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Metabolic status and first postpartum ovulation. *Butler et al. Page xxx.* This study examined whether daily oral drenching with a glucogenic precursor during the transition period would increase the proportion of cows ovulating the first postpartum follicle wave. Drenching with propylene glycol had no effect on the fate of the first postpartum follicle wave. Retrospective analysis based on follicle fate indicated that cows with ovulatory follicles had greater DMI, superior energy balance, more favorable metabolic hormone and metabolite profiles, with some differences apparent as early as 3 wk before parturition. Periparturient DMI plays a major role in determining the fate of the first postpartum follicle wave.

METABOLIC STATUS AND OVULATION

Energy Balance, Metabolic Status, and the First Postpartum Ovarian Follicle Wave in Cows Administered Propylene Glycol

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ABSTRACT

Mature Holstein cows were drenched daily with either 500 mL of water (CTL; n = 28) or propylene glycol (PPG; n = 28) from d 10 before parturition until d 25 postpartum. Follicular development was monitored thrice weekly by transrectal ultrasound. Blood samples were collected every 30 min from a subset of 10 cows per treatment on d −10, 2, and 25 to assess glucose and insulin response to treatments, and on d 10 postpartum, blood was collected every 10 min for 12 h to determine LH pulse profiles. Both insulin and glucose were elevated on d 2 and 25 following PPG administration, but only insulin was elevated on d −10. On d 10 postpartum the number of LH pulses, mean LH, and pulse amplitude were not different between CTL and PPG cows. The proportion of first postpartum dominant follicles that became ovulatory, atretic, or cystic was not different between CTL and PPG cows. Despite evidence of improved metabolic status, PPG failed to increase LH pulse frequency and failed to increase the proportion of first postpartum follicle waves resulting in ovulation. The dominant follicle of each cow was retrospectively categorized as being ovulatory (O; n = 17), non-ovulatory high estradiol (NH; n = 6), non-ovulatory low estradiol (NL; n = 24), or cystic (C; n = 8). Differences in dry matter intake and energy balance among cows in the different follicle categories were apparent as early as 3 wk before parturition. The NL cows had lower pre- and postpartum dry matter intake and energy balance compared with O cows. The NL cows also had postpartum metabolic hormone and metabolite profiles indicative of more severe negative EB.

(**Key words**: cows, energy balance, insulin, ovary, ovulation, follicle, propylene glycol)

Abbreviation key: **C** = cyst, **EB** = energy balance, **HPO =** hypothalamo-pituitary-ovarian axis, **NEB** = negative energy balance, **NH** = non-ovulatory high estradiol, **NL** = non-ovulatory low estradiol, **O** = ovulatory, **PPG** = propylene glycol, **TG** = triglyceride

INTRODUCTION

It is generally accepted that energy balance (**EB**), rather than intake of any specific class of nutrient, is the principal nutritional factor regulating the reproductive system (Butler and Smith, 1989; I'Anson et al., 1991; Wade et al., 1996). In dairy cattle, both the duration and severity of early postpartum negative energy balance (**NEB**) are correlated with the interval to resumption of ovulatory activity following parturition. The principal defect caused by NEB occurs at the level of the hypothalamus, manifested by reduced GnRH pulse frequency. This results in a parallel reduction in pulsatile pituitary LH release, with consequent compromised follicular steroid output and anovulation. In addition, follicular responsiveness to gonadotropin stimulation is blunted by the circulating hormonal and metabolite environment of the NEB state. Moreover, hypothalamic responsiveness to the negative feedback effects of estradiol is enhanced, resulting in further attenuation of already suboptimal GnRH pulse frequency (for reviews: Beam and Butler, 1999; Wiltbank et al., 2002; Diskin et al., 2003). Collectively, these lesions result in anovulation for a varying period of time, with some cows remaining anestrous beyond 80 DIM. Prolonged anovulation is associated with reduced likelihood of conception (Darwash et al., 1997; Westwood et al., 2002; Butler 2003), and thus, a prompt return to cyclicity following parturition is desirable.

Several putative hormones, growth factors, and metabolites have been identified that are stimulatory or inhibitory to the reproductive axis. In general, these factors tend to rise and fall in tandem during unfavorable nutritional conditions. Reduced circulating concentrations of insulin, IGF-I, leptin, and glucose, and elevated concentrations of β-hydroxybutyrate (BHBA), NEFA, and glucocorticoids are all associated with impaired reproductive performance. In contrast, there is an emerging paradigm that the lack of available metabolic fuels *per se* — rather than direct effects of hormones and metabolites

— is responsible for the inhibition of reproduction during NEB (Wade et al., 1996; Schneider and Wade, 2000). The precise reason for reduced GnRH pulsatility is unknown, though it is clear that pulsatile GnRH release is sensitive to glucose availability (Bucholtz et al., 1996; Ohkura et al., 2004). Low circulating insulin and IGF-I do not seem to be involved in the suboptimal GnRH and LH pulse frequency, but elevated concentrations of both are stimulatory to ovarian estradiol output (Butler et al., 2004).

Propylene glycol (1,2-propanediol - $C_3H_8O_2$) is a sweet, hygroscopic, viscous liquid that has gluconeogenic properties and is routinely used for its therapeutic effects on cows suffering from ketosis based on the premise that it will rapidly increase blood glucose. The current study was carried out to test the hypothesis that a daily increase in glucose and insulin, and reduction in NEFA, achieved via a daily propylene glycol (PPG) drench, during the period from late pregnancy through the end of the first postpartum follicle wave, would increase LH pulse frequency and the proportion of ovulatory first-wave follicles.

MATERIALS AND METHODS

Cows and Treatments

Fifty-six multiparous Holstein cows were paired on the basis of expected calving date, parity, and previous 305-d milk yield and assigned randomly to receive a daily 500 mL oral drench of either water (CTL) or PPG (Animal Medic Inc., Manchester, PA) from d 10 before expected parturition until d 25 postpartum. A dose of 500 mL was chosen based on previous studies (Grummer et al., 1994; Miyoshi et al., 2001; Pickett et al., 2003). Drenching was carried out via consecutive use of 2, 250 mL drenching guns. Cows were assigned to their experimental groups and treatments were initiated during a 5-mo period. To facilitate individual feed intake measurements, cows were housed in individual tie stalls starting on d 21 before expected parturition, moved to individual box stalls for a 2- to 4-d period around the time of parturition, and then returned to individual tie stalls until d 30 postpartum. Weekly samples of the feed offered were dried and composited on a monthly basis for nutrient analysis (Dairy One Cooperative, Ithaca, NY). Ingredient and nutrient composition of the diets fed are summarized in Table 1.

Cows were fed daily at 0900 and drenched at 1500 h. Drenching at 1500 h was chosen in an effort to extend post-prandial increases in circulating glucose and insulin. Following parturition, cows were milked three times daily at 0030, 0830 and 1630 h. During the period from d –21 until d 0 (i.e., day of parturition), blood samples were collected from all cows on Monday, Wednesday, and Friday, and then daily from parturition until d 25 postpartum. These blood samples were collected at 1200 h from the coccygeal vessels using vacutainers containing sodium heparin (Becton Dickinson, Franklin Lakes, NJ). A subset of 10 cows from each treatment was selected for periods of more intensive blood sampling and liver biopsies. More frequently obtained blood samples were collected via an indwelling jugular catheter (Tygon Microbore Tubing, Norton Performance Plastic, Akron, OH) on d –10 ± 1.2 (mean ± SEM), 2, and 25 relative to parturition (8 mL every 30 min from 0900 to 2100 h) and on d 10 postpartum (4 mL every 10 min from 0900 to 2100 h). All catheters were inserted the day before frequent blood collection. Heparinized blood samples were centrifuged at 2060 × g, plasma was harvested, and stored at –20°C for subsequent hormone and metabolite analyses. All procedures involving animals were approved by the Cornell University Institutional Animal Care and Use Committee.

Plasma Hormone and Metabolite Analysis

Plasma samples collected thrice weekly prepartum and daily after parturition were analyzed for estradiol-17β, insulin, IGF-I, glucose, NEFA, and BHBA. All frequently collected blood samples d –10, 2, and 25 relative to parturition were analyzed for insulin, glucose and NEFA. All blood samples collected at 10-min intervals on d 10 postpartum were analyzed for LH. Circulating estradiol concentrations were measured by radioimmunoassay (RIA; Serono Maia, Cortlandt Manor, NY; Butler et al., 2004) daily from day of parturition until day 25 postpartum. Insulin and glucose concentrations were determined in 3 samples collected during the period from d –10 to 0 (approx. d –9, –6, and –3), and every other day from parturition until d 24 postpartum. Insulin was measured by a double-antibody RIA (Linco Research Inc., St. Louis, MO) as described (McGuire et al., 1995). IGF-I was measured on d –7 (approx.), 0, 5, 15 and 25 relative to parturition following ethanol:acetone:acetic acid (60:30:10) extraction as described (Butler et al., 2004). Plasma LH concentrations were determined in all the frequent blood samples collected on d 10 postpartum and LH pulses were identified (Butler et al., 2004). Inter- and intra-assay coefficients of variation were 16.7 and 8.2%, 4.5 and 6.1%, 15.4 and 5.2%, and 8.1 and 2.3%, for estradiol (n = 21), insulin (n = 10), IGF-I (n = 3) and LH (n = 3), respectively.

Concentrations of NEFA were determined in 3 prepartum samples (approx. d –9, –6, and –3), daily from parturition until d 3 postpartum, and every third day thereafter until d 24 postpartum. Plasma BHBA concentrations were measured in 3 prepartum samples (approx. d –9, –6, and –3), and every third day from parturition until d 24 postpartum.

Plasma metabolites were analyzed by enzymatic colorimetric assays using procedures modified from available kits (glucose [kit 510A] and BHBA [kit 310-UV], Sigma-Aldrich Co.; NEFA, Wako Chemicals USA Inc., Dallas, TX) and validated in our laboratory. Analyses of glucose, NEFA, and BHBA were conducted in 96-well microplates (Becton Dickinson and Co., Franklin Lakes, NJ) and read using a microplate reader (Bio-Tek Instruments, EL340).

Liver Tissue Collection and Triglyceride Determination

Liver tissue was collected from 10 cows per treatment on d 5 and 25 following parturition as previously described (Butler et al., 2003). Tissue was rinsed in saline and blotted dry, snap-frozen in liquid nitrogen, and stored at –80°C. Tissue (~150 mg) was homogenized with a polytron in 2:1 chloroform:methanol for about 10 s, total lipids were extracted (Folch et al., 1957), and triglyderide (**TG**) concentration was determined by a colorimetric Hantzsch condensation method (Foster and Dunn, 1973) using glycerol trioleate (T-7140; Sigma-Aldrich Co.) to generate a standard curve. Inter- and intra-assay (n = 4) coefficients of variation were 8.4 and 6.2%, respectively.

Ovarian Ultrasonography

Ovarian follicular activity of all cows was examined by linear array ultrasonography (7.5-MHz transrectal transducer;, Aloka 210; Corometrics Medical Systems Inc., Wallingford, CT) thrice weekly (Monday, Wednesday, and Friday) beginning on d 8 to 10 postpartum and continuing until ovulation, or until d 30 for cows failing to ovulate. Diameter of dominant follicles between ultrasound examinations was calculated by linear interpolation. Follicles were considered to be dominant when a diameter of > 10 mm was reached in the absence of other large growing follicles (Savio et al., 1990). An exception to this rule occurred when co-dominant follicles were observed. In this instance, diameter of the largest co-dominant follicle was reported. Cysts were defined as anovulatory follicles > 25 mm in diameter that persisted for at least 10 d in the absence of a corpus luteum (Garverick, 1997).

Energy Balance Determination

Energy intake was computed based on daily measurements of DMI and the calculated NE_l value of the diet (Dairy One Cooperative, Ithaca, NY). Maintenance requirements were calculated based on weekly BW measurements. Energy requirements for pregnancy were based on day of gestation. Lactation requirements were based on daily measurements of milk yield, and weekly measurements of milk composition (fat, protein, and lactose). Individual estimates of daily EB were calculated as the difference between energy intake and energy requirements (NRC, 2001). Duration of declining EB was calculated as the time from the onset of declining EB until day of EB nadir.

Statistical Analyses

Data were analyzed using SAS (SAS Inst. Inc., Cary, NC). Daily measurements of DMI and EB made during the period from d –21 to –11 relative to parturition (i.e., before onset of treatments), or measurements of plasma insulin, glucose, and NEFA made during the 6-h period before drenching on days of frequent blood collection were used for covariate adjustments. Hormone, metabolite, liver TG, and production data were analyzed as repeated measures using the MIXED procedure. To accommodate the requirements of nonlinear analysis using PROC MIXED, common prepartum sampling days are needed among experimental units. Therefore, the blood samples selected for analyses of hormones and metabolites were as close as possible to the days selected for statistical analysis (e.g., d –9, –6, and –3). Fixed effects included experimental treatment, time (days or hours), and their interaction. Cow within treatment was used as a random effect, with day or hour as the repeated statement, and using a first-order autoregressive plus random effect covariance structure (Littell et al., 1998). When the interaction between treatment and time was significant ($P < 0.05$), pair-wise comparisons of individual means were carried out using the Tukey-Kramer test. The LH data were analyzed using a one-way ANOVA test.

The data were also analyzed by grouping cows on the basis of dominant follicle outcome and corresponding circulating estradiol profiles irrespective of treatment into 4 categories: ovulatory (**O**; n = 17), non-ovulatory high estradiol (**NH**; n = 6), non-ovulatory low-estradiol (**NL**; n = 24), and <u>c</u>yst (**C**; n = 8). Analysis was carried out using the MIXED procedure as described above, with the exception that follicle type was used in the model instead of treatment, cow within follicle type was the random variable, and the data were not subjected to covariate adjustment. When the interaction between follicle type and time was significant ($P < 0.05$), pair-wise comparisons of individual means were carried out using the Tukey-Kramer test. The DMI and EB profiles were divided into 3 periods for analysis purposes: d −21 to −8, d −7 to −1, and d 0 to 30 relative to parturition. Hormone and metabolite data were separated into prepartum, postpartum, and combined pre- and post-partum datasets for analysis. Blood glucose on the day of parturition was analysed using PROC MIXED with follicle type in the model, and cow within follicle type as the random variable. Pre- and post-partum DMI, EB, circulating insulin, IGF-I, glucose, and NEFA values were compared between O cows and the mean of all other cows (NH + NL + C) using the ESTIMATE statement in MIXED. Liver TG data was available for 5 ovulatory and 15 non-ovulatory cows. To minimize the large observed variance in liver TG concentrations, difference in hepatic TG accumulation between d 5 and 25 was compared for O and non-ovulatory cows using a paired t-test. Multiple logistic regression using the LOGISTIC procedure of SAS, with the SELECTION=SCORE option in the model statement to generate optimum subset models, was carried out to identify the independent variables that were most effective for predicting ovulation. Cows with an ovulatory follicle were coded 1, and all other cows were coded 0. For illustrative purposes, EB curves were smoothed using PROC LOESS with a smoothing parameter of 0.2. Significance was declared at $P < 0.05$, and trends from $P = 0.05$ to $P < 0.15$.

RESULTS

Dry Matter Intake and Milk Yield and Composition

Effects of drenching with water or PPG on DMI, milk yield, and milk composition are summarized in Table 2. No treatment effect was detected for DMI, milk yield, or milk protein concentration. Milk fat concentration tended ($P = 0.07$) to be reduced in response to PPG, and milk lactose concentration was increased ($P < 0.05$). After adding the energetic value of 500 mL of PPG (2 Mcal) to dietary energy consumed, a significant beneficial effect of PPG on EB was observed (Table 2).

Hormones, Metabolites, and Liver Triglycerides

Effect of drenching with water or PPG on the mean concentrations of glucose, insulin, and NEFA in samples collected at 30-min intervals during the 6 h post-drenching period on d −10, 2, and 25 relative to parturition is summarized in Table 3. Plasma insulin was increased ($P < 0.01$) or tended ($P \leq 0.08$) to increase in response to drenching with PPG at all time points. Glucose was increased ($P < 0.01$) and NEFA was decreased ($P < 0.05$) in response to a PPG drench postpartum, but not prepartum.

In blood samples collected at 1200 h through the transition period (3 h before daily drenching), prepartum insulin concentrations were initially lower at about d 10 prepartum, but postpartum concentrations were greater in cows given PPG (treatment × time interaction, $P < 0.05$). Circulating glucose tended ($P = 0.096$) to be greater (Figure 1), whereas plasma NEFA ($P < 0.05$) and BHBA ($P < 0\ .01$) were reduced in response to PPG (Figure 2). Drenching with PPG tended ($P = 0.08$) to reduce postpartum hepatic TG concentration (Figure 3).

Follicular Development and LH Pulse Data

Effect of drenching with water or PPG on the fate and characteristics of the first postpartum follicle wave are summarized in Table 4. No difference between treatments was detected in the number of cows with follicles ovulating, undergoing atresia, or becoming cystic. Within these 3 possible outcomes, no treatment effect was detected for maximum diameter of the dominant follicle, peak plasma estradiol concentration, or day of peak plasma estradiol concentration. Day of maximum follicle diameter was earlier ($P < 0.05$) in non-ovulatory cows drenched with PPG. No effect of the PPG drench was detected for LH pulse frequency, LH pulse amplitude, or mean circulating LH measured in blood samples collected frequently on d 10 postpartum (Table 4). These data also were analyzed by paired t-test within cow comparing the LH pulse data for the 6-h periods before and after drenching. No treatment differences were observed (data not shown).

Grouping of Follicles by Growth Pattern and Estradiol Output

Cows were grouped based on growth and fate of the first postpartum follicle wave and the associated circulating estradiol profiles. One cow had ambiguous follicular and estradiol data and was excluded from the analysis, leaving a total of 55 cows. Of these, 17 had O dominant follicles, 6 had NH dominant follicles, 24 had NL dominant follicles, and 8 cows had dominant follicles that developed into C.

All 17 of the O cows exhibited a preovulatory rise in circulating estradiol (range 3.4 to 8.0 pg/mL) and ovulation of either a single dominant follicle (n = 12) or 2 co-dominant follicles (n = 5). The 6 NH cows had a preovulatory-like rise in estradiol that exceeded 4.0 pg/mL (range 4.5 to 7.1 pg/mL), followed by either an abrupt reduction to about 1 pg/mL within 24 h (n = 3), or a slower reduction during a 3- to 4 d period (n = 3). The follicle growth pattern of the 24 NL cows was associated with low circulating estradiol; a distinct peak in estradiol was not evident, and the mean circulating estradiol on the day of maximum follicle diameter was 0.9 pg/mL (range 0.3 to 2.7 pg/mL). Among the 24 NL cows, the large follicle exerted dominance in 20 cows and a new wave did not commence until the first dominant follicle started to regress. Conversely, in 4 NL cows, the large follicle failed to exert dominance, and a new wave of follicle growth emerged while the existing dominant follicle was still growing, such that a second wave dominant follicle with a diameter of 12.4 ± 1.2 mm (range 9.5 to 15.5 mm) was present on the ovary on the day that the first-wave follicle reached its maximum diameter. Among the 8 C cows that were observed, in 6 cases the large follicle produced high circulating concentrations of estradiol (peak estradiol = 12.5 ± 2.7 pg/mL) and exerted dominance, as evidenced by preventing new follicular wave emergence. In the remaining 2 C cows, preovulatory-like estradiol concentrations (peak estradiol = 4.7 ± 0.1 pg/mL) were produced and estradiol then declined abruptly. These preovulatory-like estradiol peaks were observed on d 15 and 18 postpartum, with corresponding follicle diameters of 18 and 25 mm, respectively. The dominant follicles then continued to grow and to develop into cysts, but another wave of follicular growth commenced 3 to 4 d later in the presence of the developing cyst. Profiles of follicular development and circulating estradiol for 6 individual representative cows are illustrated in Figure 4.

Relationship Among Follicle Type, EB, DMI, and Metabolic Status

Follicle characteristics are summarized in Table 5, and EB, hormone and metabolite measurements for the different follicle types are illustrated in Figures 5 to 8. No differences were detected among follicle-type groups in milk yield (44.3 ± 0.3 kg/d; mean ± SEM), and differences in EB were reflected by differences in DMI (Table 6). When compared with all non-ovulatory cows (NH + NL + C), O cows had superior prepartum (final week) and postpartum DMI and EB, greater ($P < 001$) postpartum insulin, and tended ($P < 0.1$) to have greater postpartum glucose and IGF-I. This was primarily because of significant differences between O and NL cows for all these variables, with C cows only differing ($P < 0.05$) from O cows for glucose on day of parturition (103.6 vs. 70.7 mg/dL) and tending ($P = 0.06$) to have lower postpartum insulin (0.43 vs. 0.34 ng/mL). The NH cows were not different from O cows for any of the variables measured with the exception of tending ($P = 0.10$) to have lower postpartum concentrations of insulin (0.43 vs. 0.34 ng/mL), and having greater DMI and superior EB during late pregnancy (d −21 to −8). Within the group of cows that developed non-ovulatory follicles, NH cows had greater ($P < 0.001$) prepartum (d –21 to –8) EB, and DMI than NL or C, but postpartum differences were not significant (Table 6). Compared with O cows, NH cows had a more gradual decrease ($P < 0.001$) to EB nadir (duration of decrease in EB = 10.4 ± 1.6 vs. 24.2 ± 2.7 d; Figure 5). Compared with O cows, NL cows had lower prepartum ($P < 0.05$) and postpartum ($P < 0.005$) DMI, less ($P < 0.05$) positive prepartum and more ($P < 0.001$) negative postpartum EB (Table 6). Further, the NL cows also had postpartum metabolic hormone and metabolite profiles indicating more severe NEB [insulin, 0.43 vs. 0.30 ng/mL ($P < 0.001$); IGF-I, 63 vs. 41 ng/mL ($P < 0.05$); NEFA, 381 vs. 495 µmol/L ($P < 0.05$)]. Of those cows that developed cysts, the 2 cows that had an abrupt decline in estradiol seemed to have more ($P < 0.05$) favorable EB (average d –21 to 30: 0.7 ± 1.7 vs. -4.3 ± 1.0 Mcal/d), but the small number of cows having that type of follicle profile prohibited meaningful and reliable analysis. Of note, the glucose:insulin ratio was lowest in O cows (46.0 ± 3.5) and highest in the NL cows (68.7 ± 2.9; O vs. NL, $P < 0.001$), with NH [57.5 ± 5.7; O vs. NH ($P = 0.09$); NH vs. NL ($P < 0.08$)] and C [55.9 ± 4.9; O vs. C ($P < 0.05$); O vs. NL ($P < 0.05$)] cows being intermediate (Figure 6). Liver TG concentration was not different on d 25 compared with d 5 in cows with an O follicle (from 8.5 ± 3.0% on d 5 to 7.5 ± 2.5% on d 25), but TG concentration tended ($P = 0.08$) to increase in non-ovulatory cows during the same interval (from 9.4 ± 1.4% on d 5 to 11.5 ± 1.7% on d 25).

The multiple logistic regression model for predicting likelihood of an ovulatory follicle is outlined in Table 7. The predictors that the SCORE variable selection procedure selected were duration of declining energy balance, postpartum plasma insulin concentration, DMI in the final week before parturition, and plasma glucose on day of parturition. When all individual cow-pairs having different responses (i.e., all possible 0 and 1 combinations) were analyzed by this model, the cow with an ovulatory follicle was correctly identified in 92.9% of the cases.

DISCUSSION

Propylene glycol is a glucogenic precursor that is either rapidly absorbed from the rumen and converted to glucose, or partially metabolized to propionate in the rumen before being absorbed (Nielsen and Ingvartsen, 2004). As outlined in Table 3, drenching with PPG resulted in increased plasma glucose and insulin concentrations, and reduced NEFA concentrations, in agreement with previous studies (Studer et al., 1993; Grummer et al., 1994). In agreement with the findings of Christensen et al. (1997), PPG was more effective at increasing blood glucose and reducing plasma NEFA during NEB (lactation) than during positive EB (pregnancy). Many studies have found hypoglycemia, hypoinsulinemia, and elevated NEFA to be associated with the postpartum anovulatory condition. We considered drenching with PPG during the transition period a potentially practical and useful method to transiently increase insulin and glucose and decrease NEFA on a daily basis from d −10 to 25. Results of the current study indicate that daily drenching with PPG during the transition period had no beneficial impact on LH pulsatility, ovarian estradiol output, and the outcome of the first postpartum follicular wave, despite the improved EB status, insulin profile, and metabolite concentrations of these cows. Using a less frequent drenching schedule (300 g mixed in the diet from d −10 until day of parturition, and administered as an oral drench on d 3, 6, 9, and 12 after parturition), Formigoni et al. (1996) observed an earlier return to cyclicity. Similarly, Miyoshi et al. (2001) observed an earlier onset of ovulatory activity when cows were administered a daily 500 mL PPG drench between d 7 and 42 postpartum. In contrast, our results indicate that a single daily drench containing 500 mL of PPG did not impact any of the characteristics of the first postpartum follicle wave. This indicated that transient elevations in insulin and glucose and decreases in NEFA and the modest improvement in EB were insufficient to adequately stimulate the hypothalamic-pituitary-ovarian (**HPO**) axis.

The most interesting findings from the present study emerged after cows were categorized on the basis of follicle type. Cows with an ovulatory follicle are considered to have best adapted to lactation, as assessed by recovery of a fully functional HPO axis during recruitment and development of the first postpartum follicle wave. Cows with O follicles had the most favorable EB, DMI, and hormonal and metabolic profiles, but differences were significant compared with NL cows only for most variables. The NH cows had the greatest DMI and EB during late pregnancy (d –21 to –8), but this trend did not continue postpartum. Cows that developed cysts were not different from the other groups for most variables measured. Therefore, all non-ovulatory animals should not be considered as a similar physiological group, because there seems to be differences in the adaptive ability of these cows to the onset of lactation.

The NH cows represent an interesting group of anovulatory animals; follicle growth and estradiol output seemed identical to O cows, but they failed to ovulate following a preovulatory-like rise in estradiol. Thus, the GnRH-LH pulse frequency necessary to generate a steroidogenically competent follicle seems intact, but their ability to induce an LH surge, or to ovulate in response to an LH surge, was impaired. Similar observations of an impaired LH surge feedback mechanism during NEB have been reported in fasted beef heifers (Mackey et al., 1999) and early lactating sheep (Wright et al., 1980). Estradiol production is reduced and returns to baseline values within 8 h after the peak in the LH surge in cows having normal follicles (Haughian et al., 2004). Based on the profiles of estradiol observed in NH cows, it is tempting to speculate that cows with a slow 3 to 4 d reduction in the preovulatory-like estradiol peak failed to induce a GnRH-LH surge (hypothalamic lesion). Conversely, it is possible that the NH cows with a rapid reduction in estradiol did stimulate a GnRH-LH surge. Either the surge was inadequate to stimulate ovulation, but sufficient to shutdown estradiol synthesis, or alternatively, the surge was adequate, but ovarian responsiveness to LH was somehow blunted (hypothalamic and/or ovarian lesion). Duffy et al. (2000) reported that early lactating beef cows exhibited an LH surge, but failed to ovulate. With only a single daily blood sample available, evidence of an LH surge was not detected in any of the cows failing to ovulate after exhibiting a preovulatory-like rise in estradiol (data not shown). The NH cows had greater prepartum (d −21 to −8) EB and DMI than any of the other groups, but had a longer duration of declining EB compared with O cows.

Cows with cysts had adequate GnRH-LH pulse frequency to stimulate a preovulatory-like rise in estradiol, but failed to ovulate. Etiology of cystic ovarian disease remains unresolved, but it is clear that the positive feedback mechanism of estradiol required to stimulate an LH surge is dysfunctional (Garverick, 1997), possibly caused by low circulating concentrations of progesterone acting to antagonize estradiol action at the hypothalamus and pituitary (Silvia et al., 2002). Of the 8 cows that developed cysts in the current study, 2 cows differed from the remaining 6; both cows displayed a normal preovulatory-like rise in estradiol followed by a rapid (within 24 h) return to baseline concentrations. Similar to normal follicles, estradiol synthesis is reduced in cows with cystic follicles following an LH surge (Garverick, 1997). A new follicle wave emerged in both cows within 4 d of the peak of the preovulatory-like rise in estradiol in the presence of the growing cyst indicating that a surge in FSH also likely occurred (Figure 4E). Both cows also had an increase in progesterone to approximately 0.3 ng/mL within 7 d of the peak in estradiol implying luteinization of the cyst (progesterone < 0.05 ng/mL on day of peak estradiol). Based on this evidence, we propose that these 2 cows had a GnRH-LH surge, but either the surge was inadequate to cause ovulation, or the follicle was unresponsive to an apparently normal LH surge. Rather than ovulate or become atretic, follicles continued to grow and developed into cysts. Again, with a single daily blood sample, evidence of an LH surge was not detected.

The NL cows failed to produce any significant quantities of estradiol, and none displayed a preovulatory-like rise in circulating estradiol. This likely reflects inadequate GnRH-LH pulse frequency, and thus these cows might represent the poorest adaptation to lactation and regeneration of a functional HPO axis. The NL cows had the poorest DMI, and the least favorable EB, and hormonal and metabolic profiles. It is interesting to note that EB and DMI in the NL cows was inferior to those of the O and NH groups as early as 3 wk before parturition.

Insulin also plays a role in ovarian steroidogenesis. Insulin receptors are widely distributed throughout all ovarian compartments, including granulosa, thecal, and stromal tissues (Poretsky and Kalin, 1987). In vitro studies have shown that insulin directly stimulates both mitosis and steroid production of cultured bovine granulosa cells (Gutierrez et al., 1997) and luteal cells (Mamluk et al., 1999). In addition, insulin increases steroidogenesis in response to gonadotropins in vitro (Stewart et al., 1995; Silva and Price, 2002). Early postpartum dairy cows fed diets designed to increase circulating insulin concentrations had an earlier onset of estrous cycles and more favorable conception rates at first service (Gong et al., 2002). We have recently examined the role of circulating insulin on LH release and circulating steroids during the first postpartum follicle wave. A 2.6-fold elevation in circulating insulin resulted in increased circulating estradiol, without any apparent changes in LH pulsatility (Butler et al., 2004). Circulating insulin was elevated in O cows following parturition in the current study, and may have played a role in determining the fate of the first postpartum follicle wave.

A critical facet of successful initiation of lactation is the reduced responsiveness of muscle and adipose tissue to insulin, allowing greater nutrient uptake by the mammary gland in support of milk synthesis (Bauman and Elliot, 1983; Bell 1995; Bauman 2000). We observed little variation between the different follicle-type groups in circulating glucose during the postpartum experimental period. Circulating insulin, however, was greatest in the future O cows, resulting in this group having the lowest glucose:insulin ratio (Figure 6B and 6C). Hence, O cows had greater circulating insulin per unit of glucose, especially compared with NL cows. Pancreatic insulin release in response to propionate or glucose in dairy cows is lower during lactation than during the non-lactating state (Lomax et al., 1979; Sano et al., 1993). In the current study, O cows had greater DMI than NL cows during the duration of the study, presumably increasing propionate supply and pancreatic insulin secretion. Insulin secretion in response to intravenous infusion of glucose is greater in cows displaying normal estrous cycles than in cystic cows (Opsomer et al., 1999). Without sensitive measures of insulin secretion and glucose disposal, however, we cannot definitively state whether the differences observed in plasma insulin are attributable to differences in pancreatic insulin secretion, insulin clearance, or peripheral tissue insulin resistance.

On the day of parturition, the prospective O cows displayed a marked elevation in glucose on the day of parturition (Figure 6B). It is possible that the difference merely reflects closer proximity between collection of blood samples from this group and timing of the increase in glucose associated with parturition. Given the relatively large number of cows in the groups, however, this seems unlikely. It may also be indicative of greater stores of glycogen in these cows, or mobilization of the glycogen stores in response to glycogenolytic stimuli. A further possible interpretation is that it reflects a greater degree of peripheral tissue insulin resistance, and thus a smaller pool of tissues available to utilize the glucose in circulation.

Cows with an ovulatory follicle had reduced liver TG concentration compared with non-ovulatory cows, in agreement with preliminary findings by Marr et al. (2002). As cows with ovulatory follicles also had lower NEFA, this represents reduced mobilization of fatty acids from adipose tissue for uptake and esterification by the liver. In addition, hepatic oxidation of fatty acids may have been enhanced, and if this were the case, would present an obvious advantage. Efficacy of laying down adipose stores during nutrient excess is contingent on these stores being used for energy production during nutrient deficit. There is little advantage to mobilizing TG from adipose tissue only to re-esterify the free fatty acids back to TG in the liver. Regulation of periparturient hepatic mitochondrial and peroxisomal β-oxidation capacity is an area of growing interest (Drackley et al., 2001).

CONCLUSION

Drenching with PPG during the transition period had no positive or negative effects on the fate of the first postpartum follicle wave, despite apparent beneficial effects on metabolic status. Results of the current study indicate once again that EB is a key regulator of reproductive function. It has been suggested that DMI during late pregnancy plays a role in “programming” susceptibility to periparturient metabolic disorders (Grummer, 1995). Likewise, results of the current study indicate that DMI, and accordingly EB, during late pregnancy may play a pivotal role in predetermining the functionality of the HPO axis, and hence, fate of the first postpartum follicle wave. Further work with sensitive measures of pancreatic insulin release in response to an insulin secretagogue and peripheral insulin responsiveness are required to examine whether or not differences exist between cows with ovulatory and non-ovulatory follicles. In addition, identification of factors that stimulate DMI and upregulate hepatic β-oxidation capacity during the transition period would have immediate beneficial impact.

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Table 1. Ingredient and nutrient composition of prepartum and postpartum diets.

Ingredient (% of DM)	Prepartum	Postpartum
Corn silage	29.8	23.2
Legume haylage	14.7	15.7
Alfalfa hay	17.0	8.4
Cornmeal	-	19.0
High moisture shelled corn	25.2	7.8
Soybean meal	4.9	7.6
Homermeal[1]	4.9	6.4
Whole cottonseed	-	6.0
Yeast culture[2]	0.9	-
Ca-salts of long chain fatty acids[3]	-	1.9
Urea (45% N)	-	0.2
Mineral/vitamin premix[4,5]	2.5	1.4
Sodium bicarbonate	-	1.3
Limestone	-	0.5
Biophos[6]	-	0.2
White salt	-	0.5
Nutrient composition (DM basis)[7]		
$NE_{L,}$Mcal/kg	1.63	1.73
NDF, %	34.0	29.0
NFC, %	33.3	38.5
Starch, %	25.1	23.5
Crude fat, %	4.4	6.1
CP, %	16.4	18.3
Lignin, %	5.4	4.8
Ca, %	0.88	1.03
P, %	0.43	0.44
K, %	1.36	1.29
Mg, %	0.32	0.32

[1] Hi-bypass soybean meal, Homer Oil Co., Homer, NY.

[2] Diamond V XP, Cedar Rapids, IA.

[3] Megalac®, Church and Dwight Co., Inc., Princeton, NJ.

[4] Prepartum mineral/vitamin: 16.5% Ca, 3% P, 5% Mg, 0.3% K, 5% Na, 8% Cl, 8% S, 35 ppm Co, 450 ppm Cu, 3,700 ppm Fe, 50 ppm I, 3,100 ppm Mn, 3,500 ppm Zn, 10 ppm Se, 330,000 IU/kg Vitamin A, 88,000 IU/kg Vitamin D, 1,800 IU/kg Vitamin E.

[5] Postpartum minerals and vitamins: 11.2% Ca, 8.0% Mg, 1.0% K, 5% 13.2, 20% Cl, 8% S, 57 ppm Co, 800 ppm Cu, 2,000 ppm Fe, 90 ppm I, 5,400 ppm Mn, 6,200 ppm Zn, 20 ppm Se, 550,000 IU/kg Vitamin A, 130,000 IU/kg Vitamin D, 3,000 IU/kg Vitamin E.

[6] Agrico Feed Ingredients Products, New Wales, FL.

[7] Values represent averages of monthly composites. Analysis by Dairy One Cooperative, Ithaca, NY.

Table 2. Effect of drenching with water (control) or propylene glycol (PPG) on DMI, milk yield, and milk composition during the transition period.

	Treatment			
Item	Control	PPG	SEM	*P*-value
Day –10 to –1 prepartum				
DMI, kg/d	14.0	13.4	0.3	0.16
Energy balance, Mcal/d	6.2	7.3	0.4	0.08
Day 0 to 30 postpartum				
DMI, kg/d	19.5	18.7	0.5	0.3
Energy balance, Mcal/d	-11.4	-9.4	0.5	0.009
Milk yield, kg/d	44.9	44.3	1.0	0.7
Milk fat, %	4.47	4.23	0.09	0.071
Milk protein, %	3.21	3.23	0.08	0.8
Milk lactose, %	4.62	4.74	0.04	0.027

Table 3. Effect of drenching with water (control) or propylene glycol (PPG) on mean circulating concentration of glucose, insulin and NEFA for 6 h post-drenching on d −10, 2, and 25 relative to parturition.

	Treatment			*P*-value	
	Control	PPG	SEM	Treatment	Trt × time
Glucose, mg/dL					
d −10	61.5	62.1	1.1	0.7	0.6
d 2	46.6	52.3	1.3	0.007	0.001
d 25	52.4	56.8	0.7	0.001	0.001
Insulin, ng/mL					
d −10	1.45	1.71	0.10	0.09	0.07
d 2	0.34	0.47	0.05	0.08	0.15
d 25	0.53	0.68	0.04	0.008	0.001
NEFA, μmol/L					
d −10	100	97	6	0.7	0.4
d 2	514	391	31	0.012	0.009
d 25	306	205	30	0.026	0.001

Table 4. Characteristics associated with dominant follicles of the first postpartum follicle wave after drenching with water (control) or propylene glycol (PPG).

	Treatment			
Item	Control	PPG	SEM	*P*-value
Ovulatory	9/28	8/28		
Peak plasma estradiol, pg/mL	5.8	5.9	0.48	0.9
Day of peak estradiol	15.1	17.4	1.6	0.3
Peak follicle diameter, mm	18.6	17.5	1.2	0.5
Day of peak follicle diameter	16.6	17.9	1.6	0.6
Non-Ovulatory (atretic)[1]	15/28	16/28		
Estradiol at peak follicle diameter, pg/mL	2.3	2.1	0.6	0.8
Peak follicle diameter, mm	16.8	16.5	0.9	0.8
Day of peak follicle diameter	21.7	19.0	0.9	0.046
Cystic	4/28	4/28		
Peak plasma estradiol, pg/mL	12.4	8.8	3.4	0.5
Day of peak estradiol	19.0	19.5	1.2	0.8
Peak follicle diameter, mm	31.5	30.5	2.3	0.8
Day of peak follicle diameter	27.3	29.3	1.5	0.4
LH pulse characteristics on d 10 postpartum				
Pulse frequency, no./12 h	7.8	7.1	0.5	0.4
Pulse amplitude, ng/mL	0.56	0.50	0.07	0.5
Mean LH, ng/mL	0.56	0.46	0.05	0.2

[1] A clear peak in plasma estradiol was not apparent for most cows in the atretic group; the value on the day of peak dominant follicle diameter is reported, and day of peak estradiol is omitted.

Table 5. Summary of dominant follicle development and estradiol production in cows grouped on the basis of follicle type observed during the first postpartum wave.

	Follicle type[1]				
Item	O	NH	NL[2]	C	*P* value
No. of cows	17	6	24	8	
Peak estradiol, pg/mL	5.8^{a} ± 0.7	6.0^{a} ± 1.1	0.9^{b} ± 0.6	10.6^{c} ± 1.0	<0.001
Day of peak estradiol	16.2 ± 0.9	16.8 ± 1.6		19.3 ± 1.4	0.19
Peak follicle diameter, mm	18.0^{ab}± 0.8	20.9^{a} ± 1.3	15.6^{b} ± 0.7	31.0 ± 1.1	<0.001
Day of peak follicle diameter	17.3^{a} ± 0.9	20.0^{a} ± 1.5	20.0^{a} ±0.8	28.3^{b} ±1.3	<0.001

[1] O = Ovulatory, NH = Non-ovulatory High estradiol, NL = Non-ovulatory Low estradiol, C = Cyst.

[2] The NL cows did not have a distinct day of peak estradiol. Peak estradiol value for cows with NL follicles corresponds to circulating estradiol on day of peak follicle diameter.

^{a,b,c} Within row means with no common superscript letters differ ($P \leq 0.05$).

Table 6. Summary of transition period EB and DMI in cows grouped on the basis of type of follicle observed during the first postpartum wave.

	Follicle type			
Item	O	NH	NL	C
Day –21 to –8				
EB, Mcal/d	$10.3^{a} \pm 0.6$	$13.6^{b} \pm 0.9$	$8.0^{c} \pm 0.5$	$8.2^{ac} \pm 0.8$
DMI, kg/d	$16.3^{a} \pm 0.4$	$18.3^{b} \pm 0.6$	$14.9^{c} \pm 0.3$	$15.3^{ac} \pm 0.5$
Day -7 to -1				
EB, Mcal/d	$7.9^{a} \pm 0.8$	$8.0^{a} \pm 1.4$	$3.8^{b} \pm 0.7$	$5.7^{ab} \pm 1.2$
DMI, kg/d	$14.4^{a} \pm 0.5$	$14.3^{a} \pm 0.9$	$11.9^{b} \pm 0.4$	$13.3^{ab} \pm 0.8$
Day 0 to 30				
EB, Mcal/d	$-8.3^{a} \pm 0.8$	$-9.0^{ab} \pm 1.2$	$-12.2^{b} \pm 0.6$	$-10.1^{ab} \pm 1.1$
DMI, kg/d	$20.3^{a} \pm 0.5$	$19.7^{ab} \pm 0.8$	$18.1^{b} \pm 0.4$	$19.7^{ab} \pm 0.7$

^{abc}Within row means not sharing a common superscript letter differ ($P \leq 0.05$).

Table 7. Multiple logistic regression model predicting the probability of the first follicle wave being ovulatory.

Model for ovulatory follicles	Coefficient	*P*-value	Model Assessment	
Intercept	−14.6867	0.005	(-2LogL) Chi-Square :	35.9
Duration of declining EB	−0.3282	0.013	P > Chi-Square:	<0.0001
Insulin, d 0 to 25 AUC[1]	0.6008	0.015	% concordant:	92.9
DMI, d −7 to −1 AUC	0.0963	0.032	% discordant:	6.9
Glucose, d 0	0.0560	0.031	% tied:	0.2
			Somers' D:	0.86

[1] AUC= area under the curve

Figure 1. Circulating concentrations of insulin and glucose during the transition period in cows drenched with either 500 mL of water (CTL) or 500 mL of propylene glycol (PPG). Samples were collected before drenching. Upper panel: concentration of glucose in plasma was determined by an enzymatic assay in 3 prepartum samples, and every other day postpartum. Circulating glucose tended (*P* = 0.10) to be greater in PPG treated cows (pooled SEM = 0.83 mg/dL). No treatment × time effect was detected. Lower panel: plasma insulin concentrations were determined by RIA in 3 prepartum samples, and every other day postpartum. A significant treatment × time effect was observed (*P* < 0.05; pooled SEM = 0.028 ng/mL), but no effect of treatment.

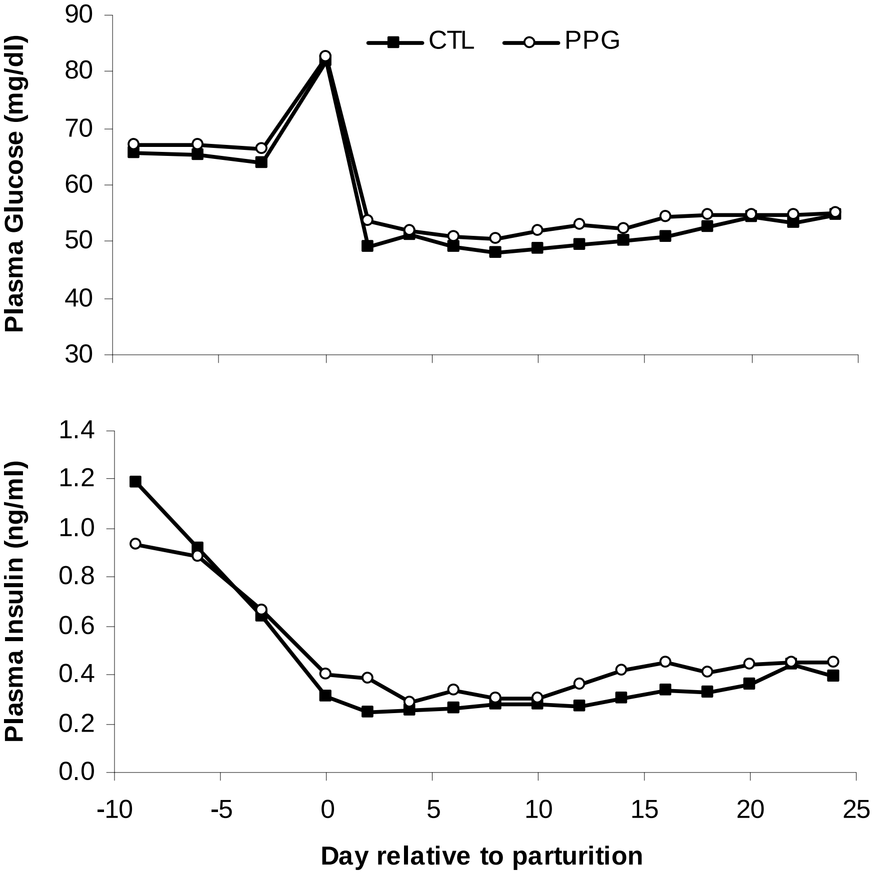

Figure 2. Circulating concentrations of NEFA and BHBA during the transition period in cows drenched with either 500 mL of water (CTL) or 500 mL of propylene glycol (PPG). Samples were collected before drenching. Upper panel: plasma NEFA concentrations were determined by enzymatic assay in 3 prepartum samples, daily from parturition until d 3 postpartum, and every third day thereafter. A significant treatment effect was observed ($P < 0.05$; pooled SEM = 21.4 µmol/l). No treatment × time interaction was detected. Lower panel: plasma BHBA concentrations were determined using an enzymatic assay in 3 prepartum samples, at parturition, and every third day thereafter. A significant treatment effect was observed ($P < 0.01$; pooled SEM = 0.56 mg/dL), but no treatment × time interaction was detected.

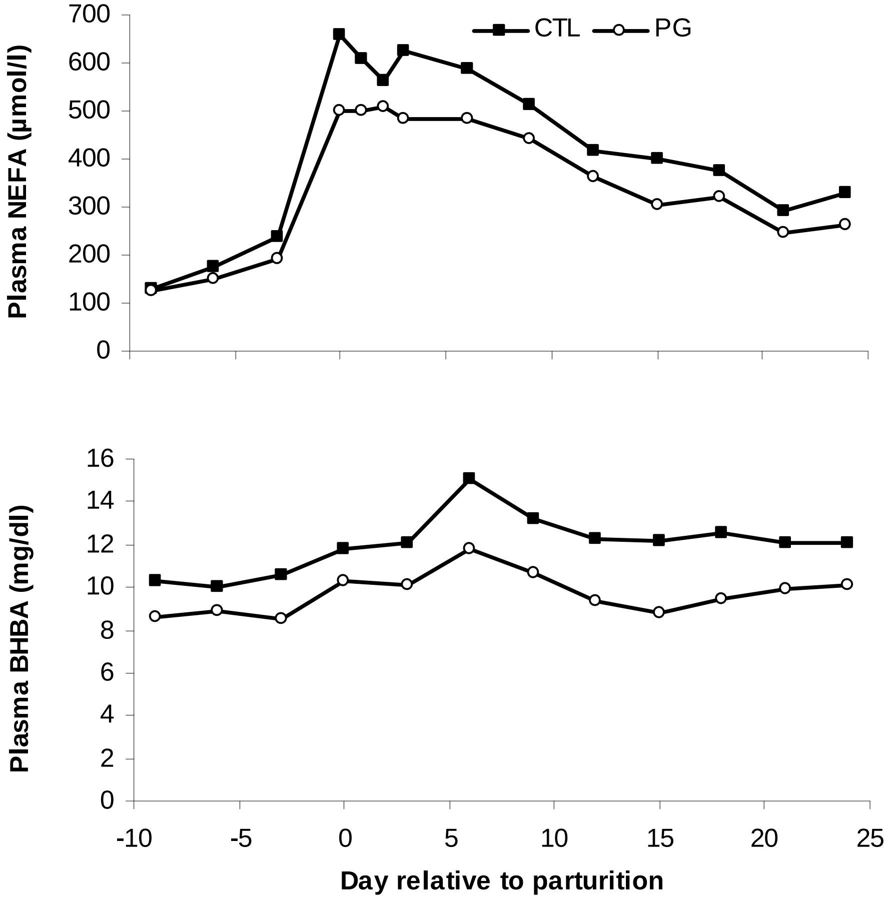

Figure 3. Triglyceride concentration in liver tissue collected on d 5 and 25 postpartum in dairy cows (n = 10 per treatment) drenched with either 500 mL of water (CTL) or 500 mL of propylene glycol (PPG). Cows were drenched from d –10 to 25. Triglyceride concentration tended (*P* = 0.08) to be lower in PPG treated cows than CTL (pooled SEM = 1.89%).

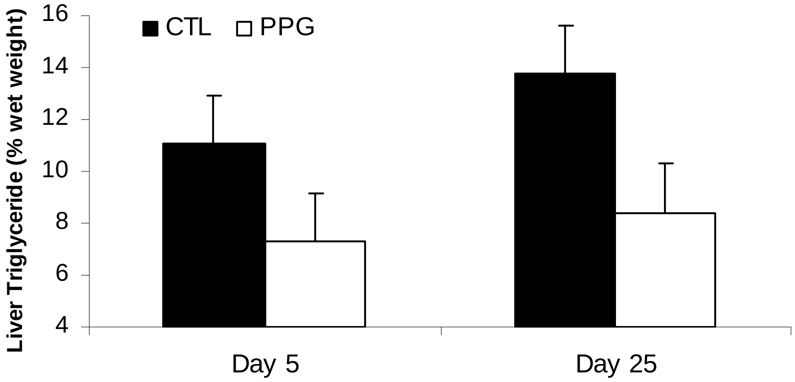

Figure 4. Patterns of follicular growth (solid lines) and circulating estradiol (lines with open circles) during the first postpartum follicle wave. (A) Growth of a single dominant follicle with a preovulatory rise in estradiol followed by an abrupt decline, and ovulation (*) of the dominant follicle. (B) Growth of a single dominant follicle with a preovulatory-like rise in estradiol followed by a rapid reduction in estradiol, failure of the dominant follicle to ovulate, and emergence of a new follicle wave while the first-wave dominant follicle underwent atresia. (C) A non-ovulatory first-wave dominant follicle that failed to exert dominance. The second wave emerged while the first-wave dominant follicle was still growing with no evidence of a preovulatory-like rise in estradiol. (D) A non-ovulatory first wave dominant follicle that exerted dominance and prevented emergence of a new wave until first-wave dominant follicle started to regress. No evidence of a preovulatory-like rise in estradiol. (E). Growth of a single dominant follicle, a preovulatory-like rise in estradiol followed by a rapid reduction, failure of the dominant follicle to ovulate, and rather than regressing, the follicle continued to grow and develop into a cyst. A new wave emerged approximately 3 d after the peak of the preovulatory-like rise in estradiol and continued to grow in the presence of the developing cyst. (F) Growth of a single dominant follicle, a preovulatory-like rise in estradiol followed by a further increase in estradiol to beyond typical LH surge inducing concentrations, failure of the dominant follicle to ovulate, and rather than regressing, the follicle continued to grow and develop into a cyst. Estradiol concentrations remained elevated, and emergence of a new follicle wave was not observed.

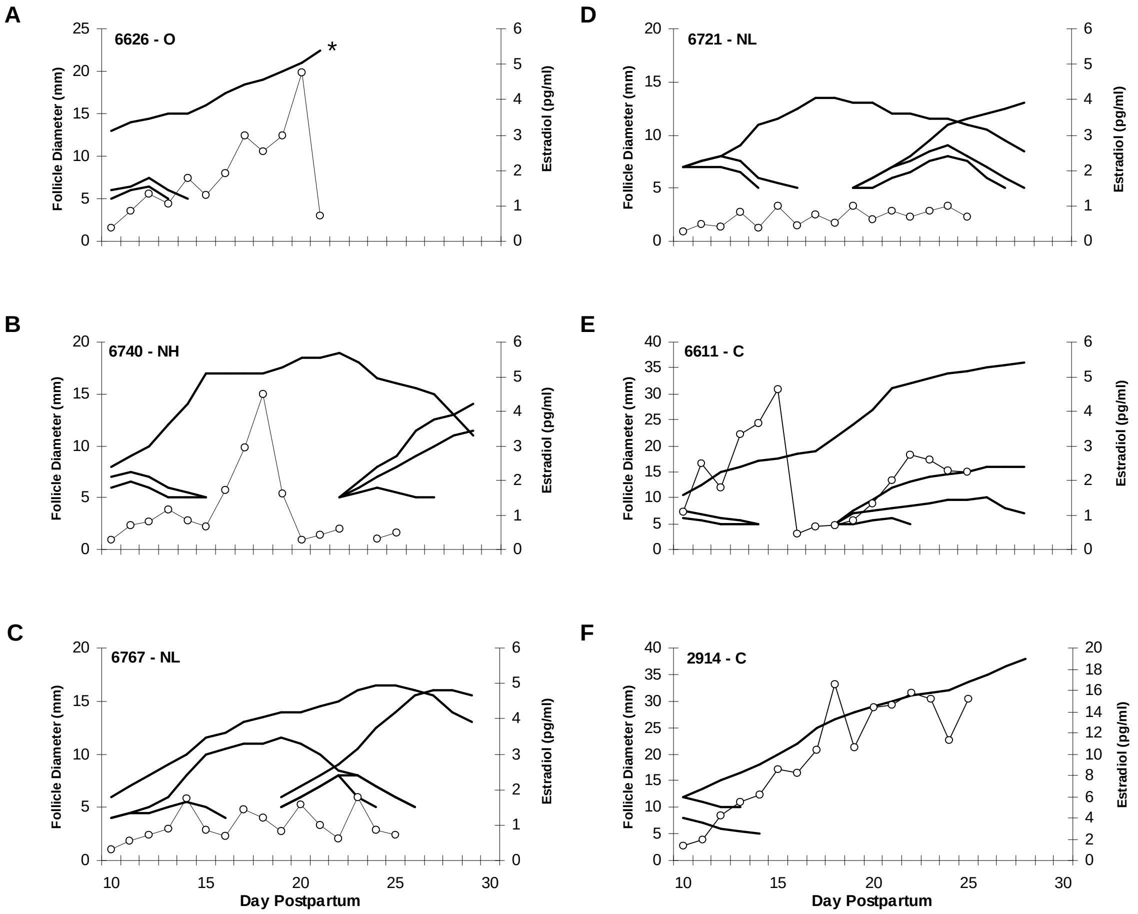
A
6626 - O
*
D
6721 - NL
B
6740 - NH
E
6611 - C
C
6767 - NL
F
2914 - C
Follicle Diameter (mm)
Estradiol (pg/ml)
Day Postpartum
10
15
20
25
30

Figure 5. The LOESS-smoothed curves of mean EB from d −10 to 30 in cows with different follicle types during the first postpartum follicle wave. Significant effects of follicle type ($P < 0.001$) and follicle type × time ($P < 0.05$) were observed. The SEM were 0.46, 0.77, 0.39, and 0.68 Mcal/d for O, NH, NL and C cows, respectively.

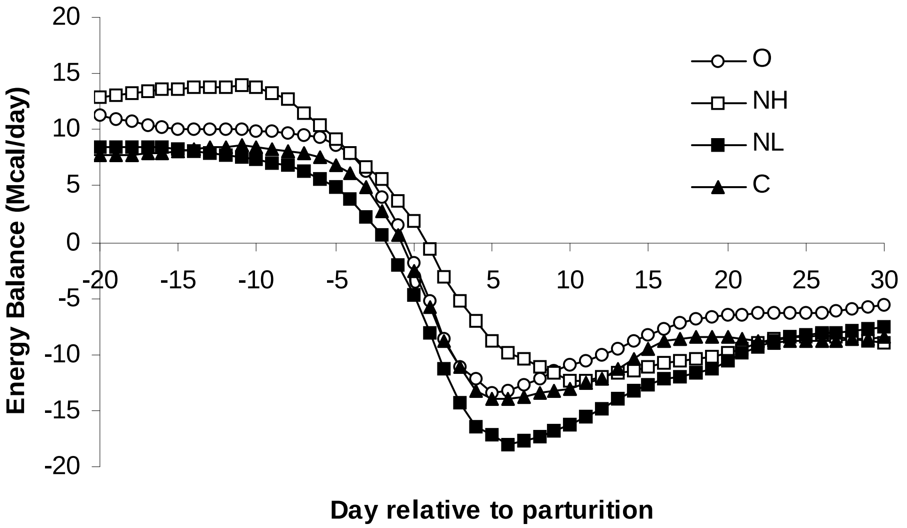

Figure 6. Profiles of circulating insulin, glucose, and the molar ratio of circulating glucose:insulin from d –10 to 25 in cows having different follicle types during the first postpartum follicle wave. A) plasma insulin concentrations were determined by RIA in 3 prepartum samples, and every other day postpartum. A significant (*P* < 0.001) effect of follicle type was observed. The SEM were 0.03, 0.04, 0.02, and 0.04 ng/mL for O, NH, NL, and C cows, respectively. B) plasma concentrations of glucose were determined in 3 prepartum samples, and every other day postpartum. Significant effects of follicle type (*P* <0.01) and follicle type by time (*P* < 0.001) were observed. The SEM were 0.7, 1.2, 0.6, and 1.0 mg/dL for O, NH, NL, and C cows, respectively. C) Molar ratio of glucose:insulin in plasma. A significant (*P* < 0.001) effect of follicle type was observed. The SEM were 3.5, 5.7, 2.9, and 4.9 for O, NH, NL, and C cows, respectively. Conversion factor used for glucose was mg/dL × 0.0554 = m*M*, and for insulin ng/mL × 0.1744 = n*M* (Mr bovine insulin = 5733).

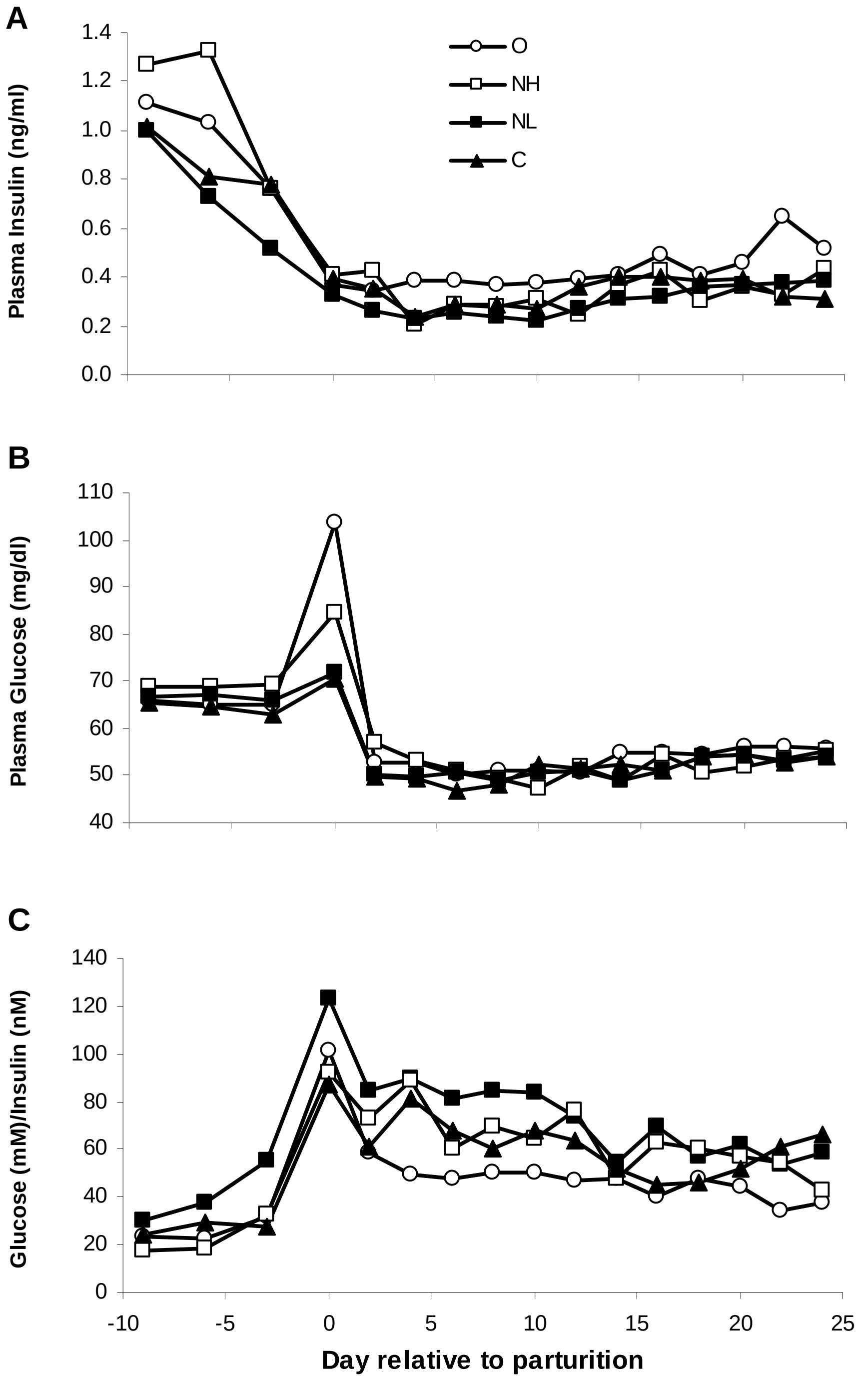
A
Plasma Insulin (ng/ml)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
O
NH
NL
C
B
Plasma Glucose (mg/dl)
110
100
90
80
70
60
50
40
C
Glucose (mM)/Insulin (nM)
140
120
100
80
60
40
20
0
-10
-5
0
5
10
15
20
25
Day relative to parturition

Figure 7. Plasma IGF-I from d -7 to 25 relative to parturition in cows having different follicle types during the first postpartum follicle wave. A significant ($P < 0.01$) effect of follicle type was observed. The SEM were 4.9, 8.5, 4.4 and 7.4 ng/mL for O, NH, NL and C cows, respectively.

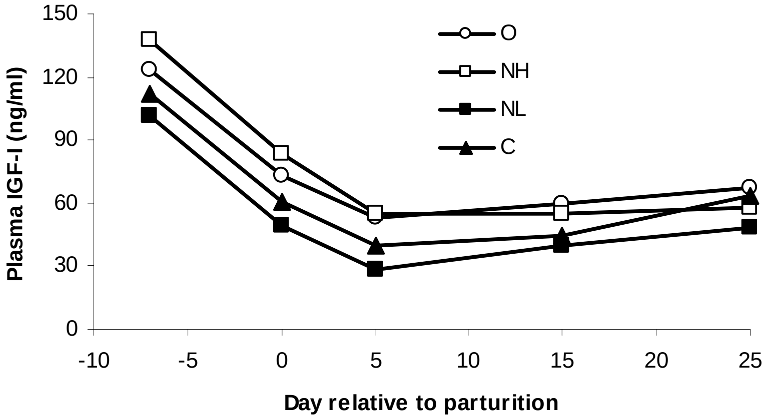

Figure 8. Plasma NEFA concentrations from d −10 to 25 relative to parturition in cows having different follicle types during the first postpartum follicle wave. A significant ($P < 0.01$) effect of follicle type was observed. The SEM were 5, 9, 4, and 7 µmol/l for O, NH, NL, and C cows, respectively.