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EFFECTS OF ENVIRONMENT ON PROGESTERONE LEVEL OF DAIRY CATTLE IN DISTRICT NOWSHERA

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ABSTRACT

Effects of environment on fertility measures early in lactation, such as the interval from calving to first luteal activity (CLA), proportion of samples with luteal activity during the first 60 days after calving (PLA) and interval to first ovulatory estrus (OOE) were studied. Traditional measurements of fertility, such as pregnancy to first insemination, number of inseminations per service period and interval from first to last insemination were studied as well as associations between the early and late measurements. Data were collected from an experimental herd during 15 years and included 1106 post-partum periods from 191 Holsteins and 325 Jersey dairy cows. Individual milk progesterone samples were taken twice a week until cyclicity and thereafter less frequently. First parity cows had 14.8 and 18.1 days longer CLA (LS-means difference) than second parity cows and older cows, respectively. Moreover, CLA was 10.5 days longer for cows that calved during the winter season compared with the summer season and 7.5 days longer for cows in tie-stalls than cows in loose-housing system. Cows treated for mastitis and lameness had 8.4 and 18.0 days longer CLA, respectively, compared with healthy cows. OOE was affected in the same way as CLA by the different environmental factors. PLA was a good indicator of CLA, and there was a high correlation (−0.69) between these two measurements. Treatment for lameness had a significant influence on all late fertility measurements, whereas housing was significant only for pregnancy to first insemination. All fertility traits were unfavorably associated with increased milk production. Regression of late fertility measurements on early fertility measurements had only a minor association with conception at first AI and interval from first to last AI for cows with conventional calving intervals, i.e. a 22 days later, CLA increased the interval from first to last insemination by 3.4 days. Early measurements had repeatability of 0.14–0.16, indicating a higher influence by the cow itself compared with late measurements, which had repeatability of 0.09–0.10. This study shows that early fertility measurements have a possibility to be used in breeding for better fertility. To improve the early fertility of the cow, there are a number of important factors that have to be taken into account.

Keywords: dairy cattle, fertility, progesterone, ovulation, ovulatory estrus.

1. INTRODUCTION

There is decreasing fertility in dairy cattle with increasing production. Royal et al. (2000) reported a decline of 1% per year in pregnancy rate to first AI, during a period from 1975 to 1998 in Great Britain. From USA, Lucy (2001) reported an increase in number of inseminations per conception from 1.75 in 1970 to 3.0 in 1999, and that the calving interval increased from 13.5 to 14.7 months.

Fertility is unfavorably genetically correlated to production traits. Therefore, it is important to include reproductive traits in the breeding programme. Fertility has been included in the breeding evaluation scheme since the 1970s but the traits included in the fertility index have very low heritabilities (Janson, 1980b; Roxstrom *et al.*, 2001). A negative genetic trend in fertility has been shown for Swedish Holsteins (SLB), whereas the Swedish Red and White breed (SRB) has been on a stable level (Philipsson and Lindh´e, 2003). There has, however, been a slightly negative phenotypic trend in some reproductive measurements and the calving interval has increased from 12.6 months in year 1974/1975 to 13.1 in year 2000/2001 (Swedish Dairy Association, 2003). Because of this, the breeding work for better fertility in dairy cows has to be improved and one way could be to use measurements of fertility with higher heritability. Currently used measurements of the reproductive capacity of dairy cows are highly influenced by management and the dairy farmer's decision. Planned extended calving intervals will delay the interval from calving to first insemination, not because the cow has a late start of cyclicity, but due to the farmer's decision. Analysis of measures that more directly reflect the cow's own physiology might yield additional information about the dairy cow's fertility. One example would be to use interval to first ovulation determined by the progesterone level in milk instead of the indirect measure of the interval to first insemination. Studies of the interval from calving to commencement of luteal activity (occurring about 5 days after first ovulation) have revealed heritabilities of 16–21%, which is considerably higher than for traditional measurements of fertility in dairy cows (Darwash *et al.*, 1997a; Royal et al., 2002; Veerkamp *et al.*, 2000). The interval to first ovulation and the interval to first ovulatory oestrus have also been shown to have higher repeatabilities than traditional measurements of dairy cow fertility, such as conception rate at first insemination and number of inseminations per service period (Berglund et al., 1989). It is likely that not only late fertility measurements (such as conception rate), but also early (endocrine) measurements of fertility, have been altered over the years with the intensive selection for higher milk production. Opsomer *et al.* (1998) observed a considerably later first ovulation in first parity compared with earlier studies (e.g. Fonseca *et al.*, 1983). An increase of atypical patterns in milk progesterone profiles has also been observed by Royal *et al.* (2000) comparing data from 1975 to 1982 with those from more recent years (1995-1998). The phenotypic association between early and late (traditional) measurements of fertility in dairy cows was studied by Darwash *et al.* (1997b). An early start of cyclicity increased the probability of an early insemination after calving, shortened the interval from calving to conception, increased the conception rate and reduced the number of services per conception. The aim of this study was to investigate the influence of various systematic environmental factors on early and late fertility measurements. We hypothesized that early fertility measures were more influenced by the cow itself compared with later measurements of fertility and that they more accurately reflect the reproductive capacity of the cow. Moreover, we also hypothesized that the early fertility measurements have an effect on the late ones, i.e. cows that start to cycle early will have a higher chance of getting pregnant at first insemination. We show that early fertility measurements are highly influenced by several environmental effects and that they have higher repeatability compared with traditional fertility measurements. We also show that early fertility measurements influence later fertility measurements but the influence decreases with time from parturition.

2. MATERIAL AND METHODS

Progesterone profiles for 1106 lactations, comprising of 30,415 progesterone samples were collected during 1992–2007. These samples were analyzed to determine the interval from calving to commencement of luteal activity (CLA), the proportion of luteal activity during the first 60 days after calving (PLA), and the interval from calving to first ovulatory oestrus (OOE).

2.1. Animals

Data was collected between April 2007 and April 1992 at the commercial dairy farms in district Nowshera. Altogether, 516 dairy cows of two breeds, Jersey (n = 325) Holstein–Friesian (n = 191) were included in the study. The milk production yield was calculated in kilogram energy corrected milk (ECM) considering fat protein and lactose contents, using a formula given by Sjaunja *et al.* (1990). The average of 305-day ECM production was 8540 kg for the Jersey cows and 9750 kg for the Holstein cows in 2007. The increase in 305-day ECM production was compared with the start of the study, 1120kg for the Jersey cows and 2090kg for the Holstein cows. Cattle were in their 1st -10th lactations but were grouped 1, 2 and ≥3. From 1994, the cows were subjected to a calving interval trial, in which they were inseminated for planned calving intervals of either 12 or 15 months. For more information about the calving interval trial, see Ratnayake *et al.* (1998) and Rehn *et al.* (2000). For more information on a selection trial for high or low fat content in the milk of Jersey cows, see Janson and Ahlin (1992). The effect of the selection trial on the various fertility measurements was found not to be significant.

2.2. Management

There were two different housing systems at the experimental farm, tie-stalls and a loosehousing system. Records of housing were available from 1997 to 2001 and 435 lactations were from the tie-stall and 295 were from the loose-housing barn. Before the year 1997, experimental herd was kept at another farm at which all animals were tied. All cows were milked twice per day, starting at 06:00 and 15:15 h, in individual stalls for the tied cows and hand milking for the loose housed cows. From calving until week 16, post-partum cows were fed according to live weight at calving and thereafter on a restricted ration based on production and body condition. Cows were fed the whole ration individually in the tie-stall, whereas only concentrates were fed individually in the loose-housing barn. From May to September each year, cows were kept on pasture during day-time. During the pasture period, additional concentrates and roughage were fed indoors. Heat detection was done visually three times daily at fixed hours, 07:30, 11:30 and 17:00 h, and the heat strength was scored according to the following scale: 0 = no heat, 1 = uncertain, 2 = weak, 3 = normal, 4 = strong, and from 1995 onwards also: 5 = very strong.

Cows were inseminated, at the earliest, at the first oestrus after days 50 and 140 for cows with calving intervals of 12 and 15 months, respectively. A maximum of five inseminations were allowed per breeding period, restricted to a maximum of 130 days, and thereafter, the cow was culled due to infertility. All inseminations were made by experienced AI technicians. Reproductive disorders were diagnosed and treated as in Ratnayake *et al.* (1998). Cows that were treated for reproductive disorders before commencement of luteal activity were not included in the study (n = 57 lactations). Incidence of other diseases was reported and treated by a veterinarian. The most common diseases, mastitis, lameness and puerperal paresis were included in the study; treatments for these diseases were made by a veterinarian. Puerperal paresis had no effect on the fertility measurements and consequently, it was excluded from further analysis.

2.3. Progesterone analyses

Milk sampling for progesterone analysis started during the second week after parturition and milk was sampled twice weekly until cyclicity was detected. Sampling was then reduced to once a week until first AI and thereafter at days 10 and 21 after each AI. Progesterone (P4) was analyzed in whole milk. The sample (about 5 ml of milk) was taken within 60 min after milking, collected into tubes containing 100 ml of preservative (Bronopol 2% + MTB 0.05%) and stored at 4 ◦C until assay. For assaying of milk samples for P4, the RIA method was used. The possible day of ovulation was merged with a corresponding P4 observation to set limits for luteal activity for different kits. Ovulation day was calculated as the day after strong heat detection (codes 3-5) or the day before a post-estrus bleeding. The value that 95% of these possible days of ovulation were below was set as the limit for luteal activity. The limits used were 8.0, 3.0 and 1.3 nmol/l for the three different kits Farmose, Spectra and Coat-A-Count, respectively.

2.4. Fertility measures

Progesterone profiles were used to derive endocrine, early fertility measurements. The first P4 value post-partum above the limit, preceded by a low P4 value, was used to define the interval from calving to commencement of luteal activity. All progesterone samples taken within the first 60 days post-partum were used to calculate the proportion of samples above the limit for luteal activity. P4 samples for each lactation were plotted together with recordings of heat observations to form a progesterone profile. From this profile, the day of first ovulatory estrus was decided as the day after a heat observation (2-5) or the day before a post-estrus bleeding, given that P4 was low and increased above the limit within 10 days.

Records of inseminations were used to calculate the fertility measurements, also referred to as late fertility measurements, such as pregnancy to first insemination (PFI), numbers of inseminations per service period (NINS) and for pregnant cows only, interval between first and last insemination (FLI). If an insemination was followed by a new insemination within 6 days, the first insemination was omitted. Measurements, such as interval from calving to first AI and interval from calving to conception were not used in this study due to the bias caused by the calving interval trial.

A measurement of CLA was required to include the lactation in the study. Further restrictions were at least four P4 measurements per cow per lactation and all of them before day 300 after calving. Five lactations were excluded due to overlapping use of RIA assays, when these were changed in 1995.

2.5. Statistical methods

The influence of various fixed effects and the random effect of cow were analyzed with mixed linear models, proc mixed of SAS (2001). The fixed effects included in the model were: breed (Jersey and Holstein); parity (from 1 to ≥3); calving interval group; season (winter and summer); year (1992-2007); housing (tied, loose or no information); interaction between year and housing; mastitis (yes or no); lameness (yes or no); a fixed regression on kg ECM produced during the first 60 days of lactation (ECM60). The calving interval group had five different classes, first lactation in the 12-month calving interval trial, later lactations in the 12-month calving interval trial, first lactation in the 15-month calving interval trial, later lactations in the 15-month calving interval trial and a group for cows not in the calving interval trial. Treatments for mastitis or lameness made before CLA were used for CLA and PLA, treatments made before OOE were used for OOE and treatments made before first insemination were used for the late fertility measurements.

Table-1. Number of lactations, overall mean, S.D., median, 75th percentile for the fertility measurements analyzed.

	n	**Mean**	**S.D.**	**Median**	**75 percentile**
CLA[a] (days)	1106	33.8	22.7	27.0	40.0
PLA[b] (%)	1106	37.2	21.3	40.0	53.8
OOE[c] (days)	776	61.3	30.9	57.0	76.0
PFI[d] (%)	912	40.0	49.0	n.i.	n.i.
NINS[f] (no.)	912	2.02	1.20	n.i.	n.i.
FLI[g] (days)	736	26.3	34.7	n.i.	47.0

a Interval from calving to commencement of luteal activity.
b Proportion of luteal activity during the first 60 days after calving.
c Interval from calving to first ovulatory estrus.
d Pregnancy to first insemination.
e Value of no interest.
f Number of inseminations per service period.
g Interval from first to last insemination.

In a separate analysis, the late fertility measures were regressed on the early measures, using residuals of the early fertility measures. The residuals were calculated with a reduced version of the model above with the random effect of cow excluded. These residuals were then included as a covariate in the model for the later measurements. The analyses were made with the early measures of fertility nested within calving intervals divided into two groups, one with cows in the 12-month calving interval group together with cows that were not in the calving interval trial (called conventional) and the other group with cows in the 15-month calving interval groups. The residuals from the reduced model were also used to calculate the correlations within the groups of early and late fertility measurements. The models used assume a normal distribution of residuals. However, CLA and OOE were both skewed (Table-1), and the natural logarithm of these two measurements was used to check that significance of estimates did not change compared with the untransformed measurements. PFI is a binary measurement, and a logistic regression, using the above model, was tested with the SAS GLIMMIX macro

(Wolfinger and O'Connell, 1993). The same fixed effects were significant when using the mixed models procedure as with the logistic regression. We, thereby, concluded that the linear mixed model was robust enough to handle the binary trait. This was also true for NINS using the GLIMMIX macro, with the assumption of a Poisson distribution of this measurement.

3. RESULTS

Overall mean, S.D., median and 75th percentile for the six analyzed fertility measurements are shown in Table-1.

Table-2 shows the mean, S.D. and number of lactations for the fertility measurements for each class of the different fixed effects analyzed. This table is given to illustrate the number of observations per class and the levels for the different fertility measurements within the fixed effects.

Table-2. Mean, S.D. and number of observations for the analyzed fertility measurements by different fixed effects.

Effect	Class	CLA[a]	S.D.	PLA	S.D.	*n*[b]	OOE	S.D.	*n*	PFI	S.D.	NINS	S.D.	*n*[c]	FLI	S.D.	*n*
Breed	Jersey	33.5	22.3	37.1	20.5	733	59.7	30.9	513	41.2	49.3	1.96	1.17	611	24.8	32.7	499
	Holstein	34.5	23.7	37.5	22.7	373	64.4	30.7	263	37.5	48.5	2.13	1.27	301	29.5	38.5	237
Parity	1	37.2	27.9	36.0	22.6	446	65.0	33.2	335	44.7	49.8	2.01	1.22	389	26.9	36.2	340
	2	29.9	15.8	39.6	20.1	297	57.4	28.9	214	38.5	48.8	2.05	1.24	257	26.6	34.2	204
	≥3	33.0	19.9	36.9	20.4	363	59.5	28.6	227	34.6	47.7	1.99	1.15	266	25.0	32.7	192
Season	Winter	39.1	26.3	31.3	22.2	557	66.5	34.0	377	38.8	48.8	2.03	1.22	469	26.3	34.5	365
	Summer	28.5	17.0	43.3	18.5	549	56.4	26.8	399	41.3	49.3	2.00	1.18	443	26.3	35.0	371
Calving interval[d]																	
	12	24.9	10.4	46.1	19.0	171	53.0	21.9	125	43.1	49.7	1.99	1.23	137	24.3	33.8	114
	15	34.0	22.8	37.8	21.5	128	71.4	36.1	95	42.0	49.6	2.03	1.21	88	22.4	31.1	72
Housing	Tied	36.7	24.0	33.9	21.4	435	67.6	32.7	297	38.0	48.6	2.01	1.18	353	25.8	32.5	279
	Loose	29.1	18.6	41.4	18.2	295	57.0	28.3	216	50.8	50.1	1.84	1.14	242	20.2	33.7	206
Mastitis	No	33.5	22.8	37.8	21.0	960	60.3	31.0	680	39.5	48.9	2.05	1.21	790	26.8	34.9	642
	Yes	36.4	22.3	33.8	22.8	146	68.4	29.6	96	43.4	49.8	1.84	1.15	122	22.7	33.4	94
Lameness	No	33.4	22.2	37.5	21.1	1078	60.4	30.3	746	39.3	48.9	2.04	1.21	867	26.8	35.0	698
	Yes	9.8	35.0	26.7	24.3	28	84.4	36.1	30	53.3	50.5	1.62	0.96	45	17.0	27.6	38

a Abbreviations as in Table-1.
b CLA and PLA have the same number of observations.
c PFI and NINS have the same number of observations.
d Second and later lactations in the calving interval trial of 12 or 15 months.

Table-3. LS-mean differences (classA-class B) and probabilities for early fertility measurements for different fixed effects in the model.

Class A	Class B	CLA[a] (days)	P	OOE (days)	P	PLA (%)	P
Parity 1	Parity 2	18.05	<0.001	19.72	<0.001	−9.55	<0.001
Parity 1	Parity≥3	14.76	<0.001	19.34	<0.001	−6.76	0.004
Winter	Summer	10.50	<0.001	9.37	<0.001	−12.16	<0.001
CI[b] 12	CI 15	−4.57	0.090	−10.98	0.008	3.24	0.193
Tied	Loose	7.50	<0.001	8.90	0.004	−6.36	<0.001
No mastitis	Mastitis	−8.37	<0.001	−12.32	<0.001	9.81	0.013

a Abbreviations as in Table-1.
b Second and later lactations in the calving interval trial of 12 or 15 months.

Parity, season, housing, mastitis, lameness and ECM60 had significant influence on all of the early fertility measurements. The effect of the calving interval group was significant on CLA and OOE. The estimated differences between classes within effects are shown in Table-3 for the early fertility measurements. The year effect was significant only for PLA and OOE of all the fertility measurements. The year effect had no trend for PLA and OOE, why these figures are not shown.

When CLA was log transformed, the difference between later lactations in the calving interval trial was significant ($P = 0.027$) and the difference between breeds ($P = 0.040$), in addition to the significant differences for the untransformed CLA in Table-3. For the log transformed OOE, the same differences were significant as for the untransformed OOE (Table-3).

Milk production and treatment for lameness had a significant influence on all late fertility measurements. Housing was significant on PFI. Cows in tie-stalls had 14.05%-units (P = 0.004) lower PFI than cows in loose-housing. Cows treated for lameness had 17.71%- units (P = 0.023) higher PFI than those that were not treated. The cows treated for lameness had also 0.60 (P = 0.001) fewer NINS than those that were not treated. Third parity cows had 0.32 (P = 0.041) fewer NINS than first parity cows. The fixed regression on ECM60 was significant for all the fertility measurements (Table-4). The average ECM60 was 1982.4kg (S.D. 422.9kg).

Table-4. Effect of ECM60a expressed as an increase of one S.D. (422.9kg).

Fertility measurement b	Change per S.D.	*P*
CLA (days)	3.73	<0.001
PLA (%)	−2.96	0.002
OOE (days)	6.28	<0.001
PFI (%)	−5.50	0.035
NINS (no.)	0.18	0.004
FLI (days)	4.47	0.036

a Kilogram ECM produced during the first 60 days of lactation.
b Abbreviations as in Table-1.

Table-5. Effect of CLA and PLA residuals on later fertility measurements, effect expressed as an increase of one S.D. of the CLA residual (22.1 days) and the PLA residual (21.3%), respectively.

Fertility a Measurement	Calving interval b	CLA change per S.D.	*P*	PLA change per S.D	*P*
OOE (days)	Conventional	15.45	<0.001	10.15	<0.001
	15 months	17.42	<0.001	15.31	<0.001
PFI (%)	Conventional	–	ns	4.78	0.030
	15 months	–	ns	–	ns
FLI (days)	Conventional	3.37	0.047	–	ns
	15 months	–	ns	–	ns

a Abbreviations as in Table-1.
b Conventional are all cows with a 12-month calving interval or not in the calving interval trial; 15 months are all cows with a 15-month calving interval.

The regression of OOE on the CLA and PLA residual was significant for both calving intervals (conventional and 15 months) (Table-5). FLI regressed on CLA residuals was significant for the group of cows with a conventional calving interval. Regression of PFI on the PLA residual was also only significant for the cows with a conventional calving interval.

The correlations between the residuals of the early fertility measurements were all significant (P < 0.001). CLA and PLA had a high negative correlation of −0.67. CLA and OOE had a correlation of 0.54 and PLA and OOE a negative correlation of −0.35. Correlations between the residuals of the late fertility measurements were significant (P < 0.001). PFI was negatively correlated with both NINS and FLI, with correlations of −0.70 and −0.74, respectively. NINS and FLI had a high positive correlation of 0.89, as expected.

The random effect of cow, tested within breed, was significant for CLA (P < 0.001), PLA (P < 0.001), PFI (P = 0.024) and NINS (P = 0.030). Repeatability estimates for these measurements were 0.14, 0.16, 0.09 and 0.10 for CLA, PLA, PFI and NINS, respectively.

4. DISCUSSION

PLA not only measures the start of cyclicity but also gives an indication of the cyclicity of the cow after the first ovulation. PLA may also reflect the early fertility of the dairy cow better than, i.e. CLA when sampling of progesterone is more infrequent. Furthermore, the correlation between PLA and CLA (−0.67) indicates that PLA might be a good measurement of the early fertility of a dairy cow. The overall mean for the interval from calving to commencement of luteal activity, CLA (33.8 days), can be used as an indication of the interval to first ovulation, which occurs on average 5 days before CLA. The interval to first ovulation, indicated by CLA, was then about 2 days later in this study compared with previous studies of Swedish cows (Larsson *et al.*, 1984; Berglund *et al.*, 1989). Royal et al. (2000) found slightly shorter CLA, 27.4 and 27.9 days for the periods 1975-1982 and 1995-1998, respectively. Lucy (2001), reporting from a selection trial for increasing milk yield, found an interval to first ovulation of 29 days for a control line maintained as it was in 1964 and an interval of 43 days for a selected line corresponding to yields of modern dairy cows. We neither observed any changes in CLA over the period of 15 years that we studied, nor in comparison with earlier studies. The median for CLA, and thereby the interval to first ovulation has, however, increased about 3 days compared with the earlier studies of Larsson *et al.* (1984) and Berglund *et al.* (1989); so, there might have been an increase in the number of animals with longer intervals in dairy cows. Overall means of the interval from calving to first ovulatory estrus, OOE (61.3 days), was considerably longer compared with earlier studies on Swedish dairy cows, where 47 days were reported (Larsson *et al.*, 1984; Berglund *et al.*, 1989). However, there was a strong effect of the calving interval trial on OOE and no effect of the calving interval trial on CLA and PLA. This might indicate less effective estrus detection for cows on the 15-month calving interval. It could also be an effect of the length of the dry period before calving, which was longer for cows on the 15-month calving interval (Rehn *et al.*, 2000) and an increased dry period has been shown to be a risk factor for delayed ovulation (Opsomer *et al.*, 2000). The mean OOE was, however, also longer for cows with the 12-month calving interval (53.0 days) compared with the earlier study (Larsson *et al.*, 1984). This might be an indication that estrus signs, rather than interval to first ovulation, have been affected by selection for increased production. A decreasing oestrus detection rate was also observed by Washburn *et al.* (2002). Oestrus detection rate was studied from 1985 to 1999, and changed from 50.9 to 41.5% for Holsteins and from 59.6 to 49.5% for Jerseys.

First parity cows had longer CLA than cows in their second, third and later parity. Bulman and Lamming (1978) and Bulman and Wood (1980) observed middle-parity cows having an earlier start of cyclicity compared with first parity cows and older cows (parity 6 and above). The effect of older cows could not be detected in our study, and neither was it detected in an initial analysis when all parities were kept separate. The effect of parity on CLA was also reflected in PLA and was thus lower in parity one compared with later parities. OOE also occurred later for the first parity cows. Lucy *et al.* (1992) showed that first lactation cows had a later first ovulation compared with later parity cows, and it seemed to be caused by a more severe negative energy balance for the first parity cows. This may possibly also be the explanation of the later CLA and OOE and lower PLA for the first parity cows in this study. Cows that calved during the winter season had longer CLA and thereby a lower PLA. The winter season also prolonged OOE. Season did not significantly influence the later fertility measurements. The winter season has, however, been shown to have a negative effect on different measures of fertility in study of Swedish cows (Janson, 1980a). An increase in the interval to first oestrus during winter compared with summer was also found by Hansen and Hauser (1983). In their study, the effect of season was concluded not to be affected by diet or management and the effect was probably due to photoperiod or temperature. In this study, we cannot exclude management or diet as probable causes of the seasonal effect because the cows were on pasture from May to September, and feed quality was different in the summer and winter seasons. In addition, results may also have been affected by temperature and photoperiod.

Tied cows had longer CLA, lower PLA and longer OOE compared with cows in loosehousing. PFI was also lower for cows in tie-stalls. Longer intervals to CLA and OOE in tie-stalls compared with loose-housing were also found by Claus *et al.* (1983) and in another study by Ratnayake *et al.* (1998) using partly the same material as in this study. 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 OOE and lower PLA. Mastitis had no effect on the later fertility measures. Possibly, due to most cases of mastitis occurring early in lactation, thereby implying a smaller effect at the times, when the cow is inseminated. Treatment for lameness had a positive effect on both PFI and NINS. These contradicting results for the effect of lameness on early and late fertility measurements might possibly be explained by differences in the interval from treatment day to CLA (27 days) or day to first AI (68 days). It seems that lameness had a negative effect on the fertility that declined with time. Perhaps, there might be a positive long-term effect of treatment on the later fertility measurements for lameness, and that treated cows got better hoof health than untreated cows. Lameness has also been suggested to have a time-dependent effect on fertility (Loeffler *et al.*, 1999). However, Melendez et al. (2003) have shown that lameness within the first 30 days after calving reduced pregnancy rates at first AI and lame cows also became pregnant later and had a higher number of services per conception. In a meta analysis of several published papers, Fourichon *et al.* (2000) found no effect of mastitis on traditional fertility measurements, whereas leg problems were associated with an average increase of 12 days to conception. All fertility traits were unfavorably associated with increased milk production. Similar results for both early and late fertility measurements have been found previously (e.g. Berglund *et al.*, 1989). No significant phenotypic association between CLA and milk yield was observed by Royal et al. (2002), but higher milk yield was genetically correlated with longer CLA. Harrison *et al.* (1989) observed a similar interval from calving to first luteal phase for low- and high-yielding cows, whereas low-yielding cows had a shorter interval from calving to first visual estrus than high-yielding cows. Lucy et al. (1992) reported the highest producing cows to have the shortest interval to first ovulation and these cows had also the highest dry matter intake and were probably in a less negative energy balance than the low-producing cows. The unfavorable association between milk yield and fertility measures that we observed may thus probably be an effect of a more negative energy balance for the cows with a higher milk yield. CLA had only a significant association with FLI, when the CLA residual was used in the regression, and PLA had only a significant association with PFI. The regression of the late fertility measurements on the early ones was made within the calving interval trial groups.

We found significant results only for the cows that had conventional calving intervals and concluded that the association between the early and late measurements decreases with an increased number of days between the time when the cow starts to cycle and when the first AI is performed.

The repeatability for CLA (0.14) was lower than reported by Darwash *et al.* (1997a) (0.26-0.28), but the repeatabilities for both CLA and PLA were higher than the repeatabilities for PFI and NINS in this study. The repeatability for CLA was the same as the repeatability for interval to first ovulation reported by Berglund *et al.* (1989).

5. CONCLUSIONS

The early endocrine measurements of a dairy cow's fertility were more influenced by the cow itself than by the more traditional measurements of fertility. However, many of the analyzed environmental factors had a large influence on the early measurements. Parity and lameness had the largest effects on CLA, OOE and PLA. Season, mastitis and housing also had substantial effects, whereas the level of milk production had somewhat less influence on the early measurements. Only housing, incidence of lameness and production had significant effects on late fertility measurements. The early fertility measures had only minor effect on the late measurements and were not significant for cows with extended calving intervals and a later start of the service period.

Our study shows that early measurements of fertility might possibly be used for improved breeding for fertility. To improve the early fertility of the cow there are a number of important environmental factors that have to be taken into account. PLA has the benefit of being easy to record and calculate automatically, but needs to be further examined. PLA turned out to be a promising measure that should be further studied. It might yield more information about the early fertility of the cow compared with CLA.

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