all dairy cows are included in this system, which already today contains information about the other effects included in the models used in this study. Information from progesterone analysis of milk samples from all cows postpartum could also become an important part of a preventive herd health programme in large, high-producing dairy herds (Sheldon, Wathes & Dobson, 2006). Further studies should be conducted to determine the exact cost-benefit of an introduction of the progesterone analysis of milk samples in the regular milk recording.

Conclusions

- Early endocrine measures of a dairy cow's fertility were more influenced by the cow itself than were the more traditional, late measures of fertility, as indicated by higher repeatability for the endocrine measures. The proportion of samples with luteal activity within 60 days after calving had a surprisingly high heritability. This was higher than that earlier reported for the interval from calving to commencement of luteal activity.

- The increased risk of atypical profiles after a cow had had an atypical profile in the previous lactation was also indicative of genetic influence on the early postpartum fertility expressed by progesterone profiles.

- The interval from calving to first ovulatory oestrus was 14 days longer in this study compared with earlier Swedish studies from the 1980s, which indicates that the strength or duration of oestrous signs has decreased in the last two decades. The proportion of atypical profiles increased in the last few years of the study.

- A higher incidence of atypical profiles in the Swedish Holsteins was found compared with the Swedish Red cows.

- Parity, season, housing and lameness considerably affected the early fertility measures and the risk for atypical progesterone profiles.

- Atypical progesterone profiles had a negative association with the traditional fertility measures, and both the interval from calving to first insemination and the interval from calving to conception were longer in lactations with an atypical profile.

- Progesterone analysis of monthly milk samples from the current Swedish milk recording can be used
  - with high accuracy to select for an earlier start of luteal activity after calving, and
  - as a management tool to predict cows with delayed cyclicity.

Future research

- The results concerning the ability to improve the genetic evaluation for fertility with inclusion of progesterone analysis of milk samples in the regular milk recording should be further examined with field data. This calls for inclusion of progesterone analysis of milk samples that are sent in for milk composition analysis in the current Swedish milk recording. However, the economics of this progesterone analysis has to be examined before being implemented in the standard procedures.

- The rather high accuracy obtained for detecting cows with delayed cyclicity suggests that the information about progesterone analysis of milk samples in the regular milk recording should be presented to farmers to be used as management tool. The interpretation of this information should be further studied taking economics into account.

- Measures based on frequent milk sampling for progesterone analysis had moderate heritabilities. This together with a breeding programme including a newly established nucleus herd for Swedish dairy cattle gives an opportunity to study inclusion of fertility in a nucleus breeding scheme.

- Because the measures based on progesterone that have been studied in this thesis are more related to the dairy cow's own physiology, they offer an opportunity to find quantitative trait loci (QTL) that affect fertility in dairy cows to a large extent. Therefore, QTL studies on progesterone-based measures could be promising to further examine the genetic background of fertility traits in dairy cattle.

- The longer interval to first ovulatory oestrus in this study compared with earlier studies is an important finding. Strength and duration of oestrous signs should be studied more in depth than in this study, noting the differences regarding fertility between the two major dairy breeds in Sweden, Swedish Holsteins and Swedish Red dairy cows.

Populärvetenskaplig sammanfattning

Syftet med denna avhandling var att studera hur mjölkprogesteron kan användas både som ett avelsverktyg och ett skötselverktyg för att förbättra mjölkkornas fruktsamhet.

Mjölkkornas fruktsamhet tycks stadigt försämras i många länder. Detta har även skett i Sverige, trots att det svenska avelsarbetet har tagit hänsyn till mjölkkons fruktsamhet sedan 1970-talet. Den försämrade fruktsamheten kan delvis bero på det ofördelaktiga genetiska sambandet som finns mellan mjölkproduktion och fruktsamhet. Försämringen beror också på att de egenskaper som används i avelsarbetet för en förbättrad fruktsamhet, till exempel intervallet från kalvning till första seminering, i allmänhet har låg arvbarhet. Detta eftersom de i hög grad påverkas av skötselbeslut, till exempel strategier för när semineringsperioden påbörjas i en besättning. Därför kan det vara intressant att undersöka mätmetoder för fruktsamhet hos mjölkkor som är mer objektiva och mindre styrda av skötsel och det är här progesteron kommer in i bilden.

Progesteron utsöndras i blodet men ”läcker” över i mjölken vilket gör det möjligt att med ett mjölkprov få ett värde på nivåerna av progesteron i blodet. Efter kalvning är progesteronhalterna låga fram till första ägglossning, då gulkroppen som bildas i äggstocken efter ägglossning växer till och börjar producera progesteron ca fyra dagar efter ägglossningen. Därefter är progesteronhalten hög i ungefär 14 dagar innan gulkroppen dör och progesteronnivån sjunker och är låg i ca tre dagar innan nästa ägglossning sker. Mätmetoder baserade på progesteron kan därmed ge ett direkt mått på när mjölkkons könsfunktioner är återställda efter kalvning och hur regelbundna brunstcyklerna är. Tidig återställning av könsfunktionerna är fördelaktigt för fruktsamheten i stort vilket bland annat beror på att ju fler brunstcykler en ko har innan första semineringen desto högre är chansen att hon ska bli dräktig på den semineringen.

Studierna i avhandlingen bygger till större delen på material insamlat vid Institutionen för Husdjursgenetiks försöksbesättning vid Jälla naturbruksgymnasium utanför Uppsala under åren 1987 till 2002. Totalt har ca 30 000 mjölkprov för progesteronanalys samlats in, från ca 1100 laktationer från ca 300 SRB- och 200 SLB-kor. Dessutom ingår analyser av ett brittiskt datamaterial som samlades in under åren 1996 till 1999. I det svenska materialet togs mjölkprover för progesteronanalys ca två gånger per vecka och i det brittiska materialet tre gånger per vecka. Utifrån progesteronanalyserna konstruerades ett antal olika mätegenskaper för tidig fruktsamhet som sedan analyserades: CLA - intervallet från kalvning till första dag med högt progesteron (luteal aktivitet), PLA - procentuell andel prov med högt progesteronvärde av alla prov tagna inom 60 dagar efter kalvning, samt intervallet från kalvning till första brunst med ägglossning, verifierat med progesteronmätningar. Både CLA och PLA beräknades också med varierande provtagningsintervall för att exempelvis efterlikna kokontrollen med ett prov per månad. I det svenska materialet

undersöktes också andelen normala och onormala progesteronprofiler. De onormala profilerna delades in i tre olika kategorier: försenad cyklicitet, avbruten cyklicitet och förlängd lutealfas (en förlängd period med högt progesteron).

Medelvärdet för CLA var 34 dagar. Eftersom den första ägglossningen inträffar fyra till fem dagar före CLA, innebär detta att första ägglossningen i genomsnitt inträffar 29 till 30 dagar efter kalvning. Detta har inte undersökts på svenskt material sedan 1980-talet då det genomsnittliga intervallet till första ägglossning var 27 dagar. Genomsnittet för intervallet från kalvning till första brunst med en verifierad ägglossning var 61 dagar i denna studie. Detta var betydligt senare än tidigare svenska studier som redovisade ett genomsnitt på 47 dagar. Detta kan betyda att kornas brunststyrka har försämrats över åren eftersom det bara var en mindre förändring i tiden till första ägglossning men en betydligt större förändring i tiden till första brunst med verifierad ägglossning. Andelen onormala progesteron-profiler var 30 % men de ökade i den sista treårsperioden (2000-2002) jämfört med de tidigare perioderna. Den vanligaste onormala typen av progesteronprofil var försenad cyklicitet vilken stod för drygt 50 % av de onormala profilerna.

Den tidiga fruktsamheten mätt med olika progesteronmått påverkade semineringsresultaten. Exempelvis så hade kor med onormal progesteronprofil i genomsnitt 15 dagar längre intervall till första seminering och 18 dagar längre intervall till dräktighetsgivande inseminering. Detta stöds av tidigare studier som visat att ju tidigare kon kommer igång efter kalvning och ju mer regelbundna cykler hon har desto bättre blir fruktsamhetsresultaten i stort.

SLB hade en högre risk för en onormal progesteronprofil jämfört med SRB. Detta har troligen ett samband med den försämrade fruktsamheten som har visats för SLB nationellt sett. Progesteronmåtten påverkades negativt av en rad olika faktorer, till exempel var de sämre för förstakalvare jämfört med äldre kor, kor som kalvade under vintern jämfört med sommaren, uppbundna kor jämfört med lösdrift och kor med mastit eller klövproblem jämfört med friska djur.

I det svenska materialet hade de progesteronbaserade fruktsamhetsmåtten en högre upprepbarhet jämfört med mått baserade på semineringsresultat. En ko som hade haft en onormal progesteronprofil i förra laktationen hade också en högre risk att visa en onormal profil igen. För skattningar av genetiska parametrar (till exempel arvbarhet) av PLA-måttet utnyttjades det brittiska datamaterialet. När PLA beräknades baserat på samtliga prover uppskattades arvbarheten till ca 30 %, vilket är högt för att vara ett fruktsamhetsmått. PLA användes därefter för att studera hur de genetiska parametrarna påverkades av förändringar i provtagnings-intervallet av mjölkprover för progesteronanalys. Som förväntat sjönk arvbarheten med ett längre provtagningsintervall och var i genomsnitt 11 % för månadsvis provtagning. Detta är emellertid högre än för de fruktsamhetsmått som används i avelsvärderingen idag. Månadsvis provtagning av progesteron, liknande prov-mjölkningen, visade sig också kunna användas för att med stor säkerhet selektera djur med tidig CLA och därmed också en tidigare första ägglossning.

Slutligen undersöktes möjligheten att diagnostisera djur med försenad cyklicitet med CLA och PLA baserat på olika provtagningsintervall av mjölkprover för progesteronanalys. Försenad cyklicitet beror i huvudsak på två olika kliniska problem, nämligen utebliven äggstocksaktivitet eller äggstockscysta. De mest frekventa provtagningsintervallen gav den största säkerheten, men även månadsvis provtagning visade på en möjlighet att diagnostisera fyra av fem kor med försenad cyklicitet redan två månader efter kalvning. Detta skulle ge en möjlighet för tidigare behandling av kor med fruktsamhetsstörningar vilket i sin tur skulle kunna minska andelen ofrivilligt förlängda kalvningsintervall.

Slutsatserna av studierna som ingår i denna avhandling är att:

- Fruktsamhetsmått baserade på progesteronmätningar har högre upprepbarhet jämfört med traditionella fruktsamhetsmått, som dräktighet vid första seminering. Om kon hade haft en onormal progesteronprofil i föregående laktation ökade risken att hon skulle uppvisa en onormal profil igen. Den procentuella andelen prov med högt progesteron av alla prov tagna inom 60 dagar efter kalvning (PLA) hade en överraskande hög arvbarhet jämfört med andra fruktsamhetsmått baserade på progesteron som har redovisats i tidigare studier.

- Intervallet från kalvning till första brunst med ägglossning var 14 dagar längre i denna studie jämfört med tidigare svenska studier från 1980-talet vilket kan bero på att brunststyrkan har försämrats eller att brunsterna blivit kortare de senaste två decennierna. Andelen onormala progesteron-profiler var dessutom högst de sista tre åren i det studerade materialet

- Andelen onormala profiler var högre hos SLB jämfört med SRB. Laktation, säsong, inhysningssystem och benproblem hade också stora effekter på risken för en onormal profil och på de andra progesteron-baserade fruktsamhetsmåtten som studerades. En SLB kviga som kalvar in under vinterhalvåret, står i ett uppbundet stall och dessutom har benproblem har en mycket stor risk för en försämrad fruktsamhet.

- Det fanns ett starkt samband mellan onormala progesteronprofiler och sämre fruktsamhetsresultat senare i laktationen, såsom längre intervall från kalvning till första seminering och från kalvning till dräktighet.

- Progesteronanalys av månadsvisa mjölkprover, liknande det sätt som man tar mjölkprover i kokontrollen, kan användas för att med hög säkerhet göra ett avelsurval för en tidigare första ägglossning och samtidigt minska andelen kor med försenad cyklicitet efter kalvning. Dessutom kan dessa analyser användas som ett skötselverktyg för att diagnostisera kor med försenad cyklicitet redan 60 dagar efter kalvning.

References

Berglund, B., Danell, B., Janson, L. & Larsson, K. 1989. Relationships between production traits and reproductive performance in dairy cattle. *Acta Agriculturae Scandinavica 39,* 169-179.

Bulman, D.C. & Wood, P.D.P. 1980. Abnormal patterns of ovarian activity in dairy cows and their relationships with reproductive performance. *Animal Production 30,* 177-188.

Båge, R., Gustafsson, H., Larsson, B., Forsberg, M. & Rodríguez-Martínez, H. 2002. Repeat breeding in dairy heifers: follicular dynamics and estrous cycle characteristics in relation to sexual hormone patterns. *Theriogenology 57,* 2257-2269.

Claus, R., Karg, H., Zwiauer, D., von Butler, I., Pirchner, F. & Rattenberger, E. 1983. Analysis of factors influencing reproductive performance of the dairy cow by progesterone assay in milk-fat. *The British Veterinary Journal 139,* 29-37.

Darwash, A.O., Lamming, G.E. & Woolliams, J.A. 1997a. Estimation of genetic variation in the interval from calving to postpartum ovulation of dairy cows. *Journal of Dairy Science 80,* 1227-1234.

Darwash, A.O., Lamming, G.E. & Woolliams, J.A. 1997b. The phenotypic association between the interval to post-partum ovulation and traditional measures of fertility in dairy cattle. *Animal Science 65,* 9-16.

DeJarnette, J.M., Sattler, C.G., Marshall C.E. & Nebel R.L. 2007. Voluntary waiting period management practices in dairy herds participating in a progeny test program. *Journal of Dairy Science 90,* 1073-1079.

Dobson, H. & Fitzpatrick, R.J. 1976. Clinical application of the progesterone-in-milk test. *British Veterinary Journal 132,* 538-542.

Dohoo, I., Martin, W. & Stryhn H. 2003. *Veterinary epidemiologic research.* AVC Inc., Prince Edward Island, Canada. 706 pp.

Etherington, W.G., Christie, K.A., Walton, J.S., Leslie, K.E., Wickstrom, S. & Johnson, W.H. 1991. Progesterone profiles in postpartum Holstein dairy cows as an aid in the study of retained fetal membranes, pyometra and anestrous. *Theriogenology 35,* 731-746.

Fagan, J.G. & Roche, J.F. 1986. Reproductive activity in postpartum dairy cows based on progesterone concentrations in milk or rectal examination. *Irish Veterinary Journal 40,* 124-131.

Fourichon, C., Seegers, H. & Malher, X. 2000. Effect of disease on reproduction in the dairy cow: a meta-analysis. *Theriogenology 53,* 1729-1759.

Friggens, N.C. & Chagunda, M.G.G. 2005. Prediction of the reproductive status of cattle on the basis of milk progesterone measures: model description. *Theriogenology 64,* 155-190.

Garbarino, E.J., Hernandez, J.A., Shearer, J.K., Risco, C.A. & Thatcher, W.W. 2004. Effect of lameness on ovarian activity in postpartum Holstein cows. *Journal of Dairy Science 87,* 4123-4131.

Greiner, M., Pfeiffer, D. & Smith, R.D. 2000. Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. *Preventive Veterinary Medicine 45,* 23-41.

Hansen, P.J. & Hauser, E.R. 1983. Genotype x environmental interactions on reproductive traits of bovine females. III. Seasonal variation in postpartum reproduction as influenced by genotype, suckling and dietary regimen. *Journal of Animal Science 56,* 1362-1369.

Janson, L. 1980. Studies on fertility traits in Swedish dairy cattle. II. Genetic parameters. *Acta Agriculturae Scandinavica 30,* 427-436.

Janson, L. & Andréasson, B. 1981. Studies on fertility traits in Swedish dairy cattle. IV. Genetic and phenotypic correlation between milk yield and fertility. *Acta Agriculturae Scandinavica 31,* 313-322.

Jensen, J. & Madsen, P. 1994. DMU: A package for the analysis of multivariate mixed models. *Proceedings 5th World Congress on Genetics Applied to Livestock Production, Guelph, Canada. Computing Strategies and Software 22,* 45-46.

Jorjani, H. 2005. Preliminary report of Interbull pilot study for female fertility traits in Holstein populations. *Interbull Bulletin 33,* 34-44.

Lamming, G.E. & Bulman D.C. 1976. The use of milk progesterone radioimmunoassay in the diagnosis and treatment of subfertility in dairy cows. *British Veterinary Journal 132,* 507-517.

Lamming, G.E. & Darwash, A.O. 1998. The use of milk progesterone profiles to characterise components of subfertility in milked dairy cows. *Animal Reproduction Science 52,* 175-190.

Larsson, K., Jansson, L., Berglund, B., Edqvist, L.-E. & Kindahl, H. 1984. Postpartum reproductive performance in dairy cows. I: Influence of animal, breed and parity. *Acta Veterinaria Scandinavica 25,* 445-461.

Lindhé, B., Danielsson, D.-A., Banos, G., Jansson, L. & Philipsson, J. 1994. Applied breeding policy 1981-1992 and its genetic effects in two Swedish dairy breeds. *Swedish University of Agricultural Sciences, Department of Animal Breeding and Genetics, Publication 111.* S-75007 Uppsala, Sweden.

Lindhé, B. & Philipsson, J. 2001. Genetic trends in the two Swedish dairy cattle breeds SRB and SLB in 1985-1999. *Swedish University of Agricultural Sciences, Department of Animal Breeding and Genetics, Publication 138.* S-75007 Uppsala, Sweden.

López-Gatius, F. 2003. Is fertility declining in dairy cattle? A retrospective study in northeastern Spain. *Theriogenology 60,* 89-99.

Lucy, M.C. 2001. Reproductive loss in high-producing dairy cattle: where will it end? *Journal of Dairy Science 84,* 1277-1293.

Nordic Cattle Genetic Evaluation. Joint Nordic fertility index. http://www.nordicebv.info; pp 2 (accessed 24-Mar-2007).

Opsomer, G., Coryn, M., Deluyker, H. & de Kruif, A. 1998. An analysis of ovarian dysfunction in high yielding dairy cows after calving based on progesterone profiles. *Reproduction in Domestic Animals 33,* 193-204.

Opsomer, G., Gröhn, Y.T., Hertl, J., Coryn, M., Deluyker, H. & de Kruif, A. 2000. Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: a field study. *Theriogenology 53,* 841-857.

Peters, A.R. & Ball, P.J.H. 1995. *Reproduction in cattle.* 2nd edition. Blackwell Science Ltd. Oxford, United Kingdom. 234 pp.

Philipsson, J. 1981. Genetic aspects of female fertility in dairy cattle. *Livestock production science 8,* 307-319.

Rehn, H., Berglund, B., Emanuelsson, U., Tengroth, G. & Philipsson, J. 2000. Milk production in Swedish dairy cows managed for calving intervals of 12 and 15 months. *Acta Agriculturae Scandinavica, Section A, Animal Science 50,* 263-271.

Research Triangle Institute 2004. *SUDAAN Language Manual, Release 9.0.* Research Triangle Inst., Research Triangle Park, NC, USA.

Roche, J.F., Mackey, D. & Diskin, M.D. 2000. Reproductive management of postpartum cows. *Animal Reproduction Science 60-61,* 703-712.

Roxström, A., Strandberg, E., Berglund, B., Emanuelsson, U. & Philipsson, J. 2001. Genetic and environmental correlations among female fertility traits and milk production in different parities of Swedish red and white dairy cattle. *Acta Agriculturae Scandinavica, Section A, Animal Science 51,* 7-14.

Royal, M.D., Darwash, A.O., Flint, A.P.F., Webb, R., Woolliams, J.A. & Lamming, G.E. 2000. Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. *Animal Science 70,* 487-501.

Royal, M.D., Flint, A.P.F. & Woolliams, J.A. 2002. Genetic and phenotypic relationships among endocrine and traditional fertility traits and production traits in Holstein-Friesian dairy cows. *Journal of Dairy Science 85,* 958-967.

Ruiz, F.J., Oltenacu, P.A. & Smith, R.D. 1992. Cost-benefit evaluation of on-farm milk progesterone testing to monitor return to cyclicity and to classify ovarian cysts. *Journal of Dairy Science 75,* 1036-1043.

SAS Institute Inc. 2002. *SAS User's Guide 9.1.* SAS Institute Inc., Cary, NC, USA.

SAS Institute Inc. 2006. *Nonparametric comparison of areas under correlated ROC curves.* http://ftp.sas.com/techsup/download/stat/roc.html (accessed 12-Oct-2006).

Sheldon, M.I., Wathes, C.D. & Dobson, H. 2006. The management of bovine reproduction in elite herds. *The Veterinary Journal 171,* 70-78.

Strandberg, E. & Oltenacu, P.A. 1989. Economic consequences of different calving intervals. *Acta Agriculturae Scandinavica 39,* 407-420.
Stronge, A.J.H., Sreenan, J.M., Diskin, M.G., Mee, J.F., Kenny, D.A. & Morris, D.G. 2005. Post-insemination milk progesterone concentration and embryo survival in dairy cows. *Theriogenology64,* 1212-1224.
Swedish Dairy Association 2006. *Husdjursstatistik (Cattle Statistics) 2006.* Svensk Mjölk, S-101 24 Stockholm, Sweden.
Sørensen, J.T. & Østergaard, S. 2003. Economic consequences of postponed first insemination of cows in a dairy cattle herd. *Livestock Production Science 79,* 145-153.
Taylor, V.J., Beever, D.E., Bryant, M.J. & Wathes, D.C. 2003. Metabolic profiles and progesterone cycles in first lactation dairy cows. *Theriogenology 59,* 1661-1677.
van der Lende, T., Kaal, L.M.T.E., Roelofs, R.M.G., Veerkamp, R.F., Schrooten, C. & Bovenhuis. H. 2004. Infrequent milk progesterone measurements in daughters enable bull selection for cow fertility. *Journal of Dairy Science 87,* 3953-3957.
Veerkamp, R.F., Oldenbroek, J.K., van der Gaast, H.J. & van der Werf, J.H.J. 2000. Genetic correlation between days until start of luteal activity and milk yield, energy balance, and live weights. *Journal of Dairy Science 83,* 577-583.
Waldmann, A., Reksen, O., Landsverk, K., Kommisrud, E., Dahl, E., Refsdal, A.O. & Ropstad, E. 2001. Progesterone concentrations in milk fat at first insemination - effects on non-return and repeat-breeding. *Animal Reproduction Science 65,* 33-41.
Wall, E., Brotherstone, S., Woolliams, J.A., Banos, G. & Coffey M.P. 2003. Genetic evaluation of fertility using direct and correlated traits. *Journal of Dairy Science 86,* 4093-4102.
Washburn, S.P., Silvia, W.J., Brown, C.H., McDaniel, B.T. & McAllister, A.J. 2002. Trends in reproductive performance in southeastern Holstein and Jersey DHI herds. *Journal of Dairy Science 85,* 244-251.
Wathes, D.C., Bourne, N., Cheng, Z., Mann, G.E., Taylor, V.J. & Coffey, M.P. 2007. Multiple correlation analyses of metabolic and endocrine profiles with fertility in primiparous and multiparous cows. *Journal of Dairy Science 90,* 1310-1325.
Whitmore, H.L., Tyler, W.J. & Casida, L.E. 1974. Effects of early postpartum breeding in dairy cattle. *Journal of Animal Science 38,* 339-346.
Wiltbank, M., Lopez, H., Sartori, R., Sangsritavong, S. & Gümen, A. 2006. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. *Theriogenology 65,* 17-29.

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