Predicting Cholesterol, Progesterone, and Days to Ovulation Using Postpartum Metabolic and Endocrine Measures¹

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ABSTRACT

The objective of this study was to examine relationships among metabolic and endocrine factors that may influence ovarian activity during early lactation. Holstein cows ($n = 19$) were bled twice each week to determine plasma concentrations of insulin (INS), glucose, cholesterol, insulin-like growth factor-1 (IGF-I), and progesterone (P_4). Feed intake and milk production were recorded daily while body weights and milk composition were recorded weekly. Relationships among plasma cholesterol and P_4 , and days to first and second postpartum ovulation were modeled with energy balance (EB), dry matter intake, milk yield and composition, plasma metabolites, and hormones using the backward elimination technique of multivariate regression analysis. Variables that contributed the most to predicting plasma cholesterol concentrations were dry matter intake $\times$ SNF using model 1 (production variables) and the square of glucose (i.e., glucose²) using model 3 (plasma hormones and metabolites). For plasma P_4 concentrations, EB (model 2, production variables) and IGF-I (model 3, plasma hormones and metabolites) were the major predictors. The production variables EB and percentage of milk lactose were the greatest contributors to the models predicting days to first and second postpartum ovulations, respectively. Of the plasma hormones and metabolites evaluated, IGF-I² was the most significant predictor of days to first postpartum ovulation, whereas glucose² and INS were the significant predictors of days to second postpartum ovulation. Plasma IGF-I, glucose, and INS have been implicated in ovarian functions and their significant contributions to these models are consistent with possible important roles in postpartum return to ovarian competence.

(**Key words:** energy balance, metabolism, lactation, reproduction)

Abbreviation key: EB = energy balance, FAT = milk fat percentage, P_4 = progesterone.

INTRODUCTION

The return to ovarian function of early postpartum cows requires optimal conditions for several metabolic and endocrine factors. Factors related to early postpartum ovarian competence include proposed relationships between energy balance (EB) and postpartum interval to first ovulation (Butler and Smith, 1989; Spicer et al., 1990). Variables that are significantly correlated with each other include milk yield with days to first postpartum ovulation (Stevenson et al., 1983; Nebel and McGilliard, 1993), plasma cholesterol with plasma progesterone (P_4), conception rate, and number of recoverable embryos (Kweon et al., 1986; Grummer and Carroll, 1988; Spicer et al., 1993b), plasma P_4 concentration with days open and pregnancy rate (Folman et al., 1973; Villa-Godoy et al., 1988), plasma IGF-I with ovarian function (Lucy et al., 1992; Spicer et al., 1993b; Beam and Butler, 1997), and plasma glucose with pulsatility of LH (Butler and Smith, 1989; Canfield and Butler, 1990). Simple correlation coefficients (r) among some of these variables are significant but range from $r = 0.06$ to 0.64 (Villa-Godoy et al., 1988; Spicer et al., 1990; Lucy et al., 1992).

Because ovarian activity is not solely influenced by any single condition or metabolite, it is necessary to integrate the relationships among various metabolic and endocrine factors. So far, few modeling studies have attempted to compare production variables with metabolic and endocrine variables of lactating dairy cows to predict postpartum ovarian competence. Stevenson and Britt (1980) utilized changes in concentrations of estradiol, LH, glucocorticoids, number of ovarian follicles, milk yield, energy intake, BW, and cross products of these variables between wk 1 and 2 postpartum to model first postpartum ovulation. The nine-variable model predicting days to first ovulation shows change

Received August 17, 2002.

Accepted April 9, 2003.

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¹This work was approved for publication by the director, Oklahoma Agricultural Experiment Station and supported by Agtech, Inc., Waukesha, WI.

and cross product of estradiol concentrations, and number of follicles as the most important factors. The scope of Stevenson and Britt (1980) model is limited because variables were measured for only a period of 14 d postpartum, thus it may not predict cows with >14 d to first ovulation. Because most dairy breeders do not routinely breed cows at first ovulation, measurement of variables over a longer period to cover second and third postpartum ovulations would be preferable. Another model (Heuer et al., 2000) predicted average herd mean EB in early lactating cows using various dietary inputs, body condition score, milk components (fat, protein, lactose), and beta-hydroxy-butyrate but did not include any endocrine variables. In this later study, fat-to-protein ratio, milk fat and milk protein concentrations substantially explained the variability in EB. In the past few years, several metabolic and endocrine modulators have been identified that affect reproductive function including IGF-I, insulin, glucose, cholesterol and P_4 (Spicer and Echternkamp, 1995; Butler, 2000). Thus, it is necessary to expand the previous models to include those reproductive modulators to delineate their contributions in predicting postpartum reproductive competence of early lactating dairy cows.

Plasma cholesterol concentrations increase between calving and wk 6 postpartum in dairy cows (Carroll et al., 1990; Spicer et al., 1993b; Francisco et al., 2002), and are correlated with plasma P_4 (Spicer et al., 1993b; Francisco et al., 2002), conception rates and number of recoverable embryos (Kweon et al., 1986; Grummer and Carroll, 1988). The association of increased plasma cholesterol concentration with increased luteal-phase P_4 secretion in early lactating dairy cows (Spicer et al., 1993b) merits further investigation. Understanding what production and hormonal factors contribute to variation in plasma cholesterol may lead to insights that may help improve reproductive efficiency in dairy cattle. Because measurement of blood cholesterol has become economical and automated via quick tests (e.g., Johnson and Johnson Advanced Cholesterol kit, BioScience 2000, CholesTrack) for assessment of health and metabolic status of humans (Koda-Kimble and Young, 1988; Lloyd, 1991; Stehbens, 2001), perhaps this practice can be extended in dairy cows to assess metabolic and/or reproductive capacity postpartum. However, further research is needed to establish the relative importance of a given metabolite for such a use. Therefore, the goal of this study was to examine relationships among production variables, and metabolic and endocrine variables that may affect ovarian function, and ultimately identify key contributors (via modeling) that may regulate plasma cholesterol, P_4 , and days to first and second postpartum ovulation in early lactating dairy cows.

MATERIALS AND METHODS

Data collection was previously described in another study (Francisco et al., 2002). Briefly, data were collected from 19 pluriparous cows from 1 to 12 wk postpartum. Cows ($n = 19$) were individually fed a TMR consisting of sorghum silage (31.9%), alfalfa hay (21.2%), cottonseed (8.0%), and concentrate (38.9%). Nine cows were supplemented with propionibacteria (17 g/d). The TMR was sampled weekly and composited monthly for analyses. Feed intake and milk production were recorded daily, whereas BW were recorded weekly. Milk was collected twice daily (0300 and 1500 h) and analyzed weekly. Blood was collected twice each week via coccygeal venipuncture, plasma harvested and assayed for plasma concentrations of P_4 , glucose, insulin, cholesterol, and IGF-I. Weekly EB was calculated using net energy of intake, for maintenance, and that secreted in milk as described previously (Francisco et al., 2002).

Multiple regression models were developed to predict plasma cholesterol and P_4 concentrations and days to first and second postpartum ovulations as indicators of metabolic and reproductive status of the cows, respectively. The basic model used to obtain the relationships of the observed variables was:

$$\text{Log } Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_k X_k + \varepsilon_i$$

where Y_i represents a dependent variable (either plasma cholesterol or plasma P_4 concentrations), β_0 represents the intercept, β_1 and β_2 are the true regressions for observed X , k is the number of independent variables, and ε_i is the random error associated with the i th observation.

All variables in the model were expressed as weekly means from wk 1 to 12 postpartum. Data from control ($n = 10$) and treated ($n = 9$; supplemented with Propionibacteria) cows were pooled because most of the variables were not found to be significantly different between treatments (see Francisco et al., 2002). Variables in the model included all the possible combinations of cross products and squares of all the variables except week postpartum. The first group of variables included the "production" variables EB, BW, DMI, FCM, and percentage of milk fat, milk protein, lactose, and SNF. The second set of variables incorporated the metabolic and hormonal variables, plasma INS, glucose, and IGF-I concentrations. These two sets of variables were used separately in obtaining the "best" model for predicting plasma cholesterol (models 1 and 3) and P_4 (models 2 and 4) concentrations during the first 12 wk postpartum. The weekly means $\pm$ SD ($n = 228$) of the various variables used in the models for predicting plasma cholesterol and P_4 concentrations are summarized in Table

Table 1. Mean, SD of the variables used in predicting plasma cholesterol and progesterone (P₄) in lactating dairy cows (models 1 to 4).

Variable	Models 1 and 3 ¹		Model 5 ²		Model 6 ³	
	Mean	SD	Mean	SD	Mean	SD
Energy balance, Mcal/d	-0.6	6.2	-4.1	3.4	-3.4	3.5
BW, kg	641.8	56.9	646.5	56	649.6	54.5
DMI, kg/d	23.7	4.0	21.4	2.6	22.2	1.7
FCM, kg/d	34.8	6.7	33.8	3.9	34.9	5.0
Milk lactose (LACT), %	5.0	0.2	4.9	0.1	5.0	0.1
Milk fat (FAT), %	3.1	0.8	3.3	0.5	3.3	0.8
Milk protein (PROT), %	3.2	0.6	3.5	0.3	3.4	0.3
SNF, %	8.9	0.6	9.2	0.4	9.2	0.4
Variable	Models 2 and 4 ¹		Model 7 ²		Model 8 ³	
	Mean	SD	Mean	SD	Mean	SD
Cholesterol, mg/dl	177.6	53.5	132.8	22.6	148.0	22.1
Progesterone (P ₄), ng/ml	1.1	1.3	—	—	—	—
IGF-I, ng/ml	106.0	60.0	78.0	39.0	98.0	41.0
Insulin (INS), ng/ml	0.4	0.2	0.3	0.1	0.4	0.1
Glucose, mg/dl	60.1	5.0	59.6	6.0	60.8	1.8

¹Means based on n = 228.²Means based on n = 15.³Means based on n = 11.

1. Plasma cholesterol and P₄ values were log transformed because it increased the coefficient of determination (R²). To determine the impact of week postpartum in predicting cholesterol (models 1 and 3) and P₄ (models 2 and 4) concentrations, it was included as one of the variables in one group of models and not included in another group of models.

The same datasets were used to predict days to first (models 5 and 7) and second (models 6 and 8) postpartum ovulations as above without cross products and squares of the variables. In the models using metabolic and hormonal variables (models 7 and 8), cross products and plasma P₄ were not included in the models because P₄ concentrations were used to determine if and when the cows had ovulated postpartum. Based on P₄ concentrations, there were 15 and 11 cows that showed first and second postpartum ovulations, respectively. Weekly means of the variables from 1 to 5 wk (n = 75) and 1 to 7 wk (n = 77) postpartum were averaged and used to generate the models for days to first and second (Table 1) postpartum ovulation, respectively, because the intervals to first and second postpartum ovulations averaged 5 and 9 wk, respectively.

The backward elimination technique of the multiple regression procedure (SAS, 1988) was used to obtain the models used in the study. Initially, the backward regression technique includes all the variables in the first model, calculating an R² for each variable. This is followed by removal of the least significant variable with the lowest R² contribution to the model, thereby producing the second model, and so forth. Only the variables that remained significant at the *P* < 0.05 or 0.01 during each permutation were included in the final

model unless stated otherwise. Models were selected based on high R², small residual variations between predicted and observed values, and a low number of variables included in the model.

RESULTS

Model Selection

Models for plasma cholesterol and P₄. Models 1 and 2 were generated using production variables (Table 1). The model that “best” predicted plasma cholesterol concentrations (model 1) contained eight variables. All of the variables in model 1 were significant at *P* < 0.01 and had a total R² = 0.685. The relative contribution of each variable, as indicated by partial R² (Table 2), showed that the interaction of DMI × SNF is the most important component in the model contributing 63% (R² = 0.433). This is followed by FCM × SNF (R² = 0.192), which explained 28% of the composite model R² (Table 2). There were other significant variables (i.e., FCM × LACT, BW × LACT, FCM², FAT², FCM × FAT, and BW) in the model but they each contributed less than 5% of the total R² (data not shown).

The “best” fit model for plasma P₄ concentrations (model 2) included three production variables all significant at *P* < 0.05 (Table 2), and had a lower R² = 0.291 compared with model 1 (plasma cholesterol). Two more variables (i.e., DMI × FAT and FCM × FAT) were significant but contributed less than 5% to the total R² of model 2. The difference in R² between models 1 and 2 indicates that the variables that predicted plasma cholesterol explained more of the variation than the model that predicted plasma P₄ (Table 2).

Table 2. Variables and proportion of R^2 in the model predicting plasma cholesterol and progesterone using weekly measurements ($n = 228$) of production variables, hormones and metabolites in lactating dairy cows.

Model	Variables ¹	Partial R^2	% Contribution to the model	Total R^2
1. Plasma cholesterol model				0.68
	DMI $\times$ SNF	0.43**	63.2	
	FCM $\times$ SNF	0.19**	28.1	
2. Plasma progesterone model				0.29
	EB	0.18**	61.1	
	EB $\times$ FAT	0.05**	17.1	
	FCM $\times$ LACT	0.04**	14.2	
3. Plasma CHOL model				0.37
	GLU ²	0.20**	55.0	
	IGF-I	0.07**	18.9	
	IGF-I $\times$ GLU	0.04**	11.8	
	IGF-I $\times$ INS	0.03**	8.8	
	INS	0.02**	5.5	
4. Plasma progesterone model				0.33
	IGF-I	0.25**	74.2	
	GLU ²	0.07**	21.8	

¹Variables included are only those that contributed $> 5\%$ of the total R^2 . EB = energy balance, GLU = glucose, INS = insulin.

** $P < 0.01$.

Models 3 and 4 included only three variables (plasma insulin, glucose, and IGF-I) and their cross products. The models for plasma cholesterol (model 3) and plasma P_4 (model 4) have similar R^2 values (Table 2) when based on this combination of mean weekly measurements of hormones and metabolites (Table 1). Model 3 indicates that glucose² contributes over half (55.0%) to the total R^2 indicating that it is the most important variable in predicting plasma cholesterol concentrations (Table 2). This is followed by IGF-I (18.9%) and

IGF-I $\times$ glucose (11.8%). The rest of the significant variables (i.e., IGF-I $\times$ INS and INS) each contributed less than 10% to total R^2 (Table 2). The R^2 of the model predicting plasma P_4 (model 4) was 0.3319 of which plasma IGF-I and glucose² contributed 74.2 and 21.8%, respectively (Table 2). The only other significant variable was IGF-I² (4.0%).

Week postpartum, when included in models 1 to 4, had the highest contribution to the total R^2 of the models, ranging from 63 to 88% (Table 3). In model 1

Table 3. Variables, R^2 and percent contribution of week postpartum when included in plasma cholesterol and progesterone models (models 1 to 4) using weekly measurements of hormonal, metabolic, and production variables in lactating dairy cows.

	Partial R^2	% Contribution	Total R^2
Production variables ¹			
Model 1, plasma cholesterol model			
Week	0.52**	71.6	0.732
EB $\times$ FAT	0.09	12.5	
FCM $\times$ LACT	0.08	10.3	
Model 2, plasma progesterone model			
Week	0.25**	67.9	0.369
BW $\times$ SNF	0.02	8.1	
EB $\times$ LACT	0.03	8.0	
Hormones and metabolic variables			
Model 3, plasma cholesterol model			
Week	0.53**	88.2	0.595
GLU ²	0.05	7.6	
Model 4, plasma progesterone model			
Week	0.26**	62.9	0.410
INS $\times$ GLU	0.12**	28.0	
IGF-I	0.03**	8.1	

¹Variables included are only those that contributed $\geq 5\%$ of the total R^2 . EB = energy balance, GLU = glucose, INS = insulin.

** $P < 0.01$.

Table 4. Variables, regression coefficients and proportion of R^2 in the model predicting days to first ($n = 15$) and second ($n = 11$) postpartum ovulation using the average of weekly measurements of production variables (wk 1 to 5, model 5; wk 1 to 7, model 6) and hormones and metabolites (wk 1 to 5, model 7; wk 1 to 7, model 8) in lactating dairy cows.

Variables ¹	Regression coefficients	SE	Partial R^2	Partial R^2	Total R^2
				(%)	
Model 5, first postpartum ovulation model					0.350
EB	-5.85	3.11	0.209*	59.78	
DMI	5.80	2.39	0.093*	26.57	
FCM	4.99	2.44	0.048*	13.67	
Model 6, second postpartum ovulation model					0.759
LACT	-51.87	13.02	0.434**	57.24	
FCM	-1.17	0.46	0.224*	29.49	
DMI	3.42	1.21	0.101*	13.28	
Model 7, first postpartum ovulation model					0.282
IGF-I	-0.14	0.063	0.282**	100.0	
Model 8, second postpartum ovulation model					0.318
GLU ²	68.64	40.94	0.23*	72.7	
INS	-0.025	0.015	0.09*	27.3	

** $P < 0.01$ for Models 5 and 6; $P < 0.05$ for Models 7 and 8.

* $P < 0.05$ for Models 5 and 6; $P < 0.10$ for Models 7 and 8.

¹EB = energy balance, GLU = glucose, INS = insulin.

(plasma cholesterol), week postpartum contributed 71.6% to model R^2 , whereas $EB \times FAT$ and $FCM \times FAT$ contributed a total of 22.8% (Table 3). Percent contribution of variables that both appeared in model 1 without and with week postpartum vary such as $DMI \times SNF$ (63.2 vs. 2.1%), $FCM \times SNF$ (28.1 vs. 2.1%), and $FCM \times LACT$ (2.7 vs. 10.3%). The other significant variables that contributed less than 5% of the model R^2 were IGF-I and $IGF-I \times glucose$. In model 2 (plasma P_4), week postpartum contributed 67.9% of total R^2 (Table 3). The variable EB appeared in model 2 with and without week postpartum but at different contributions to the total model R^2 (3.4 vs. 61.1%). Other variables (i.e., $PROT^2$, EB^2 , $EB \times FCM$, LACT, $EB \times DMI$, $PROT$, and SNF) contributed significantly but less than 5% of the total model R^2 . Week postpartum, when included in model 3 (plasma cholesterol) contributed 88.2% of the total R^2 (Table 3). The common variables of model 3 with (Table 3) and without (Table 2) week postpartum were plasma IGF-I (3.6 vs. 18.9%), glucose² (7.6 vs. 55.0%) and $IGF-I \times glucose$ (0.2 vs. 11.8%). When included in model 4 (plasma P_4), week postpartum contributed 62.9% of the total R^2 (Table 3). Variables common in model 4 (plasma cholesterol) with (Table 3) and without (Table 2) week postpartum was plasma IGF-I concentration contributing 8.1 and 74.2%, respectively. Plasma INS concentration was a significant variable in model 4, but it contributed less than 5% of total R^2 .

Models for days to first and second postpartum ovulation. Models 5 and 6 predicted days to first and second postpartum ovulation, respectively, using the average weekly means (wk 1 to 5, model 5 and wk 1 to 7, model 6) of production variables (Table 1). Model 5

showed that EB had the highest partial R^2 (0.21) while for model 6, percentage of milk LACT generated a partial R^2 of 0.43 (Table 4). Model 6 had a higher total R^2 compared with model 5 (0.76 vs. 0.35) having the same number of variables (Table 4). Both models had FCM and DMI as common variables.

models 7 and 8 were derived using the average of weekly means (wk 1 to 5, model 7 and wk 1 to 7, model 8) of plasma hormones and metabolites (Table 1) to predict days to first and second postpartum ovulation, respectively (Table 4). The square of plasma IGF-I concentrations was the only significant predictor for days to first postpartum ovulation (model 7), while days to second postpartum ovulation (model 8) showed that plasma INS and the square of plasma glucose concentrations contributed 75 and 25%, respectively, to the total R^2 (Table 4). All other variables were not significant.

If days to first and second postpartum ovulation were modeled on a weekly basis, rather than using means of wk 1 to 5 or wk 1 to 7, the production variables that influenced first postpartum ovulation were EB, FCM, and percentage of milk protein (Table 5). Plasma hormones and metabolites that influenced first postpartum ovulation were plasma cholesterol concentration that successively appeared from 2 to 5 wk postpartum and plasma IGF-I concentration that emerged 3 times (Table 6). The production variables that influenced second postpartum ovulation were FCM, DMI and percentage of milk lactose (Table 5). In the model of plasma hormones and metabolites, plasma glucose concentration emerged in 5 of 7 wk as a significant variable that influenced second postpartum ovulation (Table 6). In

Table 5. Variables and R^2 of the models predicting days to first and second postpartum ovulation in lactating dairy cows using measurements of production variables on a weekly postpartum basis.¹

Week	Production and milk component variables ²				R ²
First postpartum ovulation					
1	EB (0.15) ³				0.161
2	FAT (0.06)	PROT (0.10)			0.336
3	FCM (0.05)	PROT (0.10)	LACT (0.07)	SNF (0.12)	0.559
4	FCM (0.15)	EB (0.12)			0.251
Second postpartum ovulation					
1	FCM (0.03)	DMI (0.02)	EB (0.02)	SNF (0.04)	0.712
2	LACT (0.05)				0.391
3	LACT (0.01)				0.571
4	EB (0.01)	PROT (0.02)	SNF (0.01)		0.869
5	FCM (0.09)	DMI (0.04)			0.439
6	FCM (0.06)	LACT (0.03)	BW (0.12)	FAT (0.13)	0.652
7	FCM (0.01)	DMI (0.01)	PROT (0.01)		0.964

¹Multiple regression using backward elimination technique of variables that contributed to R^2 at $P < 0.15$.

²EB = energy balance; FAT = percentage of milk fat; PROT = percentage of milk protein; FCM = fat-corrected milk; LACT = percentage of milk lactose.

³Level of significance (P -value) for the variables that contribute at $P \leq 0.15$ to the weekly model.

several cases (Table 6, wk 4 to 7), addition of the squared values (e.g., INS^2) provided a significantly greater fit to the model than with only the linear term (e.g., INS), indicating a nonlinear relationship.

Model Prediction

Models for plasma cholesterol and P_4 . Observed plasma cholesterol concentrations and predicted values using models 1 (production variables) and 3 (plasma hormones and metabolites) are plotted by week postpartum in Figure 1 and reflects only those variables that contributed greater than 5% of the total R^2 . Predicted plasma cholesterol values derived using model 1 were more similar to observed values than were cholesterol values predicted using model 3. The weekly postpartum residuals between observed plasma cholesterol concentration and predicted values ranged from -1.0 to 0.89 (model 1, production variables) and -0.97 to 0.86 (model 3, plasma hormones and metabolites). With a similar range of residuals but higher R^2 for model 1 ($R^2 = 0.68$) vs. model 3 ($R^2 = 0.37$), model 1 was better than model 3 in predicting plasma cholesterol concentrations. Generally, the residuals between observed and predicted values using the regression coefficients of models 1 (production variables) and 3 (plasma hormones and metabolites) for predicting plasma cholesterol concentrations,

congregate within -0.5 to 0.5 except for some outliers (Figure 2).

Observed plasma P_4 concentrations and predicted values derived using models 2 (production variables) and 4 (plasma hormones and metabolites) are plotted in Figure 3. Observed values and predicted values of plasma P_4 concentrations using models 2 and 4 were similar starting from 4 wk postpartum. Residuals between observed plasma P_4 concentrations and predicted values ranged from -3.5 to 3.5 (model 2, production variables) and -3.4 to 3.8 (model 4, plasma hormones and metabolites). Even though the residuals are slightly less dispersed in model 2 than in model 4, models 2 and 4 are comparable in predicting plasma P_4 concentration as indicated by similar R^2 (Figure 3 and Table 1). Generally, the residuals for models 2 (production variables) and 4 (plasma hormones and metabolites) (Figure 4) used for predicting plasma P_4 concentrations fall between -2 to 2 , a much wider range than residuals obtained from models 1 and 3 used for predicting plasma cholesterol (Figure 2). The lower and upper diagonal borders of Figure 4 are likely due to the nature of the plasma P_4 data (i.e., 89 values were < 0.10 ng/ml).

Models for days to first and second ovulation. The residuals between observed and predicted days to first postpartum ovulation ranged from -21 to 32 d for

Table 6. Variables and R² of the models predicting days to first and second postpartum ovulation in lactating dairy cows using measurements of plasma hormones and metabolites on a weekly postpartum basis.¹

Week	Plasma hormones and metabolic variables ²						R ²
First postpartum ovulation							
1	INS (0.12) ³	GLU (0.03)	GLU ² (0.03)				0.379
2	IGF-I (0.03)	CHOL ² (0.01)	GLU ² (0.03)				0.642
3	IGF-I (0.04)	CHOL (0.14)					0.412
4	CHOL (0.001)	INS (0.001)	GLU (0.02)	CHOL ² (0.001)	INS ² (0.001)	GLU ² (0.02)	0.911
5	CHOL (0.07)	IGF-I (0.02)	CHOL ² (0.07)				0.563
Second postpartum ovulation							
1	INS (0.03)	INS ² (0.03)	GLU ² (0.11)				0.517
2	IGF (0.11)	IGF ² (0.08)					0.149
3	GLU (0.02)						0.471
4	CHOL (0.02)	INS (0.02)	CHOL ² (0.02)	INS ² (0.03)			0.711
5	GLU (0.03)	GLU ² (0.03)					0.486
6	GLU (0.05)	GLU ² (0.05)					0.391
7	CHOL (0.03)	GLU (0.01)	CHOL ² (0.03)	INS ² (0.01)	GLU ² (0.01)		0.822

^aMultiple regression using backward elimination technique of variables that contributed to R² at *P* < 0.15.
²INS = plasma insulin; GLU = plasma glucose; IGF-I = plasma insulin-like growth factor-1; CHOL = plasma cholesterol.
³Level of significance (*P*-value) for the variables that contributed at *P* ≤ 0.15 to the weekly model.

model 5 (production variables) and –22 to 36 d for model 7 (plasma hormones and metabolites, Figure 5). Because the dispersion of the residuals for the two models is equivalent and the R² of model 5 is greater than that of model 7, model 5 (production variables) appears

Figure 1. Predicted weekly means (*n* = 228) of log cholesterol with week postpartum using models 1 (production variables) and 3 (plasma hormones and metabolites) compared with actual observed values. SD = model 1 (0.26), model 3 (0.13), and Observed (0.18).

slightly better predictor of days to first postpartum ovulation than model 7 (plasma hormones and metabolites).

The difference between predicted and observed values for days to second postpartum ovulation ranged from –6 to 11 d for model 6 (production variables) and –15 to 9 d for model 8 (plasma hormones and metabolites) (Figure 5). Because the dispersion of the residuals for model 6 is less than model 8 and the R² of model 6

Figure 2. Plot of residuals of observed-predicted log cholesterol vs. predicted log cholesterol values using regression coefficients of model 1 (production variables) and model 3 (plasma hormones and metabolites).

Figure 3. Predicted weekly means ($n = 228$) of log progesterone with week postpartum using models 2 (production variables) and 4 (plasma hormones and metabolites) compared with actual observed values. SD = Model 2 (0.79), model 4 (0.69), and Observed (1.12).

is over threefold that of model 8, days to second postpartum ovulation is best predicted by model 6 (production variables).

DISCUSSION

Prediction of plasma cholesterol levels was better using model 1, based on production and milk composition variables, than using model 3, based on endocrine and metabolic variables. This implies that DMI and milk production and its components are more important factors driving changes in plasma cholesterol than are hormones and metabolites. In model 1, the product of SNF with DMI and FCM accounted for a significant percentage of the variation in plasma cholesterol but those variables appeared to be less important when week postpartum was included in the model. This is not surprising because during early postpartum the increase in plasma cholesterol concentrations coincides with the increase in DMI and FCM with week postpar-

Figure 4. Plot of residuals of observed-predicted log progesterone vs. predicted log progesterone values using model coefficients of model 2 (production variables) and model 4 (plasma hormones and metabolites).

Figure 5. Plot of residuals of observed-predicted days to first postpartum ovulation versus predicted days to first ($n = 15$) and second ($n = 11$) postpartum ovulation values using regression coefficients of models 5 and 6 (production variables) and models 7 and 8 (plasma hormone and metabolites).

tum (Carroll et al., 1990; Spicer et al., 1993b; Francisco et al., 2002). In addition, the percentage of SNF in milk decreases with increasing milk production during the early postpartum period (McDonald et al., 1995; Francisco et al., 2002). These interactions in model 1 (i.e., $\text{DMI} \times \text{SNF}$, $\text{FCM} \times \text{SNF}$) indicate that the response of plasma cholesterol may be dependent on DMI and FCM but this dependence changes depending on the level of SNF. Previously, plasma cholesterol concentrations were consistently important in predicting nutritional status of lactating and nonlactating dairy cows (Kronfeld et al., 1982), and the fact that cross products that included DMI and EB were significant contributors to variation in plasma cholesterol levels in model 1 of the present study support the results of Kronfeld et al. (1982). In model 3, the variables that significantly contributed to variation in plasma cholesterol concentration were plasma glucose, INS, and IGF-I concentrations. This is consistent with reports that plasma INS and IGF-I concentrations increase concomitantly with plasma cholesterol during early lactation (Carroll et al., 1990; Spicer et al., 1993b; Francisco et al., 2002). Model 3 was even more precise in predicting plasma cholesterol when the week postpartum was included along with plasma hormone and metabolite variables. Previous studies have documented the importance of cholesterol as a precursor of ovarian steroidogenesis (Kronfeld et al., 1980; Grummer and Carroll, 1988; Spicer et al., 1993a) and thus understanding the variables that contribute to changes in plasma cholesterol concentrations

may help understand factors that may contribute to reproductive success in postpartum dairy cows.

Models 2 and 4 appeared to be equivalent at predicting plasma P_4 concentrations using production variables and plasma hormones and metabolites, respectively, because of their similar R^2 and range of residuals. In model 2, weekly EB accounted for about 18% of the variation in plasma P_4 and was the most important predictor of the model. Energy balance had been shown in several studies to modulate plasma P_4 concentrations during early postpartum (Villa-Godoy et al., 1988; Spicer et al., 1990, 1993b). Concentrations of P_4 have been associated positively with fertility (Folman et al., 1973) and pregnancy rates (Sklan et al., 1991) and negatively with EB (Villa-Godoy et al., 1988; Spicer et al., 1990, 1993b) and days open (Sklan et al., 1991). In addition, a negative EB reduces the weight of corpus luteum (Apgar et al., 1975) and decreases steroidogenic activity of luteal tissue (Villa-Godoy et al., 1990). Thus, the weekly energy status of the cows measured as the difference between net energy intake and energy expended for lactation plus maintenance in the present study, served as a good predictor of return to normal ovarian activity as measured by P_4 concentrations. In model 4, IGF-I accounted for 25% of the variation in plasma P_4 and served as the best predictor of plasma P_4 levels in early postpartum cows. Plasma IGF-I concentrations increase with week postpartum as does plasma P_4 concentrations (Abribat et al., 1990; Spicer et al., 1990) and are positively correlated with energy status and P_4 production in early postpartum cows (Spicer et al., 1990, 1993b; Lucy et al., 1992). Also, IGF-I stimulates bovine luteal cell P_4 production (Sauerwein et al., 1992; Liebermann et al., 1996). Thus, it is not surprising that IGF-I is a good predictor of plasma P_4 concentrations in early postpartum cows. Values predicted by both models 2 and 4 closely matched observed plasma P_4 concentrations after wk 4 of lactation suggesting that both models were more precise from 4 to 12 wk postpartum period. Because the dispersion of the residuals for model 2 were slightly less than for model 4, but R^2 of model 4 was slightly greater than that of model 2, both models appear similar in their ability to predict plasma P_4 concentrations. Thus, plasma P_4 concentration could be predicted as well by EB as by plasma IGF-I concentrations, and further imply that IGF-I may in part mediate the effect of EB on luteal function as previously suggested (Spicer et al., 1990). The cholesterol models were more accurate models (i.e., lower residuals and greater R^2) than the P_4 models, probably because plasma cholesterol concentrations steadily increase with week postpartum and then plateau after 8 wk postpartum compared with plasma P_4 concentrations that start low initially and then increase

and decrease in a cyclic pattern after the first postpartum ovulation. Adding week to the model marginally increased the R^2 in the P_4 models (models 2 and 4) compared with a large effect of week in predicting cholesterol concentrations (models 1 and 3) associated with the more consistent increase in plasma cholesterol as the postpartum period progressed.

Week postpartum when included in the model was the best single predictor of plasma P_4 and cholesterol in models 1 to 4. The main predictors of the models without week postpartum became secondary predictors when week postpartum was in the model. For instance, in model 3 (plasma cholesterol), the variable glucose² largely predicted the model (55% of total R^2) without week postpartum but accounted for only 7.6% of total R^2 when week postpartum was included in the model. Also, most variables that correlate to the number of days or weeks postpartum were normally overshadowed by week postpartum. For example, IGF-I accounted for 74.2% of the variability of the P_4 model (model 3) without week postpartum but only 8.1% when week postpartum was included in the model. However, running the models without week helped to determine which production, endocrine, and metabolic variables contributed more to each model independent of postpartum intervals.

Predicting days to first postpartum ovulation using model 5 (production variables) generated a higher R^2 (0.35) compared to model 7 (0.28) (plasma hormones and metabolites) although model 7 contained only 1 variable. The production variable in model 5 that "best" predicted days to first postpartum ovulation was EB, but DMI and FCM were also significant contributors of the model. Models predicting first postpartum ovulation on a weekly basis also gave EB as one of the most influential variables (significant in 2 of 4 wk modeled) but FCM was also significant in 2 of 4 wk modeled. The small sample size (i.e., $n = 11$) may have contributed to the variation in the weekly models. This model supports the findings of others that milk production and DMI and hence EB during the early postpartum period is a critical factor to the return to reproductive cyclicity (Butler and Smith, 1989; Spicer et al., 1993b; Staples et al., 1998).

In model 7, the squared term of plasma IGF-I accounted for the most variation in days to first postpartum ovulation. Because plasma IGF-I concentrations steadily increase with week postpartum, plasma IGF-I may reach a certain concentration that may serve as a signal that first postpartum ovulation may occur. Generally, plasma IGF-I peaks around 7 to 8 wk postpartum long after which the first postpartum ovulation normally takes place (Abribat et al., 1990; Spicer et al., 1990; Francisco et al., 2002). Furthermore, IGF-I is

positively correlated with P_4 (as mentioned earlier), which is necessary to maintain pregnancy after ovulation. On a weekly basis, models for first postpartum ovulation appeared to be influenced most by plasma cholesterol (significant in 4 of 5 wk modeled) and IGF-I (significant in 3 of 5 wk modeled) concentrations the latter of which coincides with the results of model 7. Plasma cholesterol is a known precursor of P_4 (Savion et al., 1982; Grummer and Carroll, 1988), and necessary to maintain pregnancy after ovulation. Also, IGF-I is a potent stimulator of HDL and LDL metabolism and P_4 biosynthesis by ovarian cells (Veldhuis et al., 1987; Veldhuis and Gwynne, 1989).

Models 6 (production variables) and 8 (plasma hormones and metabolites) used to predict days to second postpartum ovulation generated R^2 values of 0.76 and 0.32, respectively. Although the greater R^2 in model 6 can be partially explained by the greater number of variables generated compared with model 8 (3 vs. 2 variables), model 6 had smaller dispersion of residuals than model 8. In model 6, percentage of milk lactose, FCM, and DMI influenced the interval of return to second postpartum ovulation. This finding is similar to the results generated when first postpartum ovulation was modeled on a weekly postpartum basis: percentage of milk lactose, FCM, and DMI were significant factors in 3 of 7 wk, 4 of 7 wk, and 3 of 7 wk modeled, respectively. This result is consistent with the fact that return to reproductive competence is influenced by the EB of the cow, and that EB is partially explained by the DMI, amount of milk produced and variability of milk components according to the models of Heuer et al. (2000).

Model 8 (plasma hormones and metabolites), showed that concentrations of plasma glucose and INS had important associations with return to second postpartum ovulation. Similar results were obtained when second postpartum ovulation was examined weekly in the postpartum period: glucose or glucose² were significant effects in 4 of 7 wk modeled, and INS or INS² were significant effects in 3 of 7 wk modeled. Because glucose is the main source of energy for ovarian function (Rabiee et al., 1997), and influences bovine thecal cell steroidogenesis in vitro (Stewart et al., 1995) it may play a major role in achievement of postpartum ovulation. Rabiee and Lean (2000) found that there was a positive and highly significant cross correlation ($r = 0.5$) between the uptake of glucose and cholesterol and suggested that glucose may promote cholesterol uptake into ovarian cells or vice versa. Also, low plasma INS concentrations were associated with decreased LH pulsatility (Butler and Smith, 1989; Canfield and Butler, 1990). Moreover, INS administered in vivo increases estradiol concentrations in follicular fluid of superovulated cattle (Simpson et al., 1994). In cultured granulosa cells, INS

augments FSH-induced P_4 production (Langhout et al., 1991) and LDL metabolism (Veldhuis and Gwynne, 1989), increases proliferation (Langhout et al., 1991; Spicer et al., 1993a) and stimulates aromatase activity (Spicer and Francisco, 1997; Spicer and Chamberlain, 1998; Spicer et al., 2002). Thus, lower INS that is normally observed in early lactating cows (Koprowski and Tucker, 1973; Smith et al., 1976; Francisco et al., 2002) likely affects days to return to postpartum ovulation in part by the lack of sufficient direct action on the ovary.

During early postpartum where lactation overrides other physiological processes (Bauman and Curie, 1980; Swanson, 1989), glucose, a precursor of milk lactose, may become limiting to other physiological processes such as postpartum ovulation (Staples and Thatcher, 1990). Interestingly, milk lactose was the most important production variable whereas plasma INS and glucose were the most important hormone/metabolite variables modeling days to second postpartum ovulation. The energetic efficiency of converting glucose to milk lactose is 0.98 (McDonald et al., 1995). If early postpartum DMI is low and if a cow produces more milk, then glucose supply to the ovary may not be sufficient for a cow to return to postpartum ovulation. Results of model 5 are consistent with this latter statement because EB, DMI, and FCM all accounted for significant variation in days to first postpartum ovulation. Contrary to this, Stevenson and Britt (1980) showed that energy intake and milk yield during the first 2 wk of lactation provided small contributions to the model predicting days to first ovulation. However, energy intakes and EB are low and milk production is increasing during the first 2 wk postpartum. In contrast, in the current study, models covered a longer postpartum period (5 to 12 wk) and a greater range of physiological conditions than in the model of Stevenson and Britt (1980). In the present study, some of the same variables (i.e., IGF-I, cholesterol, glucose, and insulin) emerged in the models predicting days to first and second postpartum ovulation when values for each week were modeled, and their significant contribution to these models are consistent with a possible important role in postpartum return to ovarian competence. Further research will be needed to ascertain whether some of those variables can be used in an applied way to monitor reproductive competence in early lactating dairy cows.

CONCLUSIONS

Relationships of plasma cholesterol and P_4 with EB, DMI, milk yield and composition, metabolites and hormones, and week postpartum were modeled using multivariate regression analysis. Models using EB, DMI,

FCM, BW, and milk composition as independent variables consistently predicted both plasma cholesterol and P_4 quite well as shown by the minimal residuals between predicted and observed values. Models using metabolites and hormones as independent variables predicted plasma cholesterol more accurately than they predicted plasma P_4 based on the magnitude of residuals. The primary variables involved with predicting plasma cholesterol were $DMI \times SNF$ (model 1, production variables) and the square of glucose (model 3, plasma hormones and metabolites) contributing most to the total model R^2 (63 and 55%, respectively). For P_4 , EB (model 2, production variables) and IGF-I (model 4, plasma hormones and metabolites) were the primary contributors to the total model, providing 61 and 74% of the total model R^2 values, respectively. Week postpartum when included in the model was the major predictor in models 1 to 4, although cross products of EB were significant contributors to models 1 and 2 and IGF-I and (or) cross products of glucose were significant contributors of models 3 and 4.

Days to first and second postpartum ovulation were "best" predicted by production variables EB and milk lactose percentage (models 5 and 6). Plasma metabolic and hormonal predictors for days to first and second postpartum ovulations were IGF-I² (model 7, plasma hormones and metabolites) and INS (model 8, plasma hormones and metabolites), respectively, and support previous studies that have implicated both IGF-I and insulin as important regulators of ovarian function. Data analysis from other herds or studies should be used to further validate these results.

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