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Factors Influencing Fertility of Holstein Dairy Cows: A Multivariate Description

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ABSTRACT

Eighty-two multiparous cows of high and low genetic merit were fed one of two isonitrogenous (19.3% crude protein), isoenergetic (11.3 MJ of metabolizable energy) diets that differed in ratio of rumen-undegradable protein to rumen-degradable protein. Factors that influenced reproductive performance were investigated using logistic regression and survival analysis. Significant associations were identified between reproductive performance and indicators associated with nutrient balance. Cows with higher dry matter intake were more likely to show signs of estrus at first ovulation and to become pregnant by d 150 of lactation. Increased ratio of plasma glucose to 3-hydroxybutyrate was associated with a greater probability of estrous expression at first ovulation. Concentrations of plasma cholesterol were positively associated with expression of estrus at first ovulation, interval from calving to conception, and likelihood of conception and pregnancy. Greater concentrations of nonesterified fatty acids in plasma were associated with a lower probability of conception by d 150 of lactation. Increased yield of fat-corrected milk during early lactation was negatively associated with expression of estrus at first ovulation and probability of pregnancy by d 150 of lactation. Cows of high genetic merit were less likely to show signs of estrus at first ovulation. Cows fed the high rumen-degradable-protein diet that also lost more body weight during early lactation were less likely to conceive at first service and to have a prolonged interval from calving to conception. Continued selection for increased production of milk and a more negative nutrient balance during early lactation may reduce reproductive performance particularly for cows fed high concentrations of rumen-degradable protein.
(**Key words:** fertility, genetic merit, multivariate, protein degradability)

Abbreviation key: ABV = Australian breeding value, **HD** = highly degradable, **LD** = low degradability, **MJME** = megajoules of metabolizable energy, **NEB** = negative energy balance, **3-OH** = 3-hydroxybutyrate, **UDP** = undegradable protein.

INTRODUCTION

Early reestablishment of ovarian activity after calving has been identified as a significant modifier of reproductive efficiency of dairy cows. Intervals from calving to first ovulation, first service, conception, and calving are extended when resumption of ovarian activity is delayed. Efficiency of reproduction is further modified by factors that influence probability of conception, including dietary protein (Jordan and Swanson, 1979), genetic merit of cows (MacMillan et al., 1996), milk yield (Laben et al., 1982; Lean et al., 1989), energy balance (Haresign et al., 1981), number of estrous cycles, progesterone concentrations in cycles preceding insemination (Fonseca et al., 1983), and parity. Extended calving-to-calving intervals lower profit because efficiency of milk production is reduced, fewer calves are born, and rate of genetic gain in the herd is slowed.

In a previous paper, Westwood et al. (2000) investigated the univariate associations between dietary protein and the genetic merit of cows, and the concentrations of metabolites, body condition, and the reproductive performance of lactating dairy cows. Farm management factors including sensitivity and specificity of estrous detection, voluntary waiting period, semen storage, and semen placement in the uterine tract will also modify reproductive performance. Given the potential for these management factors to confound univariate associations, the second paper of this series uses multivariate analytical methods to further investigate the reproductive response to dietary protein degradability and the genetic merit of cows. The objective of this study was to evaluate the factors that influenced the fertility of cows maintained under tightly controlled experimental conditions. We considered that by investigating several different measures of reproductive outcome, mechanisms that lower reproductive performance may be better understood, and the hierarchical level at which each

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factor may affect reproductive performance may be identified.

MATERIALS AND METHODS

Cows and Feeding

Eighty-two multiparous Friesian dairy cows of high or low genetic merit were fed one of two isonitrogenous (19.3% CP), isoenergetic (11.3 MJ of metabolizable energy; **MJME**) diets: 1)a low-degradable (**LD**) diet, of composed 37% undegradable protein (**UDP**) and 63% RDP; and a highly-degradable (**HD**) diet, composed of 15% UDP and 85% RDP. Rations were composed of chaffed alfalfa and oaten hay, and pelleted concentrate, as described by Westwood et al. (2000). Diets were fed ad libitum as TMR in a Calan-Broadbent feeding facility (American Calan Inc., Northwood, NH) for the first 150 d of lactation. Cows were maintained on a shaded loafing area adjacent to the feeding facility without access to other feeds.

Cows of high genetic merit were defined as those with an Australian breeding value (**ABV**) fat plus protein greater than or equal to 31. Cows of low genetic merit had an ABV less than or equal to 15.

The study was conducted over a 2-yr period with cows calving year round. A maximum of 44 cows were present in the herd at any time, and herds were balanced for dietary and genetic merit group. All work was carried out with the approval of the Animal Care and Ethics Committee of the University of Sydney, Australia.

Data and Sample Collection

Daily DMI were calculated for each cow. Cows were milked twice daily and individual yields recorded. Cows were weighed and scored for body condition every 7 d. Body condition was scored using a five-point scale of 0.25 increments, where 1 = thin and 5 = obese (Edmondson et al., 1989).

Whole-milk samples for milk progesterone concentration were collected from each cow at 3-d intervals, beginning 7 d after calving. Weekly milk samples were collected until wk 10 of lactation for milk fat, protein, and lactose percentage analysis. Blood samples were taken weekly from wk –3 to wk 10 of lactation and analyzed for plasma urea, glucose, cholesterol, 3-hydroxybutyrate (**3-OH**), and serum NEFA. Plasma progesterone concentrations were determined with heparinized blood collected from each cow twice weekly for 24 d after each breeding.

Calculation of Energy Balance

Weekly energy balance was calculated for each cow using mean daily DMI/wk, mean daily milk production per week, weekly change in BW, and weekly percentage of milk fat, protein, and lactose. Daily metabolizable energy intake was calculated using formulated values for metabolizable energy for each ration.

Analytical Procedures

Plasma urea, glucose, and cholesterol concentrations were determined with commercial kits using a Cobas Mira autoanalyzer. Concentrations of plasma 3-OH were determined by auto analyzer according to the method of Zivin and Snarr (1973). NEFA concentrations were determined with Wako NEFA C kits (Wako Pure Chemical Industries, Osaka, Japan), with modifications described by Rabiee (1995).

Milk progesterone concentration was determined with commercial liquid- and solid-phase ^{125}I-labeled progesterone kits (Orion Diagnostica, Espoo, Finland); a solid-phase ^{125}I-labeled solid-phase kit (Orion Diagnostica) was used to assay plasma progesterone concentrations.

Reproductive Measures

A detailed description of the reproductive performance evaluation was given by Westwood et al. (2000). The reproductive health of all cows that had not been inseminated during the previous 42 d was monitored every 3 wk by palpation of the reproductive tract per rectum, commencing 21 ± 3.5 d after calving. Cattle were observed for estrus for at least 14 h/d. Sensitivity of estrous detection was increased by placing KaMaR heatmount detectors (Steamboat Springs, CO) on the tailhead of each cow. Cows were bred on the observation of primary and/or secondary heat signs. Cows were not bred before d 45 of lactation.

Calving to first ovulation interval was defined by the first increase in milk progesterone >6 nmol/L after calving; ovulation was assumed to have occurred 5 d before elevation of progesterone concentration. Intervals from calving to first estrus and first service were defined as intervals to estrus or service events accompanied by ovulation (progesterone concentration <6 nmol/L at the time of event). Conception and pregnancy rate to first service were calculated only for services accompanied by ovulation.

Early establishment of pregnancy (initial conception) was defined by an elevated progesterone concentration that persisted beyond d 24 after insemination. Successful pregnancy was defined by pregnancy palpable per rectum at d 42 after mating. Interval from calving to initial conception was calculated with progesterone concentrations; time to successful pregnancy was calculated with palpation records. Early embryonic death was defined as loss of conceptus between initial conception diagnosis and confirmation of pregnancy by rectal palpation.

Table 1. Categorical variables evaluated in models.

Name	Description	Values
Diet	Diet, isonitrogenous, isoenergetic containing protein of low or high degradability	1 = LD; 2 = HD[1]
Metritis	Metritis and/or retained fetal membrane, that required veterinary intervention, including use of uterine and/or parenteral antibiotics	0 = no; 1 = yes
Season calved	Season in which cow calved	Winter, Spring, Summer, Autumn[2]
Season 1st Ovulation	Season during which first ovulation occurred	Winter, Spring, Summer, Autumn[2]
Season 2nd Ovulation	Season during which second ovulation occurred	Winter, Spring, Summer, Autumn[2]

[1]LD = Low-degradable diet; HD = highly-degradable diet.

[2]Winter = June 1 through August 31; Spring = September 1 through November 30; Summer = December 1 through February 28; Autumn = March 1 through May 31.

Statistical Analyses

Univariate analysis of the relationships between the genetic merit of cows, the degradability of dietary protein, and reproductive performance indicated a number of significant associations (Westwood et al., 2000). Reproductive failure is unlikely to be the result of a single dominant factor and is more likely the result of a combination of factors. Multivariate analyses permit the simultaneous evaluation of a large number of explanatory variables to examine their effect on the outcome variable and allow for the examination of interactions between these variables and control confounding. Therefore the data were further explored using multivariate analyses.

Categorical and continuous variables that were investigated as potential predictors are listed in Table 1 and Table 2. Univariate analyses were performed using survival analysis (BMDP 1L; BMDP Statistical Software Inc., Los Angeles, CA) to assess the association between a prognostic variable and time to a reproductive event. Continuous variables were categorized into top, middle, and lower quartiles to examine the effect of each stratum of prognostic variable on time to an event. Associations between categorical prognostic variables and expression of estrus at ovulation, conception to first service, and probability of conception or pregnancy by d 150 of lactation were assessed using χ^2 analysis (BMDP 4F). Effects of continuous variables on estrous expression and probability of conception or pregnancy were assessed using one-way analysis of covariance (BMDP 1V).

Variables that had a univariate P-value less than or equal to 0.25 were included for evaluation using stepwise regression. Regression coefficients for time to first ovulation, first estrus, first service, first conception, and first pregnancy were determined using Cox's regression model (BMDP 2L). Backwards stepwise logistic regression (BMDP LR) was used to model probability of expression of estrus, conception to first service, and conception or pregnancy by d 150 of lactation.

Only the most significant univariate relationship among a group of highly correlated variables was retained for multivariate analysis. Variables that had a univariate P-value less than or equal to 0.25 were included for evaluation using Cox's regression model (BMDP 2L) (time to an event) or backwards stepwise logistic regression (BMDP LR; probability of an event occurring). For logistic regression models, change in maximal log likelihood values were used to indicate the R_L^2 for each model. Because models for time to a reproductive event were partial maximal log likelihood models, R_L^2 were not calculated.

Linearity between dependent and independent variables was assessed graphically by examining quartile cutpoints for continuous independent variables plotted against the proportion of individuals with the outcome of interest. Interactions between variables were assessed by examining correlation matrices, by examination of coefficients, and by testing the significance of interactions among variables. Colinearity between variables was tested by evaluation of the underlying biological relationships between variables, by examination of correlation matrices, and by examination of changes in coefficients as new variables entered or were removed from stepwise models. Confounding was assessed by evaluating changes in coefficients as new terms were entered into multivariate models. Methods used in model development are described by Hosmer and Lemeshow (1989).

All multivariate models included diet and genetic merit as significant effects or as potential confounders. Neither variable was a significant confounder for any final regression model.

One cow was culled at d 77 of lactation as a result of chronic vagal indigestion. Milk progesterone concentrations were not determined, in error, for that cow. Reproductive data for that cow was, therefore, excluded from analysis, and the results for 81 cows presented.

RESULTS

Interval from Calving to First Ovulation

Cows that calved during winter months were 6.8 times more likely to have a delayed interval from calving to

Table 2. Continuous variables evaluated in models.[1]

Name	Description	Value
ABV	ABV for fat + protein yield	
BW precalving	BW, 1 wk before calving. Includes fetus and fluids	kg
BW at calving	BW, < 7 d after calving	kg
BW change	Change in BW, wk –1 to 6 of lactation	kg
BCS precalving	BCS, 1 wk before calving	1 to 5 scale
BCS at calving	BCS, < 7 d after calving	1 to 5 scale
BCS change	Change in BCS, wk –1 to 6 of lactation	1 to 5 scale
Cholesterol, wk -3, 0, 6, 10 and nadir	Plasma cholesterol, mean, wk –3 to 10, 0 to 6, or 0 to 10 of lactation, and nadir during early lactation	mmol/L
Cholesterol at ovulation	Plasma cholesterol during the week of first or second ovulation	mmol/L
Days to ovulation	Interval from calving to first and second ovulation	d
DMI % of BW	Daily DMI as % BW, mean, wk 0 to 6, 0 to 10, or 0 to 17 of lactation	BW %
DMI42	Cumulative DMI, d 0 to 42 and d 0 to 120 of lactation	kg
DMI at ovulation	Mean DMI intake during wk of first or second ovulation	kg
DMI, % at ovulation	Daily DMI as % BW, mean, during the week of first or second ovulation	
Energy balance	Cumulative energy balance, d 0 to 42 and d 0 to 120 of lactation	MJME
Energy balance at ovulation	Mean energy balance during the week of first or second ovulation	MJME
Energy balance nadir	Energy balance nadir, wk –3 to wk 17 after calving	MJME
FCM cumulative	Cumulative FCM yield, d 0 to 42 and d 0 to 120 of lactation	L
FCM peak	FCM peak, d 0 to 120 of lactation	L
FCM at ovulation	Mean daily FCM yield during the week of first or second ovulation	L
Glucose; wk –3, 0, 6, 10 and nadir	Plasma glucose, mean, wk -3 to 10, 0 to 6, or 0 to 10 of lactation, and nadir during early lactation	mmol/L
Glucose ovulation	Plasma glucose at the week of first or second ovulation	mmol/L
Glucose:3-OH ratio	Plasma glucose:3-OH ratio, mean, wk –3 to 10, 0 to 6, or 0 to 10 of lactation, and nadir	Ratio
Glucose:3-OH ovulation	Plasma glucose:3-OH ratio during the week of first or second ovulation	Ratio
3-OH	Plasma 3-OH, mean, wk –3 to 10, 0 to 6, or 0 to 10 of lactation, and peak during early lactation	mmol/L
3-OH ovulation	Plasma 3-OH at the week of first or second ovulation	mmol/L
Luteal	Length of luteal phase, indicated by progesterone >6 nmol/L, subsequent to first ovulation	d
Milk cumulative	Cumulative milk yield, uncorrected, d 0 to 42 and d 0 to 120 of lactation	L
Milk peak	Peak daily milk yield, uncorrected, d 0 to 120 of lactation	L
Milk ovulation	Mean daily uncorrected milk yield during the week of first or second ovulation	L
NEFA; wk -3, 0, 6, 10	Serum NEFA, mean, wk –3 to 10, 0 to 6, or 0 to 10 of lactation	μmol/L
NEFA peak	Serum NEFA, peak, wk –3 to 10 of lactation	μmol/L
NEFA ovulation	Serum NEFA during wk of first or second ovulation	μmol/L
Parity	Parity of cow	Lactations
Ovulation prevalence	Prevalence of cows in herd that ovulated in herd during the week of first or second ovulation	No. of ovulations/wk
Urea; wk -3, 0, 6, 10 and peak	Plasma urea, mean, wk –3 to 10, 0 to 6, or 0 to 10 of lactation, and peak	mmol/L
Urea ovulation	Plasma urea during the week of first or second ovulation	mmol/L

[1]ABV=Australian breeding value; 3-OH = 3-hydroxybutyrate; MJME= megajoules of metabolizable energy.

first ovulation compared with cows that calved during other seasons. Cows with a cumulative yield of FCM from d 0 to 42 of lactation greater than 1623.9 L (or greater than 38 L/d) were 2.6 times more likely to ovulate later than cows with a cumulative yield of FCM less than 1212.5 L (or less than 29 L/d).

Interval from Calving to First Estrus

Cows that had a first ovulation later than d 53 of lactation were 1.6 times more likely to have an increased interval from calving to first estrus compared to cows that ovulated before d 21 of lactation (P = 0.001).

Expression of Estrus at First Ovulation

The model contained six variables (Table 3) that explained almost 50% of the variance for estrous display at first ovulation. The model was a good predictor of expression of estrus at first ovulation. With a cutpoint to optimize allocation to groups (silent estrus, visual

Table 3. Factors affecting risk of estrous expression at first ovulation (Hosmer–Lemeshow goodness of fit χ^2 = 0.850).

Step[1]	Prognostic variable	Regression coefficient (SE*)	Significance	Max log likelihood	OR[2]	95% CI	Relative risk Fav	Relative risk Unfav	OR[3]
0				–39.81					
1	Glucose:3-OH ratio, first 6 wk of lactation[4]	+1.047 (0.464)	0.022	–37.17	2.85	1.13 to 7.19	2.65	0.56	4.71
2	DMI, wk of ovulation[4]	+0.4002 (0.149)	0.007	–33.53	1.49	1.11 to 2.01	3.10	0.12	26.20
3	FCM, wk of ovulation[4]	–0.2168 (0.0822)	0.005	–29.58	0.81	0.68 to 0.95	2.77	0.25	10.98
4	Urea, wk –3 to 10 of lactation[4]	–1.519 (0.548)	0.018	–26.77	0.22	0.07 to 0.65	2.99	0.32	9.19
5	Cholesterol, wk of ovulation[4]	+0.7162 (0.282)	0.005	–22.76	2.05	1.17 to 3.60	3.58	0.50	7.17
6	ABV	–0.0557 (0.0267)	0.020	–20.06	0.95	0.89 to 1.00	2.73	0.46	5.94
				R_L^2 = 0.496					

[1]Step refers to the step at which each variable entered the statistical model by producing the greatest change in log likelihood relative to a model not containing that variable.

[2]OR = odds ratio; risk associated with increase in variable by one unit.

[3] Risk, favorable vs unfavorable.

[4]Mean during period; favorable characteristics: glucose: 3-hydroxybutyrate (3-OH) ratio first 6 wk of lactation > 3.26; DMI, week of ovulation > 28.68 kg; FCM, week of ovulation < 20.53; urea, wk –3 to 10 of lactation < 7.97; cholesterol, week of ovulation > 5.38; ABV < 6. Unfavorable characteristics: Glucose:3-OH first 6 wk of lactation < 1.78; DMI, week of ovulation< 20.53; FCM, week of ovulation > 38.25; urea, wk –3 to 10 of lactation > 9.43; cholesterol, week of ovulation < 2.63; ABV > 38.

estrus, and total ovulations) from the model, approximately 86% of cows were correctly classified.

Expression of Estrus at Second Ovulation

Inclusion of five variables within the model (Table 4) explained 36% of the variation in the probability of a cow showing estrus at second ovulation. With a cutpoint of 0.53, each group is approximately 76% correctly classified.

Interval from Calving to First Service

Calving to first ovulation interval was the only significant predictor of calving to first service interval (P = 0.003). Cows with a calving-to-first-ovulation interval of between 31 and 53 d were 1.46 times more likely to be served for the first time later than cows that ovulated before d 21 of lactation.

Initial Conception to First Service

We defined initial conception to first service as a mating to first true service (that is, service accompanied by ovulation) that resulted in an elevated milk or plasma progesterone concentration of greater than 6 nmol/L that persisted beyond d 24 after mating. Cows that lost less than 51 kg of BW between wk 1 before calving and wk 6 of lactation were 3.7 times more likely to conceive at first service compared with herdmates that lost more

Table 4. Factors affecting risk of estrous expression at second ovulation (Hosmer–Lemeshow goodness of fit χ^2 = 0.849).

Step[1]	Prognostic variable	Regression coefficient (SE*)	Significance	Max log likelihood	OR[2]	95% CI	Relative risk Fav	Relative risk Unfav	OR[3]
0			–49.80						
1	Calving to 2nd ovulation interval[4]	+0.0595 (0.0189)	<0.0001	–41.25	1.06	1.02 to 1.10	2.10	0.42	5.06
2	BW at week before calving[4]	+0.0124 (0.0053)	0.005	–37.38	1.01	1.00 to 1.02	1.89	0.62	3.07
3	Prevalence of ovulation during week of ovulation[4]	+0.3364 (0.153)	0.022	–34.75	1.40	1.04 to 1.89	1.96	0.51	3.84
4	Diet (HD or LD)	+1.282 (0.691)	0.090	–33.31	3.60	0.93 to 14.01	1.00	0.28	3.60
5	Length of luteal phase before 2nd ovulation	+0.0679 (0.0435)	0.085	–31.83	1.07	0.98 to 1.17	1.23	0.67	1.84
				R_L^2 = 0.361					

[1]Step refers to the step at which each variable entered the statistical model by producing the greatest change in log-likelihood relative to a model not containing that variable.

[2]OR = odds ratio; risk associated with increase in variable by one unit.

[3]Risk, favorable vs unfavorable.

[4]Mean during period; favorable characteristics: calving to second ovulation interval >64.5 D; BW at week before calving >715.5 kg; mean prevalence of ovulation during week of ovulation > 7; diet = highly-degradable (HD); length of luteal phase before 2nd ovulation > 21d. Unfavorable characteristics: calving to second ovulation interval <37.3 d; BW at week before calving <625.0 kg; mean prevalence of ovulation during week of ovulation <3; diet = low-degradable (LD); length of luteal phase before 2nd ovulation < 12d.

than 109 kg (Table 5). Cows in the LD dietary group were 3.2 times more likely to conceive at first service than were cows fed the HD diet. The model explained 13.1% of the variance in probability of conception to first service. Sixty-seven percent of cows were correctly allocated to groups when a cutpoint of 0.41 was used.

Interval from Calving to Initial Conception

Interval from calving to initial conception was defined as time from calving to initial conception, with conception defined as for the model for initial conception to first service. Cows that were not served for the first time until after d 87 of lactation were 1.3 times more likely to have a delayed interval from calving to initial conception compared with herdmates served for the first time before d 56 of lactation (Table 6). An interaction between change in BW and dietary protein degradability significantly predicted time to initial conception. Cows fed the HD diet and losing more than 76 kg of BW between 1 wk before calving and wk 6 of lactation were almost twice as likely to have a longer interval from calving to conception than cows fed the LD diet and that lost less than 76 kg during the same period (Figure 1). The model did not fit the criteria for the Cox's proportional hazards model because plots overlapped, therefore, data were reassessed using the Weibull accelerated failure time model.

Interval from Calving to Successful Pregnancy

The interval from calving to successful pregnancy was defined as the number of days from calving to a mating that resulted in a palpable pregnancy at d 42 after mating. Cows with a plasma cholesterol nadir of less than 0.9 mmol/L were 1.4 times more likely to have a delayed calving to successful pregnancy interval than were cows with a plasma cholesterol nadir greater than 1.8 mmol/L (Table 7). Cows that lost more than 76 kg of BW between precalving and wk 6 of lactation, and that were slow to initiate luteal activity after calving were 4.4 times more likely to have an extended calving-to-successful-pregnancy interval than were cows that lost less than 76 kg during the same period, and ovulated before d 31 of lactation.

Probability of Conception Before d 150 of Lactation

Probability of conception before d 150 was defined as the likelihood of recording a persistently elevated milk or plasma progesterone of greater than 6 nmol/L that persisted for more than 24 d subsequent to any mating to a service occurring before d 150 of lactation. Cows that had a prolonged calving to first ovulation interval and had high mean serum NEFA concentrations—greater than 313.3 μmol/L—during the first 10 wk of lactation were 20 times less likely to conceive by d 150 of lactation than were herdmates that also had a prolonged calving-to-first-ovulation interval, but that had mean concentrations of NEFA of less than 313.3 μmol/L (Table 8). Cows with a higher plasma cholesterol nadir were 2.3 times more likely to conceive by d 150 of lactation than cows with a low nadir of plasma cholesterol. Cows fed the LD ration were three times more likely to conceive by d 150, than were those fed the HD diet. The final model explained 29.1% of the variance. With a cutpoint of 0.19, approximately 74% of each group (conceived, failed to conceive, and total) were correctly classified.

Probability of Pregnancy Before Day 150 of Lactation

Probability of pregnancy before d 150 of lactation was defined as probability of retaining a successful pregnancy (palpable pregnancy at d 42 after mating) to any mating before d 150 of lactation. Differences between data describing failure to conceive and failure to become pregnant reflect cows that conceived before d 150, but

Table 5. Factors affecting probability of conception to first service (Hosmer–Lemeshow goodness of fit χ^2 = 0.495).

Step[a]	Prognostic variable	Regression coefficient (SE*)	Significance	Max log likelihood	OR[2]	95% CI	Relative risk Fav	Relative risk Unfav	OR[3]
0				–53.68					
1	Change in bodyweight during first 6 wk of lactation	+0.0225 (0.00749)	0.003	–49.26	1.02	1.008 to 1.038	1.76	0.48	3.69
2	Diet (HD or LD)	–1.159 (0.521)	0.022	–46.65	0.31	0.113 to 0.874	1.00	0.31	0.31
				R_L^2 = 0.131					

[1]Step refers to the step at which each variable entered the statistical model by producing the greatest change in loglikelihood relative to a model not containing that variable.

[2]OR = Odds ratio. Risk associated with increase in variable by one unit.

[3]Risk, favorable vs unfavorable. Favorable characteristics: change in BW during first 6 wk of lactation >–51 kg; low-degradable (LD) diet. Unfavorable characteristics: change in BW during first 6 wk of lactation <–109 kg; highly-degradable (HD) diet.

Table 6. Factors affecting calving to initial conception interval, using the Weibull accelerated failure time model.

Step[1]	Prognostic variable	Regression coefficient (SE*)	Significance	Max log likelihood	HR[2]	95% CI	Relative risk Fav	Relative risk Unfav	HR[3]
0				–65.75					
1	Calving-to-first-service interval	+0.0083 (0.1762)	<0.00001	–60.94	1.02	0.71 to 1.43	1.12	0.87	1.29
	Interaction between BW loss and diet: Groups are expressed relative to the reference group of "low BW loss, fed LD"								
2	High BW loss, fed HD[4]	+0.6070 (0.1131)	<0.00001	–56.44	1.83	1.47 to 2.29			
3	High BW loss, fed LD[4]	+0.5150 (0.1148)	<0.00001	–50.21	1.67	1.34 to 2.10			
4	Low BW loss, fed HD[4]	+0.2696 (0.1110)	0.0330	–47.35	1.31	1.05 to 1.63			

[1]Step refers to the step at which each variable entered the statistical model by producing the greatest change in loglikelihood relative to a model not containing that variable.

[2]HR = Hazard ratio. Risk associated with increase in variable by one unit.

[3]Risk, favorable vs unfavorable.

[4]In reference to cows with low BW loss (BW loss >–76 kg) during the first 6 wk of lactation, and fed the low degradable (LD) diet. Favorable characteristics: low BW loss (BW change >– 51 kg) and feeding LD ration. Calving to first service interval <56 d. Unfavorable characteristics: high BW loss (BW change < –109 kg) and feeding high degradable (HD) ration. Calving to first service interval > 87 d.

failed to maintain a successful pregnancy. Twenty-three percent of the variance was explained by the model for probability of pregnancy (Table 9). Approximately 73% of each group were correctly classified using a cutpoint of 0.31. A significant interaction term entered the model. Cows that produced more than 1393 L of cumulative FCM by d 42 of lactation and that were slow to resume ovulation after calving were 10 times less likely to be pregnant by d 150 of lactation than were cows that also produced more than 1393 L of FCM during early lactation, but had a short interval from calving to first ovulation.

Plasma cholesterol nadir during the first 10 wk of lactation > 1.8 mmol/L was associated with a 2.7 times greater probability of successful pregnancy by d 150 of lactation. Cows that ate more than 4.03% of their BW each day during the first 17 wk of lactation were almost twice as likely to be pregnant at d 150 of lactation than cows that ate less than 3.45% of BW during the same period.

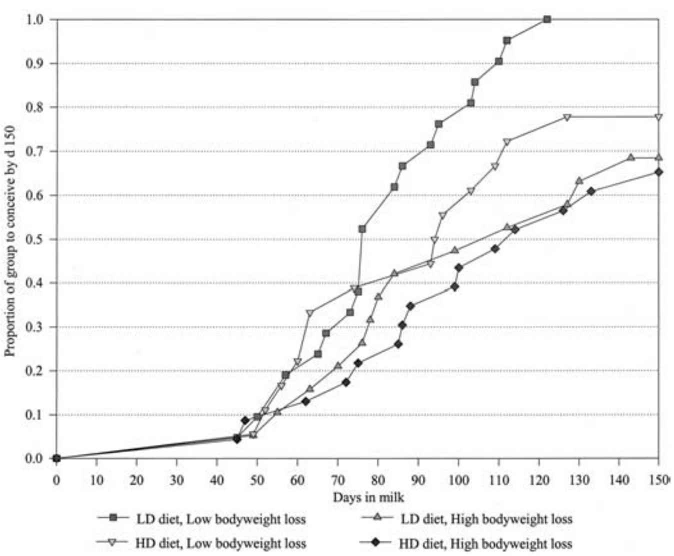

Figure 1. Interval from calving to initial conception for cows fed low-degradable (LD) or high-degradable (HD) diets and losing greater or less than 76 kg during early lactation.

DISCUSSION

A number of interrelated factors that influenced reproductive success in a group of high-producing dairy cows maintained under controlled experimental conditions were identified. The term net energy balance is a calculated estimate of the deficit between energy intake and energy output. This term, however, also reflects BW loss; this loss includes irreversible losses of protein, minerals, vitamins, and energy from the body. While energy balance was calculated and assessed, factors that influence energy balance, feed intake, and milk solids production were examined as individual factors influencing fertility. The multivariate models that evaluated display of estrus explained much of the variation and are, to our knowledge, the first multivariate models to examine estrus display. The models evaluating conception and pregnancy at first service explained a moderate amount of the variance, and all models developed had good fit. Discussion of the results is presented in terms of the factors that influenced fertility in the study.

Dietary Protein Degradability

Diet was not accepted in the model evaluating expression of estrus at first ovulation; however, a proxy for diet, plasma urea, did significantly predict expression of estrus at first ovulation because cows with higher concentrations of urea were less likely to show estrus.

Table 7. Factors affecting calving to successful pregnancy interval.

Step[1]	Prognostic variable	Regression coefficient (SE*)	Significance	Max log likelihood	HR[2]	95% CI	Relative risk Fav	Relative risk Unfav	HR[3]
0				–217.06					
1	Cholesterol concentration nadir during first 10 wk of lactation	–0.4016 (0.2082)	0.051	–215.15	0.67	0.44 to 1.01	0.85	1.22	0.70[2]
	Interaction between calving to first ovulation and BW loss								
2	Time to first ovulation<31, low BW loss[4]	–1.4670 (0.4537)	0.050	–213.24	0.31	0.12 to 0.76			
3	Time to first ovulation < 31, high BW loss[4]	–1.3033 (0.4506)	0.035	–211.02	0.28	0.12 to 0.68			
4	Time to first ovulation> 31, low BW loss[4]	–1.1237 (0.4662)	0.012	–207.89	0.23	0.09 to 0.56			

[1]Step refers to the step at which each variable entered the statistical model by producing the greatest change in loglikelihood relative to a model not containing that variable.

[2]HR = Hazard ratio. Risk associated with increase in variable by one unit.

[3]Risk, favorable vs unfavorable.

[4]In reference to cows with time to first ovulation > 31 d and cows with BW loss of more than 76 kg during the first 6 wk of lactation. Favorable characteristics: low BW loss (BW change > –51 kg) and time to first ovulation <21 d; cholesterol concentration nadir during first 10 wk > 1.8 mmol/L. Unfavorable characteristics: high BW loss (BW change <–109 kg) and time to first ovulation > 53 d. Cholesterol concentration nadir during first 10 wk <0.9 mmol/L.

Feeding the HD diet was associated with higher concentrations of plasma urea than for the LD group during the first 10 wk of lactation (Westwood et al., 2000). In contrast, at second ovulation, cows fed the HD diet were more likely to show estrus, despite the fact that these cows tended to mobilize more body condition after calving (Westwood et al., 2000). While the interval from calving to NEB nadir did not differ significantly between dietary groups (Westwood et al., 2000), the HD group regained positive energy balance more rapidly than the LD group. A more favorable energy balance may have favored estrus expression at second and subsequent ovulations for the HD group.

Reproductive measures of conception success were negatively influenced by the HD diet, a finding consistent with previous reports (Folman et al., 1981; Bruckental et al., 1989; Son et al., 1996). The negative effects of increased protein degradability on conception were, however, accentuated by concurrent loss of BW during early lactation. Relatively high conception rates (> 50% to first service) have been reported for Australian and New Zealand dairy cows fed large amounts of rapidly

Table 8. Factors affecting probability of conception before d 150 of lactation (Hosmer–Lemeshow goodness of fit χ^2 = 0.808).

Step[1]	Prognostic variable	Regression coefficient (SE*)	Significance	Max log likelihood	OR[2]	95% CI	Relative risk Fav	Relative risk Unfav	OR[3]
0				–42.91					
1	Interaction between NEFA concentration and calving to first ovulation interval	-0.112×10^{-3} (0.3×10^{-4})	<0.0001	–33.25					
	NEFA < 313.3 and calving to first ovulation <31[4]				8.0	1.6 to 46.8			
	NEFA >313.3 and calving to first ovulation <31[4]				9.6	1.5 to 78.6			
	NEFA <313.3 and calving to first ovulation > 31[4]				20.4	2.1 to 486.5			
2	Cholesterol concentration nadir during first 10 wk of lactation	+0.9169 (0.540)	0.092	–31.83	2.50	0.87 to 7.23	1.44	0.63	2.28
3	Diet (HD or LD)	–1.0106 (0.687)	0.095	–30.43 R_L^2 = 0.291	0.33	0.09 to 1.28	0.33	1.00	0.33

[1]Step refers to the step at which each variable entered the statistical model by producing the greatest change in log-likelihood relative to a model not containing that variable.

[2]OR = Odds ratio. Risk associated with increase in variable by one unit.

[3]Risk, favorable vs unfavorable.

[4]In reference to cows with mean NEFA during first 10 wk of lactation > 313.3 mmol/L, and calving to first ovulation interval > 31 d. Favorable characteristics: NEFA < 331.3 mmol/L and calving-to-first-ovulation interval <31 d; cholesterol nadir > 1.8; diet = low-degradable (LD). Unfavorable characteristics: NEFA10 > 331.3 mmol/L and calving-to-first-ovulation interval > 31 d; cholesterol nadir <0.9 mmol/L; diet = highly-degradable (HD).

Table 9. Factors affecting probability of pregnancy before d 150 of lactation (Hosmer–Lemeshow goodness of fit $\chi^2 = 0.620$).

Step[1]	Prognostic variable	Regression coefficient (SE*)	Significance	Max log likelihood	OR[2]	95% CI	Relative risk		OR[3]
							Fav	Unfav	
0				– 50.84					
1	Interaction between calving to first ovulation and cumulative FCM yield to d 42 of lactation	-0.23×10^{-4} (0.69×10^{-4})	0.001	–45.78					
	Calving to first ovulation < 31d and FCM < 1393[4]				4.7	1.1 to 21.1			
	Calving to first ovulation <31 d and FCM > 1393[4]				10.4	1.6 to 84.5			
	Calving to first ovulation< 31 d and FCM < 1393[4]				2.38	0.6 to 10.7			
2	Cholesterol concentration nadir during first 10 wk of lactation	+1.112 (0.472)	0.005	–41.83	3.04	1.20 to 7.69	1.56	0.57	2.72
3	DMI, % BW during first 17 wk of lactation	+1.678 (0.745)	0.017	–38.97	3.21	0.74 to 13.90	1.47	0.75	1.97
				$R_L^2 = 0.233$					

[1] Step refers to the step at which each variable entered the statistical model by producing the greatest change in loglikelihood relative to a model not containing that variable.

[2]OR = Odds ratio. Risk associated with increase in variable by one unit.

[3]Risk, favorable vs unfavorable; mean during period.

[4] In reference to cows with calving to first ovulation interval > 31 d and cumulative FCM (FCM42) during first 42 d of lactation > 1393 L. Favorable characteristics: calving to first ovulation <31 d and FCM42 <1393 L; cholesterol nadir > 1.8 mmol/L; DMI as % BW, to wk 17 of lactation > 4.03%. Unfavorable characteristics: Calving to first ovulation >31 d and FCM42 > 1393 L; cholesterol nadir <0.9 mmol/L; DMI as % BW, to wk 17 of lactation <3.45%.

RDP (Williamson and Fernandez-Baca, 1992); however, lower conception efficiency has been reported for American and European herds fed diets high in RDP. Despite a similar genetic base, Australasian dairy cows generally calve in low body condition, lose less BW during early lactation (Abe et al., 1994; Mackle et al., 1996), and produce less milk than cows in North America. These observations suggest that the depth and duration of nutrient deficit is less severe in Australasian than in North American cattle. Blood concentrations of NEFA and 3-OH are lower and appetite is less depressed during early lactation than in cows that calve with greater reserves of body fat, and that mobilize more body tissue during early lactation. Australasian dairy cows may, therefore, better tolerate ingestion of rapidly degradable dietary proteins than North American cows, because negative effects of feeding more RDP on reproduction are not exacerbated by marked body tissue loss.

Effect of Genetic Merit

This study had limited power to detect subtle effects of genetic merit on fertility, and negative results should not be assumed to be no effect. Genetic merit of cows was not a significant predictor for interval from calving to first ovulation or for calving to first estrus. This finding contrasted with our hypothesis that, because improved genetic merit is associated with greater mobilization of body tissue in cows (Veerkamp et al., 1995; Westwood et al., 2000), and more mobilization of body tissue delayed resumption of ovarian activity (Butler and Smith, 1989), cows of higher genetic merit would be at greater risk of prolonged interval from calving to first ovulation. While the energy balance for cows of superior genetic merit was significantly lower than for those of lower merit (Westwood et al., 2000), the interval from calving to NEB nadir did not differ significantly between genetic groups. The interval from calving to NEB nadir, rather than magnitude of NEB, has been significantly associated with the onset of ovarian activity (Canfield and Butler, 1991), a finding that may reflect the absence of association in this study.

Cows of superior genetic merit were less likely to display estrus at first ovulation and may reflect the lower nutrient balance of those cows in early lactation (Westwood et al., 2000). Spicer et al. (1990) found that expression of estrus was reduced for cows with a more negative energy balance.

Relationship with Blood Metabolites

Relationships between blood metabolites and the reproductive performance of dairy cows reported previously (Eldon et al., 1988; Huszenicza et al., 1988) have been substantiated by our findings. Ratios of glucose:3-OH ratio and concentrations of plasma cholesterol were positively associated with expression of estrus at first ovulation. A lower glucose:3-OH ratio reflects a relative shortage of gluconeogenic substrates, and blood glucose concentrations are lower and 3-OH concentrations are higher during a period of NEB (Herdt et al., 1981; Canfield and Butler, 1990; Harrison, 1990). Time series analysis (Lean et al., 1992) found that plasma cholesterol concentrations were positively correlated with energy balance for dairy cows in early lactation.

Higher concentrations of plasma cholesterol were associated with a shorter interval from calving to conception, and with greater probabilities of conception and successful pregnancy by d 150 of lactation, a finding consistent with those of Kappel et al. (1984) and Ruegg et al. (1992). The mechanisms by which cholesterol may influence the fertility of dairy cattle are unclear. Improved fertility for cows with higher concentrations of plasma cholesterol may reflect other aspects of a more positive energy balance, rather than a causal relationship between higher cholesterol and fertility per se. However, Rabiee and Lean (2000) found that uptake of glucose and cholesterol by the ovary are strongly correlated in sheep and cattle. Both of these metabolites are vital to ovarian function and may provide evidence of a mechanism whereby a negative nutrient balance can influence ovarian metabolism. In vitro studies also showed a regulatory role for blood cholesterol concentrations in steroid production by ovarian tissue (Gwynne and Strauss, 1982).

Higher concentrations of plasma cholesterol may also reflect less severe hepatic lipidosis and improved hepatic function. Concentrations of plasma cholesterol and lipoproteins lipids are highly correlated (Van den Top et al., 1995), and higher concentrations of lipoprotein lipids were associated with less lipid infiltration of liver (Rayssiguer et al., 1988). Hepatic lipidosis during early lactation has been associated with reduced reproductive efficiency and an increased incidence of metabolic and health disorders (Reid and Roberts, 1983). Higher serum concentrations of NEFA lowered the probability of conception by d 150, a finding that may reflect excessive mobilization of body fat reserves and consequent perturbation of hepatic function and hormonal metabolism.

Effect of Milk Production, DMI, BW, and BCS

Higher production of FCM during early lactation was associated with a longer interval from calving to first ovulation and with a reduced likelihood of expression of estrus and successful pregnancy by d 150. Cows with higher DMI had a greater probability of expression of estrus at first ovulation, and improved likelihood of pregnancy by d 150 of lactation. DMI and milk yield are major determinants of energy balance, but also reflect the ingestion and loss of other nutrients. It is probable that protein and mineral balances also influence fertility; hence, the term nutrient balance should be preferred to energy balance in general discussion of the influence of nutrition on fertility.

Mechanisms by which more severe nutrient deficits may affect reproductive performance require further definition. Follicular development begins during early lactation when nutrient balance is lowest. It has been hypothesized that metabolic conditions associated with NEB may perturb ovarian follicular development and reduce oocyte quality (Britt, 1992) and lower concentrations of plasma progesterone (Villa-Godoy et al., 1988; Spicer et al., 1990; Britt, 1992; Ljøkjel et al., 1995), possibly reflecting reduced luteal viability, impaired synthesis of progesterone, or altered clearance of progesterone by the liver. Quantitative studies of nutrient balance, ovarian and hepatic flux of progesterone, and fertility in dairy cows are required to elucidate these relationships.

Effect of Season of Calving

Winter-calved cows had a significant delay in time to first ovulation compared with those that calved during other seasons. These findings are consistent with previous studies (Bulman and Lamming, 1978; Montgomery et al., 1980; Peters and Riley, 1982; Eldon and Olafsson, 1986). Inconsistent management practices, particularly with regard to nutrition, have confounded some studies investigating the relationship between season and time to first ovulation. In this study, rations did not change significantly with season. The mechanisms by which season influenced the resumption of ovarian activity after calving remain unclear, but may reflect photoperiodic effects that have been identified in cattle (Peters and Riley, 1982).

Effect of Interval from Calving to First Ovulation

The negative effects of anestrus on reproductive performance have been reported previously (Thatcher and Wilcox, 1973; Lucy et al., 1992; Senatore et al., 1996). Early resumption of normal ovarian function can modify reproductive outcome because luteal function is improved and concentrations of plasma progesterone increase from first to subsequent estrous cycles after calving (Stevenson and Britt, 1979; Staples et al., 1990). Conception success is positively associated with progesterone concentration in the preceding luteal phase.

The findings in all cases indicated that cows with a shorter interval to ovulation and less metabolic stress, as indicated by lower milk production, lower serum NEFA, or higher DMI, had better reproductive performance. Cows that have successfully adapted to lactation by eating enough, by controlling tissue mobilization or with lower milk yields are more fertile if they also have an early resumption of cyclicity. Univariate studies will not have detected this pattern of response and this response may explain, in part, differences between studies.

CONCLUSIONS

The multivariate survival and logistic regression models used provided useful insights to the mechanisms influencing reproductive behavior in high-producing dairy cows. Interactions between dietary protein degradability and indicators of body tissue mobilization were associated with lower reproductive performance.

FCM production and DMI, factors that reflect energy, protein, and mineral balance, influenced reproductive outcomes. Availability of specific substrates for metabolism and those that are associated with mobilization of body tissue may mediate associations between NEB and fertility previously identified in other studies, because concentrations of serum NEFA and plasma cholesterol significantly predicted reproductive outcomes in this study.

Cows of higher genetic merit in this study had lower estimated energy balances. The inclusion of variables associated with body tissue mobilization in predictive models suggests that continued genetic selection for higher milk yield and greater partitioning of nutrients towards production, at the expense of body tissue reserves, will place dairy cows at greater risk of reproductive failure. The identification and implementation of practical strategies to address these concerns are essential objectives for future management practices.

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REFERENCES

Abe, N., I. J. Lean, A. Rabiee, J. Porter, and C. Graham. 1994. Effects of sodium monensin on reproductive performance of dairy cattle. II. Effects on metabolites in plasma, resumption of ovarian activity and oestrus in lactating cows. Aust. Vet. J. 71:277–282.

Bruckental, I., D. Drori, M. Kaim, H. Lehrer, and Y. Folman. 1989. Effects of source and level of protein on milk yield and reproductive performance of high-producing primiparous and multiparous dairy cows. Anim. Prod. 48:319–329.

Britt, J. H. 1992. Nutrition, weight loss affect reproduction, embryonic death. Feedstuffs. 12, 13, 17.

Bulman, D. C., and G. E. Lamming. 1978. Milk progesterone levels in relation to conception, repeat breeding and factors influencing acyclicity in dairy cows. J. Reprod. Fertil. 54:447–458.

Butler, W. R., and R. D. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci. 72:767–783.

Canfield, R. W., and W. R. Butler. 1990. Energy balance and pulsatile LH secretion in early postpartum dairy cows. Domest. Anim. Endocrinol. 7:323–330.

Canfield, R. W., and W. R. Butler. 1991. Energy balance, first ovulation and the effects of naloxone on LH secretion in early postpartum dairy cows. J. Anim. Sci. 69:740–746.

Edmondson, A. J., I. J. Lean, L. D. Weaver, T. Farver, and G. Webster. 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72:68–78.

Eldon, J., and T. Olafsson. 1986. The postpartum reproductive status of dairy cows in two areas of Iceland. Acta Vet. Scand. 27:421–439.

Eldon, J., T. Olafsson, and T. Thorsteinsson. 1988. The relationship between blood and fertility parameters in postpartum dairy cows. Acta Vet. Scand. 29:393–399.

Folman, Y., H. Neumark, M. Kaim, and W. Kaufmann. 1981. Performance, rumen, and blood metabolites in high yielding cows fed varying protein percents and protected soybean. J. Dairy Sci. 64:759–768.

Fonseca, F. A., J. H. Britt, B. T. McDaniel, J. C. Wilk, and A. H. Rakes. 1983. Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open. J. Dairy Sci. 66:1128–1147.

Garnsworthy, P. C., and J. H. Topps. 1982. The effect of body condition of dairy cows at calving on their food intake and performance when given complete diets. Anim. Prod. 35:113–119.

Gwynne, J. T., and J. F. Strauss. 1982. The role of lipoproteins in steroidogenesis and cholesterol metabolism in steroidogenic glands. Endocr. Rev. 3:299–330.

Haresign, W. 1981. Body condition, milk yield and reproduction. Pages 1–16 in Recent Developments in Ruminant Nutrition, W. Haresign and D.J.A Cole, ed. Butterworths, London, United Kingdom.

Harrison, R. A. 1990. Increased milk yield versus reproductive energy status of high producing dairy cows. J. Dairy Sci. 73:2759–2758.

Herdt, T. H., J. B. Stevens, J. Linn, and V. Larson. 1981. Influence of ration composition and energy balance on blood *B*-hydroxybutyrate (ketone) and plasma glucose concentrations of dairy cows in early lactation. Am. J. Vet. Res. 42:1177–1180.

Hosmer, D. W., and S. Lemeshow. 1989. Pages 63–68 in Applied Logistic Regression. John Wiley & Sons, New York, NY.

Husenicza, G., J. Haraszti, L. Molnár, L. Solti, S. Fekete, K. Ekés, and A. C. Yaro. 1988. Some metabolic characteristics of dairy cows with different postpartum ovarian function. J. Vet. Med. 35:506–515.

Jones, G. P., and P. C. Garnsworthy. 1988. The effects of body condition at calving and dietary protein content on dry matter intake and performance in lactating dairy cows given diets of low energy content. Anim. Prod. 47:321–333.

Jordan, E. R., and L. V. Swanson. 1979. Effect of crude protein on reproductive efficiency, serum total protein, and albumin in the high-producing dairy cow. J. Dairy Sci. 62:58–63.

Kappel, L. C., R. H. Ingraham, E. B. Morgan, L. Zeringue, B. Wilson, and D. K. Babcock. 1984. Relationship between fertility and blood glucose and cholesterol concentrations in Holstein cows. Am. J. Vet. Res. 45:2607–2612.

Laben, R. L., R. Shanks, P. J. Berger, and A. E. Freeman. 1982. Factors affecting milk yield and reproductive performance. J. Dairy Sci. 65:1004–1015.

Lean, I. J., J. C. Galland, and J. L. Scott. 1989. Relationships between fertility, peak milk yields and lactational persistency in dairy cows. Theriogenology 31:1093–1103.

Lean, I. J., T. B. Farver, H. F. Troutt, M. L. Bruss, J. C. Galland, R. L. Baldwin, C. A. Holmberg, and L. D. Weaver. 1992. Time series cross correlation analysis of postparturient relationships among serum metabolites and yield variables in Holstein cows. J. Dairy Sci. 75:1891–1900.

Ljøkel, K., G. Klemetsdal, E. Prestløkken, and E. Ropstad. 1995. The effect of energy balance on ovarian activity in a herd of Norwegian cattle. Acta Vet. Scand. 36:533–542.

Lucy, M. C., C. R. Staples, W. W. Thatcher, P. S. Erickson, R. M. Cleale, J. L. Firkins, J. H. Clark, M. R. Murphy, and B. O. Brodie. 1992. Influence of diet composition, dry matter intake, milk production and energy balance on time of postpartum ovulation and fertility in dairy cows. Anim. Prod. 54:323-331.

Mackle, T. R., C. R. Parr, and A. M. Bryant. 1996. Nitrogen fertiliser effects on milk yield and composition, pasture intake, nitrogen and energy partitioning, and rumen fermentation parameters of dairy cows in early lactation. N. Z. J. Agric. Res. 39:341–356.

McDougall, S. 1994. Postpartum anoestrum in the pasture grazed New Zealand dairy cow. Ph.D. Thesis, Massey Univ., New Zealand.

MacMillan, K. L., I. J. Lean, and C. T. Westwood. 1996. The effects of lactation on the fertility of dairy cows. Aust. Vet. J. 73:141–147.

Montgomery, G. W., G. H. Davis, and G. A. Hurrell. 1980. Interval from calving to first estrus in autumn and spring-calving herds in the same locality. Proc. N.Z. Soc. Anim. Prod. 40:280–284.

Peters, A. R., and G. M. Riley. 1982. Is the cow a seasonal breeder? Br. Vet. J. 138:533–537.

Peters, A. R. 1986. Hormonal control of the bovine oestrus cycle. I. The natural cycle. Br. Vet. J. 142:564–575.

Rabiee, A. R. 1995. Transovarian uptake of metabolites in sheep and cattle. Ph.D. Thesis, Univ. Sydney, Australia.

Rabiee, A. R., and I. J. Lean. 2000. Uptake of glucose and cholesterol by the ovary of sheep and cattle and the influence of arterial LH concentrations. Animal Reprod. Sci. 64:199–209.

Rayssiguier, Y., A. Mazur, E. Gueux, I. M. Reid, C. J. Roberts. 1988. Plasma lipoproteins and fatty liver in dairy cows. Res. Vet. Sci. 45:389–393.

Reid, I. M., and C. J. Roberts. 1983. Subclinical fatty liver in dairy cows. Irish Vet. J. 37:104–110.

Ruegg, P. L., W. J. Goodger, C. A. Holmberg, L. D. Weaver, and E. M. Huffman. 1992. Relation among body condition score, serum urea nitrogen, and cholesterol concentrations, and reproductive performance in high producing Holstein dairy cows in early lactation. J. Am. Med. Assoc. 53:10–14.

Senatore, E. M., W. R. Butler, and P. A. Oltenacu. 1996. Relationships between energy balance and post-partum ovarian activity and fertility in first lactation dairy cows. Anim. Sci. 62:17–23.

Son, J., R. J. Grant, and L. L. Larson. 1996. Effects of tallow and escape protein on lactational and reproductive performance of dairy cows. J. Dairy Sci. 79:822–830.

Spicer, L. J., W. B. Tucker, and G. D. Adams. 1990. Insulin-like growth factor-1 in dairy cows: Relationships among energy balance, body condition, ovarian activity and estrous behavior. J. Dairy Sci. 73:929–937.

Staples, C. R., W. W. Thatcher, and J. H. Clark. 1990. Relationship between ovarian activity and energy status during the early post partum period of high producing dairy cows. J. Dairy Sci. 73:938–947.

Stevenson, J. S., and J. H. Britt. 1979. Relationships among luteinising hormone, oestradiol, progesterone, glucocorticoids, milk yield, body weight and post partum ovarian activity. J. Anim. Sci. 48:570–577.

Thatcher, W. W., and C. J. Wilcox. 1973. Postpartum estrus as an indicator of reproductive status in the dairy cow. J. Dairy Sci. 56:608–610.

Van Den Top, A. M., T. Wensing, M. J. H. Geelen, G. H. Wentink, A. T. Van't Klooster, and A. C. Beynen. 1995. Time trends of plasma lipids and enzymes synthesising hepatic triacylglycerol during postpartum development of fatty liver in dairy cows. J. Dairy Sci. 78:2208–2220.

Veerkamp, R. F., G. Simm, and J. D. Oldham. 1995. Genotype by environment interactions: Experience from Langhill. Pages 59-65 in Breeding and Feeding the High Genetic Merit Dairy Cow. Occ. Publ. No. 19, Br. Soc. Anim. Sci.

Villa-Godoy, A., T. L. Hughes, R. S. Emery, L. T. Chapin, and R. L. Fogwell. 1988. Association between energy balance and luteal function in lactating dairy cows. J. Dairy Sci. 71:1063–1072.

Westwood, C. T., I. J. Lean, J. K. Garvin, and P. Wynn. 2000. Effects of genetic merit and varying dietary protein degradability on lactating dairy cows. J. Dairy Sci. 83:2926–2940.

Williamson, N. B., and E. Fernandez-Baca. 1992. The role of dietary protein and energy in dairy herd fertility. Pages 39–48 in Proc. Soc. Dairy Cattle Vet. N. Z. Vet. Assoc.

Zivin, J. A., and J. F. Snarr. 1973. An automated colorimetric method for the measurement of 3-hydroxybutyrate concentration. Anal. Biochem. 52:456–461.