Phenotypic attributes of postpartum ovulation and other fertility measures in Friesian-Jersey crossbred cows

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ABSTRACT

Reproductive performance is an important factor contributing to on-farm efficiency and profitability. The purpose of this study was to define and quantify a set of fertility phenotypes for 352 Friesian-Jersey crossbred heifers, and determine key factors contributing to these indicators. Heats were observed and recorded prior to herd start of mating, and CIDRs were used to treat non-cycling heifers (51) once mating started. Progesterone samples were collected twice weekly, and luteal activity was identified where concentrations were >0.9 ng/ml for blood and >3.0 ng/ml for milk samples. Commencement of luteal activity was defined by either one elevated sample (CLA1) or two consecutive elevated samples (CLA2). For untreated heifers, the average intervals to CLA1 and CLA2 were 29.6 days (sd=14.3) and 34.1 days (sd=16.9) respectively. Other phenotypes were evaluated for conception and intervals to first heat, first service and successful service. Significant relationships were established between body condition score (BCS) at calving and the intervals to CLA. For every unit decline in BCS at calving there was an increase of 7.4 days to CLA1 and 8.8 days to CLA2. The evaluated fertility phenotypes, and their key contributing factors will be used in another study to identify related quantitative trait loci (QTL).

Keywords: fertility; reproductive performance; luteal activity; progesterone; oestrus; condition score.

INTRODUCTION

Pasture-based feeding systems and high milk production in New Zealand dairy herds demands maintenance of seasonal calving patterns. Maintenance of an approximately 365-day calving interval aids in ensuring that lactational peak coincides with peak spring pasture growth, so that cow production is maximised. It also assists with herd management efficiencies, and helps to avoid losses resulting from fewer days in milk. Cows that start cycling, and express oestrus within a short period post-calving have more conception opportunities, and are more likely to calve soon after the planned start of calving. This will improve their reproductive performance in successive seasons, and help maximise overall herd performance (Morton, 2000).

Research on fertility traits in dairy cows to date has been extensive, from both the perspective of traditional traits, and those based on endocrine measurements. Traditional fertility measures such as days to first mating and days to successful mating are a combined representation of endocrine, behavioural and management effects. Endocrine fertility measures such as the interval to CLA differ in that they do not include any management bias. Phenotypic correlations between CLA and other traditional fertility measures are well documented (Darwash *et al.*, 1997b; Royal *et al.*, 2002a; 2002b), and high genetic correlations with production traits have also been established (Royal *et al.*, 2002b). Unfavourable phenotypic, environmental and genetic relationships between fertility and measures of energy balance have also been presented in a number of different studies (Butler *et al.*, 1981; Butler & Smith, 1989; Canfield & Butler, 1990; Villa-Godoy *et al.*, 1990; Veerkamp *et al.*, 2000).

The Boviquest Friesian-Jersey Crossbred trial (Spelman *et al.*, 2001) was initiated as a joint venture between Livestock Improvement Corporation and Via Lactia Biosciences in 1998. Calves were generated over a two-year period with the first cohort born in 2000, and the second cohort born in 2001. Animals are being extensively phenotyped over their lifetime for traits related to milk characteristics, health and disease, and reproduction.

The primary purpose of this study was to define and evaluate a set of fertility phenotypes for animals in the trial. It is intended that these phenotypes will be used in a QTL analysis. The secondary purpose was to evaluate phenotypic relationships between a subset of key fertility indicators and measures of body condition score, liveweight and production.

MATERIALS AND METHODS

Data

Composite milk samples and blood samples were collected and analysed for cohort one (352 animals) during their first lactation. These samples were used to evaluate progesterone concentrations over a 26 week period from the week following start of calving through to the end of mating. Milk samples were collected on the Monday of each week, and blood samples were collected on the Thursday of each week. Collection of milk samples started a week later than for blood, and results for the milk samples collected in the tenth week after start of calving were missing. This resulted in a difference between the number of milk and blood samples available to be analysed. A total of 12253 samples are included in the analysis, 6408 blood and 5845 milk.

Observed heats prior to and during the herd mating period were monitored daily using tail paint. Tail paint was applied within 10 days of calving, and where there was evidence that the animal had been ridden, this was recorded and the paint reapplied. Artificial breeding (AB) took place in the first six weeks of the herd mating period, followed by an additional three weeks of natural matings. Overall there were 328 pre-mating heats recorded for 215 animals, and 543 matings (510 to AB and 33 natural) recorded for 352 animals. Animals with no observed pre-mating heat as at seven days prior to the herd start of mating were treated intravaginally with a CIDR insert (CIDR® Cattle Insert, Pharmacia Animal Health, Auckland, NZ). The CIDR inserts were removed after seven days, and each animal was injected intramuscularly 24 hours later with 1mg oestradiol benzoate (CIDIROL, Bomac Laboratories Ltd., Auckland, NZ). The 51 animals treated with a CIDR insert were on average 58 days (sd=13) postpartum at the time of insertion.

Body condition score (BCS) was recorded once weekly (1=emaciated, 10=obese; Macdonald & Macmillan, 1993), and liveweight (kg) at least twice weekly from calving to the end of mating. Production data was collated from fortnightly herd tests, and a pregnancy diagnosis was carried out (by a veterinarian) 68 days after the end of AB.

Progesterone enzyme-linked immunosorbent assay (ELISA)

Milk progesterone concentrations were measured in preserved whole milk samples using ELISA plates (Ridgeway Science Ltd, Alvington, Gloucestershire, UK), verified for use in dairy cattle by Sauer *et al.* (1986). Single samples were analysed once per week using up to five plates with one control sample per plate. Serum progesterone concentrations were measured in frozen aliquots of blood samples using the same assay and method as for milk samples. The minimum detectable concentration for the assay was 0.5 ng/ml, with standards between 0.5 and 15 ng/ml for the milk assays, and between 0.5 and 20 ng/ml for the blood assays. The interassay coefficient of variation for control milk samples was 29.3% for an average concentration of 2.2 ng/ml. For control blood samples, the interassay coefficient of variation was 35.8% for an average concentration of 1.8 ng/ml.

Progesterone profiles

Elevated progesterone levels for an animal indicate luteal activity, and can be used to identify start of cycling. Various cut-off levels for determining elevations in progesterone have been used in other research, but are typically defined as 0.5 to 1 ng/ml for blood samples (Zurek *et al.*, 1995) and >3 ng/ml for milk samples (Darwash *et al.*, 1997a). By using these cut-off levels as a guideline, and analysing individual animal profiles, progesterone concentrations were classified into three groups corresponding to zero, marginal and elevated progesterone; ≤0.6, 0.6 to 0.9, or >0.9 ng/ml for

blood, and ≤1.5, 1.5 to 3.0, or >3.0 ng/ml for milk samples. Individual animal profiles were checked to insure that critical points of zero or elevation had not been missed due to timing of sampling. Where this was suspected, the start and end points for cycles were manually determined from progesterone concentrations in the marginal range and confirmed by heat detection or mating records.

Phenotype definitions based on progesterone profiles

Studies by Darwash *et al.* (1997a) and Royal *et al.* (2000) use the postpartum day of the first elevated reading (of two consecutive elevated readings) to define the interval to CLA. Both these studies use milk samples collected in a thrice-weekly sampling routine, and have a progesterone concentration cut-off for identifying elevated samples of 3 ng/ml. In a thrice-weekly sampling routine, luteal activity defined by two consecutive elevated progesterone samples equates to a minimum cycle length of 8 days. When samples are taken twice weekly, as in this study, one elevated progesterone sample equates to a minimum cycle length of 8 days. Two consecutive elevated readings equates to a minimum cycle length of 11-12 days. For this reason, intervals to CLA in this study have been defined in two different ways as follows: Commencement of luteal activity characterised by at least one elevated result and followed thereafter by a cyclical pattern (CLA1); commencement of luteal activity characterised by two or more consecutive elevated results (CLA2).

Phenotype definitions for oestrus expression and traditional fertility measures

Progesterone results inclusive of the oestrus prior to CLA2 were scanned to identify the number of oestrus for each animal, and whether or not oestrus was detected at appropriate times. A detected oestrus has been identified where there is a pre-mating heat observation or mating within four days of the zero result prior to the commencement of luteal activity. The number of detected oestrus has been expressed as a % of total oestrus (POD).

Traditional fertility measures in this study are defined as follows: Days from calving to first detected heat (CFH), days from calving to first service (CFS), days from calving to successful service (CSS). A successful service was confirmed where both the calving date and progesterone levels following the service are consistent with conception being established.

Phenotype definitions for pregnancy and calving rates

Overall pregnancy and calving rates were evaluated as a binary response based on pregnancy diagnosis and calving records. Pregnancy and calving rates to a first mating or within the first 21 days of AB were identified where the concentrations of progesterone increased and remained elevated for at least 18 days following a correctly-timed (valid) mating. Valid matings were defined as those taking place at zero or marginal levels

TABLE 1: Means (and standard deviations) of fertility and other traits

Trait[1]	All animals	Untreated	CIDR-Treated
CLA1 (days)	33.3 (17.1)	29.6 (14.3)	55.3 (16.1)*
CLA2 (days)	37.4 (18.6)	34.1 (16.9)	57.2 (15.7)*
CFH (days)	52.8 (23.6)	50.6 (24.1)	65.9 (14.8)*
CFS (days)	75.8 (16.4)	77.2 (16.2)	67.6 (14.7)*
CSS (days)	84.9 (21.1)	85.1 (20.9)	84.0 (22.8)
POD (%)	51.7 (18.8)	51.3 (19.5)	53.7 (14.3)
PD (%)	92.0 (324/352)	93.0 (280/301)	86.3 (44/51)
PF (%)	63.6 (224/352)	67.4 (203/301)	41.2 (21/51)
P21 (%)	68.5 (241/352)	70.4 (212/301)	56.9 (29/51)
C (%)	88.4 (311/352)	89.4 (269/301)	82.4 (42/51)
CF (%)	61.4 (216/352)	64.8 (195/301)	41.2 (21/51)
C21 (%)	58.8 (207/352)	61.5 (185/301)	43.1 (22/51)
BCSC (1 to 10)	4.96 (0.34)	4.97 (0.33)	4.88 (0.38)
ΔBCS	-0.54 (0.37)	-0.54 (0.37)	-0.53 (0.38)
LWTC (kg)	389 (35)	389 (34)	388 (39)
ΔLWT (kg)	-40 (19)	-39 (19)	-43 (18)
MY56 (litres/day)	14.1 (2.4)	14.0 (2.4)	14.8 (2.7)
FY56 (kg/day)	0.73 (0.13)	0.72 (0.13)	0.74 (0.13)
PY56 (kg/day)	0.51 (0.08)	0.51 (0.08)	0.52 (0.09)

[1] CLA1: commencement of luteal activity (1 elevated); CLA2: commencement of luteal activity (2 elevated); CFH: days from calving to first heat; CFS: days from calving to first service; CSS: days from calving to successful service; POD: % of oestrus detected; PD: pregnant; PF: pregnant to 1st mating; P21: pregnant to matings in 1st 21 days; C: calved; CF: calved to 1st mating; C21: calved to matings in 1st 21 days; BCSC: body condition score within 1 week of calving; ΔBCS: change in body condition score from calving to time of nadir; LWTC: liveweight within 1 week of calving; ΔLWT: change in liveweight from calving to time of nadir; MY56: average daily milk volume; FY56: average daily milkfat yield; PY56: average daily protein yield.

* These phenotypes will be highly confounded with the timing of the CIDR treatment

of progesterone. Binary response phenotypes defined were: Pregnant as at the pregnancy diagnosis (PD), pregnant to first confirmed valid mating (PF), pregnant as a result of matings in the first 21 days of AB (P21), calved (C), calved to first confirmed valid mating (CF), and calved as a result of matings in the first 21 days of AB (C21). Definitions of CFS, PF and CF in this study differ from traditional measures because progesterone profiles enabled the exclusion of incorrectly-timed first matings. Incorrectly-timed matings took place where there were elevated levels of progesterone, and were caused by falsely detected heats, thus making them unlikely to result in conception.

STATISTICAL ANALYSIS

Basic summary statistics were evaluated for all defined phenotypes. A subset of fertility traits (CFH, CFS, PF and the intervals to CLA) for untreated animals were analysed using ASREML (Gilmour *et al.*, 1998).
The models fitted to the subset of fertility traits were as follows:

$$Y_{ijkl} = \mu + \alpha_i + p_j + w_k + e_{ijkl}$$

Where for trait *l*, μ =overall mean; α_i = random effect of cow; p_j = random permanent environment effect; w_k = fixed effect for week of calving; e_{ijkl} = random error term.

Relationships between the fertility traits and BCS were evaluated by including BCS at calving (BCSC) and change in BCS to time of nadir (ΔBCS) as covariates in the model above. Additional covariates were also fitted for age at calving (in weeks), liveweight at calving (LWTC), change in liveweight to time of nadir (ΔLWT), and average daily yields for milk volume (litres), milkfat (kg) and protein (kg) over the first 56 days of lactation (MY56, FY56, PY56). Nadir for BCS and liveweight were evaluated from values in the 56 days following calving, and prior to any matings for the animal. Average daily yields were estimated from a model of phenotypic yield using a Wilmink function (Wilmink, 1987).

RESULTS

Means and standard deviations of defined fertility phenotypes, and other traits are shown in Table 1. Phenotypes for intervals to CLA, first heat and first

service will be highly confounded with the timing of time of treatment for the CIDR-treated animals. For this reason, they will not be a true indication of cycling commencement or luteal activity. Phenotypes for pregnancy, calving, the days from calving to successful service and % of oestrus detected are not highly confounded with the timing of CIDR treatments. This makes them more meaningful indications of fertility for the CIDR-treated animals. Pregnancy and calving rates have all been expressed as the % of total animals that were either diagnosed as pregnant or calved. Overall pregnancy and calving rates were in the range 82-93% with rates lower for the CIDR-treated animals across all phenotypes analysed. BCS averaged 4.96 in the first week following calving, and reduced to 4.42 at nadir. Liveweight averaged 389kg in the first week following calving, and reduced to 349kg at nadir. Average daily yields for the first 56 days of lactation were 14 litres for milk volume, 0.7kg for milkfat yield and 0.5kg for protein yield.

Regression estimates of selected fertility traits on BCS, liveweight and estimated yields are shown in Table 2. No significant relationships were established between ΔBCS and the fertility traits. However, significant relationships were identified between BCS at calving and the intervals to CLA. Every unit decline in BCS at calving resulted in an increase of 7.4 days to CLA1 and 8.8 days to CLA2. The only other significant relationship was identified for average daily milkfat yield on CFS.

The phenotypic correlation between CLA1 and CLA2 was 0.91. For PF, phenotypic correlations with CFH, CFS and intervals to CLA were low (–0.10 to -0.01). The correlation between CFH and CFS was 0.29; correlations between CFH and the CLA intervals were 0.42 for CLA1 and 0.39 for CLA2; correlations between CFS and the CLA intervals were 0.20 for CLA1 and 0.23 for CLA2.

DISCUSSION

Data collected from cohort one Friesian-Jersey Crossbred trial animals in their first lactation has been used to define and quantify a set of fertility phenotypes. Analysis of progesterone samples has enabled definition and measurement of phenotypes associated with endocrine traits such as intervals to CLA. Additionally, the collation of progesterone data with pre-mating heats and matings has enabled the evaluation of a phenotype associated with oestrus expression, and accurate evaluations of other traditional fertility measures such as days to first heat, first service and successful service. For untreated animals, the mean interval to CLA defined by one elevated progesterone sample (CLA1) was 29.6 days (sd=14.3). This is similar to the 29.4 days (sd=18.4) reported by Royal *et al.* (2002b) in their study based on a thrice-weekly sampling routine. The mean interval to CLA defined by two consecutive elevated progesterone samples (CLA2) for untreated animals was 34.1 days (sd=16.9). A comparable study based on a twice-weekly sampling routine by Veerkamp *et al.* (1997) reported a mean interval to CLA of 36.5 days (sd=25). Other studies reporting varying intervals to CLA are Darwash *et al.* (1997a; 27.0 days (sd=12.1)) and Royal *et al.* (2002a; 27.9 (sd=15.4)).

The average days to first heat and first service for untreated animals in this study were 50.6 days (sd=24.1) and 77.2 days (sd=16.2) respectively. Other studies of different sizes report varying intervals from calving to first heat and first service. Pryce *et al.* (2001) reported an interval of 47.9 days (sd=27.4) to first heat and 77.4 days (sd=21.3) to first service. Grosshans *et al.* (1997) reported a higher interval to first service of 84.8 days (sd=22.0) for first lactation data, and 75.0 days (sd=20.7) for second lactation data. In the present study, the average days to successful service over both untreated and CIDR-treated animals was 84.9 days (sd=21.1), lower than the 99.0 days (sd=32.0) reported by Grosshans *et al.* (1997) for first lactation data.

Conceptions confirmed by calvings to first inseminations occurred for 64.8% of untreated animals and 41.2% of CIDR-treated animals. These rates are lower than those reported by Royal *et al.* (2000), 65.4% for untreated and 52.0% for CIDR-treated animals. Overall conceptions confirmed by calvings to inseminations in the first 21 days of AB occurred for 58.8% of animals, higher than that reported by Grosshans *et al.* (1997; 48.5%).

TABLE 2: Regression estimates (and standard errors) of fertility traits on BCS, liveweight and estimated yields

Trait[1]	CFH (days)	CFS (days)	PF (%)	CLA1(days)	CLA2(days)
BCSC	-9.0(5.4)	-2.4(2.4)	-0.05(0.1)	-7.4(3.3)**	-8.8(3.8)**
ΔBCS	-8.6(5.0)	-3.7(2.2)	0.03(0.11)	-5.7(3.0)	-5.4(3.6)
LWTC	-0.06(-0.06)	-0.04(0.02)	-0.00(0.00)	0.04(0.03)	0.03(0.04)
ΔLWT	-0.11(0.09)	-0.04(0.04)	-0.00(0.00)	0.02(0.05)	0.03(0.06)
MY56	2.7(1.8)	-1.0(0.8)	-0.01(0.04)	1.3(1.1)	1.2(1.2)
FY56	22(25)	22(11)*	0.5(0.5)	11(15)	26(17)
PY56	-67(52)	-10(23)	-0.8(1.1)	-32(31)	-43(36)

[1]   See Table 1 for a list of trait definitions
*   *P < 0.05; **P < 0.025.*

Phenotypic relationships between the intervals to CLA and BCS at calving were identified as significant in this study. Although no significant relationships were established between the selected traits and ΔBCS, given a larger study the p-values may increase. This differs from Pryce *et al.* (2001) where the most significant relationships were reported between fertility traits and change in BCS (measured on a five-point scale) from week 1 to 10. The suggestion from their study was that because changes in BCS could be closely related to energy balance, BCS measured at week 10 (close to the start of the herd mating period) may be a better indicator of reproductive performance.

Fertility traits and their key relationships with other commonly measured traits have been evaluated from a phenotypic perspective only in this study. It is intended that the set of fertility phenotypes will be used in a further study to identify related QTL. Other studies have shown that endocrine fertility parameters are heritable, in particular, measurements of intervals to CLA (h^2=0.14 to 0.28; Darwash *et al.*, 1997a; 1997b; 1998; Royal *et al.*, 2002a; 2002b; Veerkamp *et al.,* 1998; 2000). These heritabilities give a positive indication of the possibilities for identifying QTL from traits defined in this study.

This study has resulted in quantification of key fertility traits for a group of animals that will have genotype information available. The phenotypes reported have mostly been reported in other studies, and the values from this study have been found to be comparable. Further evaluation of phenotypes for cohort one based on their second lactation, and cohort two for their first lactation is well under way. These will also be utilised in the planned analysis aimed at identifying fertility related QTL.

ACKNOWLEDGEMENTS

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