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Relationships among metabolic hormones, luteinising hormone and anoestrus in periparturient dairy heifers fed two nutritional levels prepartum

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ABSTRACT

A mathematical model that describes the dynamics of reproductive hormones was developed to estimate the duration of the postpartum anoestrus interval (PPAI) in cows. The model identified two relatively separate mechanisms: one influenced PPAI through luteinising hormone (LH) release; and it is suggested that another one controlled ovary sensitivity to LH. Examining the role played by these mechanisms in the relationships between nutrition and PPAI involved analysing the data from an experiment that measured the effects of prepartum nutrition on PPAI in which levels of insulin-like growth factor-I (IGF-I), insulin, glucose, leptin and growth hormone (GH) were recorded. Five months before calving, 30 Holstein-Friesian heifers were randomly allocated to a high (HP) or low plane (LP) of nutrition. Body condition scores at calving were 6.3 and 4.1 for the HP and LP treatments respectively. Both groups were fed *ad libitum* after calving. Secretion patterns of LH were measured at 2, 5 and 8 weeks after calving. PPAI differed between treatments (HP 62.0 ± 7.95 days; LP 83.1 ± 6.95 days; P < 0.05), as did the level of GH at calving (HP 5.8 ± 1.23 ng/ml; LP 10.3 ± 1.23 ng/ml; P < 0.001). The within treatment variation in PPAI was partitioned into that associated with LH frequency post calving and that orthogonal to LH pulse frequency. Within each treatment, GH was associated with the LH frequency and insulin was associated with the component orthogonal to LH frequency. These results link nutrition to the PPAI through the dynamics of insulin and GH.

Keywords: mathematical model; anoestrus; dairy cows; nutrition; metabolites; LH.

INTRODUCTION

Prolonged postpartum anoestrous interval (PPAI) is a major problem limiting the reproductive performance among dairy cows in New Zealand. Anoestrous cows are more likely to conceive later in the breeding season, affecting their productivity. Recovery of cyclicity after calving is influenced by body condition score at calving and nutritional status during early lactation. A factor known to affect the duration of postpartum anoestrus is the level of nutrition (pre- and postpartum). It is recognized that first postpartum ovulation is delayed in cows when the energy demands for milk production and maintenance are greater than energy available from food intake and energy reserves (i.e. negative energy balance) (Smith *et al.*, 2001; Chagas *et al.*, 2001). However, the nature of the relationship between nutrition before and after calving and anoestrus has never been clear. Chagas (2003) demonstrated metabolic differences between cows of different genotypes and suggested that this was associated with varying status in energy balance which affected the period of anoestrus.

A mathematical model describing the dynamics of the reproductive hormones was developed to estimate the duration of PPAI in cows (Smith *et al.*, 2005). This model identified two relatively separate mechanisms operating close to calving: one influenced PPAI through luteinising hormone (LH) release and another controlled the ovary sensitivity to LH. In an attempt to link the effects of nutrition to the PPAI it was suggested that these mechanisms were directly related to the levels of

metabolic hormones close to calving. This paper supports the work of Smith *et al.* (2005) by investigating the relationship between anoestrus in the dairy cow and the blood levels of metabolites associated with energy management close to calving: growth hormone (GH), glucose, insulin, insulin-like growth factor-I (IGF-1), leptin and non-esterified fatty acids (NEFA). The analyses of these data sought to identify the relationships between these hormones and PPAI that would support the model of Smith *et al.* (2005).

MATERIALS AND METHODS

Data used in these analyses were from a trial conducted at Dexcel, Hamilton, New Zealand previously described by Chagas (2003). Thirty Holstein-Friesian heifers (2 years old) that conceived to a synchronised first insemination were selected and balanced for breeding worth and live weight and randomly allocated to treatment groups. During the last five months of gestation they were managed in two groups, one well-fed group calved at an average body condition score of 6.3 (high plane; HP; n = 13) and a restricted group (low plane; LP, n = 17) calved at body condition score of 4.1 using a 10-point body condition score scale. The animals were monitored for live weight and condition score weekly from 19 weeks before to 10 weeks after calving. Following calving, all cows were offered a generous pasture allowance. From 10 weeks before until 10 weeks after calving blood samples were collected weekly to measure plasma leptin, insulin, GH, IGF-I, glucose and NEFA concentration. Pulsatile LH

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release was measured by collecting blood samples every 15 minutes for 16 hours from jugular catheters. Profiles of LH secretion for each were taken at 2, 5, 8, 11, 14, 19 and 21 weeks after calving where measurements continued while a cow had not had oestrus. However, only the results for 2, 5 and 8 weeks are used in this analysis because of low numbers in subsequent weeks as cows experienced oestrus. The time to first ovulation was estimated from milk progesterone concentrations measured in samples taken twice a week.

Statistical analyses

An analysis of variance tested the effect of nutritional treatment on anoestrus, the LH frequency at 2, 5 and 8 weeks after calving and the metabolites measured at calving. The period of anoestrus was regressed on the LH pulse frequency, partitioning this variable into the estimate (associated with the LH frequency) and the residual, the component of anoestrus uncorrelated with the LH frequency. These two orthogonal components of anoestrus were then related to measurements of the metabolites taken close to calving and to the first principal component of each metabolite time series after calving. The first principal component, being that linear function of the observations accounting for greatest variance, provides a better estimate of the 'size' of a nonlinear time series than the mean, which is difficult to interpret in this situation (Ramsay & Silverman, 1997).

The ensemble of relationships between the metabolite and the components of anoestrus over the 3 sets of LH measurements were tested by combining the separate inference tests using union–intersection arguments taking account of the correlation between the LH frequencies measured over time (Silvey, 1975; Freund & Walpole, 1980). The point is that while the probability of a type 1 error on an individual correlation may be high, the probability of a consistent sequence of similar observations based on the same population is low.

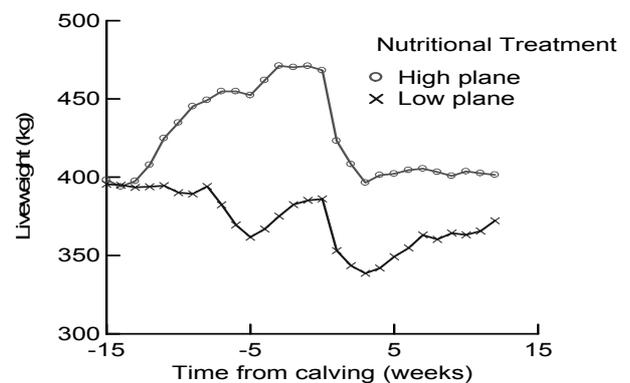
RESULTS

The live-weight change over time for cows on each treatment group is shown in Figure 1. HP cows were heavier (470 kg) at calving than LP cows (423 kg). Both groups lost live weight until about 3 weeks after calving. Following this HP cows maintained their live weight while LP cows gained live weight.

The least squares means and standard errors for the anoestrous period, the LH frequencies (pulses/16 hours) measured at 2, 5 and 8 weeks after calving and the metabolites measured at about calving for each of the nutritional treatments are presented in Table 1. The LP of nutrition was associated with an increase in the average period of anoestrus by 21 days compared to the HP (HP 62 ± 7.9 days, LP 83 ± 6.9 days, $P < 0.05$). Cows on the HP nutritional treatment had significantly less growth hormone (ng/ml) in their blood than cows on the LP (HP 5.8 ± 1.23 , LP 10.3 ± 1.08 , $P < 0.0005$), significantly more leptin (ng/ml) (HP 1.05 ± 0.056 , LP

0.83 ± 0.049 , $P < 0.02$) and marginally more NEFA and insulin ($P < 0.10$). Levels of IGF-1 and glucose did not show significant differences. Levels of insulin are higher and IGF-1 lower than generally observed (Gong et al., 2002; Taylor et al., 2004). However, IGF-1 levels in the heifers in this trial fell sharply (3x) over the last 3 weeks before calving, suggesting that the dynamics of these hormones are not fully understood. The level of LH frequency within nutritional treatment group shows lower LH frequency for the LP cows though this difference was not significant.

FIGURE 1: Live-weight profile of the cows during the experimental period, from 15 weeks before calving until 12 weeks after calving.



For the LP group there were significant ($P < 0.05$) correlations between the period of anoestrous and the LH frequency at 2 weeks ($r = -0.42$) and 5 weeks ($r = -0.49$) after calving. For the HP group the correlations were higher at 2 weeks ($r = -0.60$, $P < 0.05$) and 5 weeks ($r = -0.77$) after calving ($P < 0.01$).

FIGURE 2: Growth hormone profile after calving of cows on HP and LP nutritional treatments.

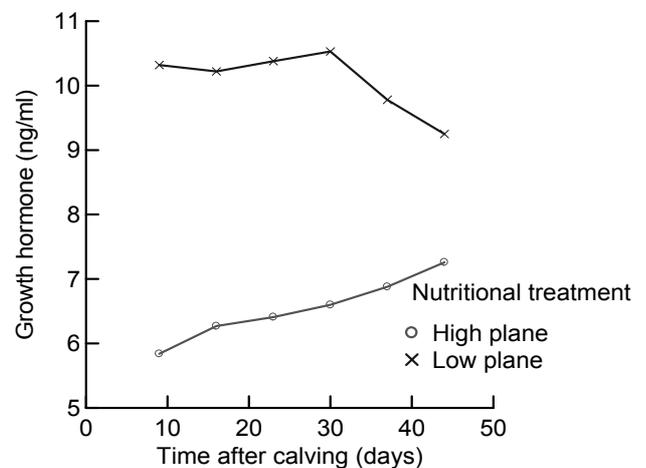


TABLE 1: The least squares means and SE for the anoestrous interval, the LH frequency at 2, 5 and 8 weeks and the metabolites measured in the blood at calving.

Variable	Low plane	High plane
Anoestrous interval (days)	83.1 ± 6.95 ¹	62.0 ± 7.95 ²
LH frequency at 2 weeks (pulses/16 hours)	3.0 ± 0.49	3.3 ± 0.59
LH frequency at 5 weeks (pulses/16 hours)	3.1 ± 0.56	4.3 ± 0.60
LH frequency at 8 weeks (pulses/16 hours)	5.1 ± 0.66	5.3 ± 0.79
Growth hormone (ng/ml)	10.3 ± 1.08 ²	5.8 ± 1.23 ¹
IGF-1 (ng/ml)	10.5 ± 1.71	12.8 ± 1.96
Insulin (ng/ml)	7.8 ± 0.93 ²	11.0 ± 1.07 ¹
NEFA (ng/ml)	0.43 ± 0.067 ²	0.92 ± 0.077 ¹
Leptin (ng/ml)	0.83 ± 0.049 ²	1.05 ± 0.056 ¹
Glucose (ng/ml)	3.1 ± 0.07	3.2 ± 0.08

Estimates with different superscripts are significantly different (P < 0.05)

The variation in PPAI was partitioned into a component associated with the LH frequency and the component orthogonal to LH frequency. This partitioned variable was examined for relationships with the measured metabolites within each treatment group at calving. In addition, the relationships with the metabolites measured after calving were examined using the scores of the first principal component of the 6 measurements taken over the 6 weeks following calving. This provided a measure of the between-cow within-treatment variation in these variables during this time.

Within nutritional treatment group, only growth hormone and insulin showed a consistent relationship with PPAI. Growth hormone in the blood close to calving was significantly correlated with the LH frequency (Table 2), but not with the component of anoestrus orthogonal to LH frequency (Table 3). Insulin in the blood close to calving was also correlated with

the component of anoestrus associated with LH frequency, although not as strongly or consistently as growth hormone (Table 2). However, insulin in the blood at calving was related to the component of anoestrus orthogonal to LH frequency (Table 3). While the insulin correlations considered individually are not significant, a test should recognise the information generated by the ensemble, which shows a consistent pattern of relationships. When considered as an ensemble over the 3 LH frequency measurements the relationship between insulin and the components of anoestrus was significant (P < 0.01). Similarly, for growth hormone and the component of anoestrus associated with LH frequency (P < 0.01). Table 4 shows the correlations between the component of anoestrous orthogonal to LH frequency and the scores of the first principal components. The relationship with insulin is stronger, though similar, to that observed close to calving.

TABLE 2: The correlations between the components of anoestrus associated with LH frequency measured at 2, 5 and 8 weeks after calving with the measurements of GH and insulin taken close to calving

Level of nutrition	Metabolite	Correlation with LH frequency 2 weeks after calving	Correlation with LH frequency 5 weeks after calving	Correlation with LH frequency 8 weeks after calving
LP	GH	0.3	0.5*	0.3
	insulin	-	-0.4	-
HP	GH	0.4	-	0.7**
	insulin	-0.3	-	-0.3

* P < 0.05 (one tailed t-test)

** P < 0.01

TABLE 3: The correlations between the components of anoestrus orthogonal to LH frequency measured at 2, 5 and 8 weeks after calving with the measurements of GH and insulin taken close to calving.

Level of nutrition	Metabolite	Correlation with LH frequency 2 weeks after calving	Correlation with LH frequency 5 weeks after calving	Correlation with LH frequency 8 weeks after calving
LP	GH	-	-	-
	insulin	-0.3	-	-0.3
HP	GH	-	-	-
	insulin	-0.3	-0.4	-0.3

TABLE 4: The correlations between the components of anoestrus orthogonal to LH frequency measured at 2, 5 and 8 weeks after calving with the factor scores of the first principal components of the time series measurements of GH and insulin over 6 weeks following calving. Cells with (-) represent correlations less than absolute value 0.15.

Level of Nutrition	Metabolite	Correlation with LH frequency 2 weeks after calving	Correlation with LH frequency 5 weeks after calving	Correlation with LH frequency 8 weeks after calving
LP	GH	-	-	-
	insulin	-0.3	-0.2	-0.5*
HP	GH	-	-	-
	insulin	-0.4*	-0.3	-0.3

* $P < 0.05$ (one tailed *t*-test)

DISCUSSION

It is notable that although the HP cows lost live weight and condition after calving they still achieved a shorter anoestrus interval than the LP cows. This implies that in this trial the nutritional path to calving was important in determining the onset of oestrus, not just the way the energy levels were changing. The main physiological component associated with the difference in prepartum nutritional levels was GH.

The partitioning of anoestrus within each nutritional treatment into two components, one associated with LH frequency and another with that part of anoestrus not associated (uncorrelated) with LH frequency suggests that the level of GH at about calving appears to act to mediate the level of LH frequency during the time following calving. The model of anoestrus constructed by Smith *et al.* (2005) describes changes in the pattern of the reproductive hormones leading to ovulation postpartum. The value of the parameter *b* in this model sets the pattern of hypothalamic sensitivity to oestrogens and thus the pattern of LH pulsatility and the *potential* for the length of anoestrus. The implication of these results is that this parameter (*b*) is associated with the level of GH in the blood of the cow close to calving. That is, the level of GH in the blood of the cow close to calving relates to the development of the frequency of the LH generator over time, and thus affects the period of anoestrus. The large difference in GH arising from the pre-calving nutritional treatments and the association of this with a large difference in anoestrus supports this role. The

within treatment correlations between the LH frequency and GH for individual cows demonstrate the same phenomena on a smaller scale.

GH may continue to affect anoestrus during the period following calving, but it is suggested that this could be through mechanisms other than its relationship with the parameter *b*. Lucy (2004) showed the links between growth hormone and other metabolites that could influence the period of anoestrus. For example, changes in GH could effect changes in insulin through a series of nonlinear relationships.

The level of insulin in the blood appears to be associated with both aspects of the reproductive physiology postpartum, i.e. the LH pulse frequency and thus by implication the central nervous system (CNS) and also at the ovarian level independent of LH pulse frequency. Insulin receptors (IR) in the CNS are distinctly located in the olfactory bulb, the hypothalamus and the pituitary gland. Neuron-specific disruption of the IR gene in mice causes hypothalamic dysregulation of LH secretion (Brüning *et al.*, 2000). Similarly, deletion of the 'IR substrate -2' gene in mice reduces plasma concentrations of LH (Burks *et al.*, 2000). In diabetic sheep, administration of physiological levels of insulin into the lateral cerebral ventricle of the brain increases the pulsatile secretion of pituitary LH (Tanaka *et al.*, 2000). Using a hyperinsulinemic-euglycemic clamp procedure in postpartum dairy cows, Butler *et al.* (2004) demonstrated that while plasma oestradiol concentrations were elevated, reflecting increased

follicular aromatase activity, there was no effect on the pulsatile secretion of LH. At the thecal and granulosa cell level there is positive cross-talk between the insulin and LH signalling pathways suggesting a mechanism to augment the activities of both hormones regulating steroidogenesis (Carvalho *et al.*, 2003).

Increasing plasma insulin concentrations during the early postpartum period in dairy cows by increasing the starch content of the diet significantly reduced anoestrus (Gong *et al.*, 2002). Thus, insulin plays a pivotal role in the postpartum cow – it is a major component in controlling the pulsatile secretion of LH and it increases the sensitivity of the ovary to this gonadotrophin. In the latter case it might be expected that insulin would increase its effect over time following calving rather than at calving. Indeed, this is the case as the correlations in Table 4 compared with Table 3 show.

The different actions of growth hormone and insulin on anoestrus could explain the interactions between pre- and post-calving energy treatments on this variable observed in a wide range of experiments (Wiltbank *et al.*, 1962; Nicoll, 1979; Pleasants & Barton, 1992). Insulin responds rapidly to changes in energy intake as it acts to maintain blood glucose within narrow limits. GH appears to change over a longer period in response to persistent levels of nutrition. For example, Figure 2 shows that GH falls 1 ng/ml (about 10%) in the LP cows in response to improved energy intake over 15 days.

In this respect, changes in the level of GH due to energy intake pre-calving would set the environment within which insulin would act as a result of post-calving nutrition. For example, if post-calving nutrition was high following poor pre-calving nutrition then GH would be high, but falling, and insulin levels rising. Alternatively, if pre-calving nutrition was good GH levels would already be low, and insulin levels would be rising promoting a short anoestrus period. That is, different nutritional paths would result in various levels of GH and insulin in the blood of the cow. This would provide a range of physiological environments acting on anoestrus.

If indeed variation in anoestrus is the result of variation in these hormones acting on the reproductive system as the model of Smith *et al.* (2005) suggests then this lays the basis for designing management strategies (nutritional and genetic) to deal with this problem.

ACKNOWLEDGEMENTS

This joint AgResearch and Dexcel project was funded by New Zealand Dairy Farmers through Dairy InSight.

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