Blood metabolites and hormone-based programmed breeding treatments in anovular lactating dairy cows

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ABSTRACT

The objectives were to (1) compare blood metabolites and reproductive outcomes in lactating dairy cows not inseminated before (early) and after (late) 100 d in milk (DIM) because of prolonged anovulation or anestrus; and (2) evaluate reproductive responses of cows ≤ 100 DIM to GnRH + PGF_{2 α} treatments after a fixed-time artificial insemination (AI; Ovsynch) or after induced estrus (Select Synch). In blood samples collected before initiating hormone-based breeding programs, anovular cows ≤ 100 DIM had the greatest serum total protein and globulin concentrations and the lowest tri-iodothyronine concentrations. Anovular and ovular cows > 100 DIM had the greatest serum urea concentrations. Ovaries in cows ($n = 40$) > 100 DIM were examined by transrectal ultrasonography, and those without a detectable corpus luteum (CL; anovular) were given GnRH and then PGF_{2 α} 7 d later (Select Synch), whereas cows with a CL (ovular) were given 2 PGF_{2 α} injections 12 d apart. Cows were inseminated at observed estrus after the second or only PGF_{2 α} injection. More ovular (79%; 15/19) than anovular cows (24%; 5/21) were detected in estrus. No differences were detected between ovular and anovular cows in DIM at first AI, conception rate to first AI, cumulative pregnancy rates, number of services per conception, or days open. Cows ($n = 93$) ≤ 100 DIM were assigned randomly to 3 treatments: (1) control ($n = 20$) AI at estrus; (2) GnRH and then PGF_{2 α} on 7 d (Select Synch; $n = 42$) and monitored for signs of estrus for 5 d and AI accordingly; or (3) 2 GnRH injections 9 d apart with PGF_{2 α} given 48 h before second GnRH injection and AI at 16 h after the second GnRH injection (Ovsynch; $n = 31$). Among cows ≤ 100 DIM, controls had more days to first service (149 ± 16 d) than Select Synch cows (117 ± 7 d). Ovsynch cows had the fewest days to first service (84 ± 10 d) compared with control (149 ± 16 d) and Select Synch (117 ± 7 d) cows. Conception rates

in control (25%) and Select Synch (26.2%) cows did not differ from those in Ovsynch cows (29%). Ovsynch cows had greater cumulative pregnancy rates and fewer days open than control (161 ± 20 vs. 258 ± 29 d), but did not differ from Select Synch (233 ± 19 d). Timed AI produced comparable fertility and superior cumulative pregnancy rates, fewer days to first service, and fewer days open than AI at observed estrus in cows inseminated ≤ 100 DIM.

Key words: anovulation, blood metabolite, hormone-based insemination

INTRODUCTION

Anestrus is a major contributor to infertility because a significant proportion of cows, ranging from 8 to 56% (El-Zarkouny et al., 2004; Silva et al., 2007), have not initiated ovulatory estrous cycles before an AI program is implemented. A major challenge for AI of dairy cows is the absence of estrus or such a subtle display of estrual symptoms that cows may not be detected in estrus for prolonged periods after calving. Therefore, DIM at first service and days open are extended.

Anovulation in lactating dairy cows may be associated with disease, low DMI, negative energy balance, and low body condition (Stevenson et al., 1997). Metabolic markers may provide some information about the causes and correlative effects of various factors associated with prolonged anovulation. Efficiency of reproduction is influenced by factors that affect the establishment of pregnancy, such as dietary protein (Elrod and Butler, 1993; Elrod et al., 1993), milk yield (Laben et al., 1982), and energy balance (Haresign, 1981). High protein diets can be detrimental to expression of estrus (Orihuela, 2000). Greater dietary protein intake will increase milk yield, which is not emulated entirely by energy intake, subsequently exacerbating negative energy balance. Negative energy balance will increase the risk of prolonged anestrus (Ferguson, 1991) resulting from reduced follicular development, which will reduce estradiol production and subsequent expression of estrus.

Anovular cows that were retrospectively identified after synchronization of ovulation and timed AI (TAI)

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had reduced fertility and greater pregnancy loss than their cycling herd mates (Sterry et al., 2006). Treatment of anovular cows with GnRH 5 d after TAI improved fertility for anovular cows but not for cycling cows. Strategies developed to resolve anovulation before or after TAI may be best achieved by identifying and targeting those anovular cows that may benefit from treatment rather than treating all cows (Silva et al., 2007). Cartmill et al. (2001) reported that 16.2% of cows were anovular by examining concentrations of progesterone in 3 blood samples collected before initiation of Ovsynch (2 GnRH injections 9 d apart with PGF_{2α} given 48 h before second GnRH injection and AI at 16 h after the second GnRH injection), whereas Gümen et al. (2003) used 2 consecutive weekly ultrasound examinations and blood samples to detect the presence of luteal tissue and serum progesterone and reported that 20.2% of cows were anovular.

Ovulation synchronization programs using only PGF_{2α} or combined with GnRH have been used widely to improve reproductive outcomes of dairy cows (Caraviello et al., 2006). Programs that regulate follicular development and luteal lifespan with sequential injections of GnRH and PGF_{2α} improve estrus detection rates and synchrony of estrus (Pursley et al., 1997).

To determine potential relationships between various blood metabolites and anovulation, the objectives were to (1) compare blood metabolite concentrations and reproductive outcomes in lactating dairy cows inseminated before (early) and after (late) 100 DIM; and (2) evaluate reproductive responses of cows ≤ 100 DIM to GnRH + PGF_{2α} treatments after a fixed-time AI (Ovsynch) or after induced estrus (Select Synch).

MATERIALS AND METHODS

Cow Management

The study was conducted in a dairy herd that consisted of 700 lactating Holstein cows in Egypt with an average daily milk yield of 25 kg per cow. Cows were housed in open barns with shades and machine-milked thrice daily. Cows were offered green artichoke (7% of BW) from January to April, artichoke silage (3.5% of BW) from May to June, chopped green corn (4% of BW) from July to August, and corn silage (2% of BW) and berseem (5% of BW) from September to December. A concentrate mix (16% CP) consisting of ground yellow corn, soybean meal, wheat bran, cottonseed cake, molasses, rumen-protected fat (5%), and minerals (premix) was offered to cows every 6 h to meet or exceed NRC (1989) recommendations for lactating dairy cows. The total amount of the concentrate mix was 1 kg per

2 kg of milk (3.5% fat) or 2.5% of BW of cows. Cows had access to fresh water ad libitum.

Synchronization of Estrus in Previously Noninseminated Cows with Extended Anovulation (>100 DIM)

Transrectal ultrasound was implemented to identify anovulation in 40 cows having extended postpartum intervals (mean = 220 ± 10 and median = 193 DIM) by subjecting them to one ovarian examination using a Dynamic Imaging scanner (Concept MLV, Livingston, UK) with a B-mode, real-time, 5.0- and 7.5-MHz dual probe. The 5.0-MHz frequency feature of the probe was used to scan deeper structures such as uterine contents, whereas the 7.5-MHz feature was used to scan ovaries when greater resolution was needed. Both ovaries were scanned to detect the number and diameter of the largest follicles, and number and diameter of each corpus luteum (CL). Based on the presence of certain ovarian structures, cows were allotted to 2 treatments (Figure 1A). Select Synch (anovular, $n = 21$) cows were those having no CL present at the time of ovarian examination with or without a follicle ≥ 10 mm capable of ovulation in response to GnRH. Select Synch cows were treated with 8 μ g of buserelin (GnRH analog; Receptal, Intervet International B.V., Boxmeer, Holland) followed in 7 d with 500 μ g of PGF_{2α} analog (Estrumate, Schering-Plough Animal Health, Friesoythe, Germany). Estrus was monitored closely twice daily for 1 h each for 5 d after PGF_{2α} injection. Detection of estrus was done visually in the morning and afternoon by herds-men. No estrus detection aids were used. Cows detected in estrus in the morning were inseminated with frozen-thawed semen in the evening of the same day and those detected in estrus in the evening were inseminated early the next morning. A second treatment ($2 \times$ PGF_{2α}) was applied to the remaining cows having a CL (ovular; $n = 19$). These cows were given 2 injections of PGF_{2α} 12 d apart. Estrus was monitored closely twice daily for 1 h each for 5 d after each PGF_{2α} injection. Cows observed in estrus were inseminated using frozen-thawed semen. Cows exhibiting estrus and inseminated after the first PGF_{2α} injection did not receive the second PGF_{2α} injection. Body condition score (1 = emaciated and 5 = obese; Wildman et al., 1982) was evaluated by the same individual at the time of enrollment.

Synchronization of Ovulation or Estrus in Previously Noninseminated Cows (≤ 100 DIM)

Eight clusters of at least 10 lactating cows each ($n = 93$; mean and median = 64 ± 2.3 DIM) were assigned

Figure 1. Synchronization of estrus or ovulation in dairy cows with (A) extended (≥ 100 DIM; late) or (B) normal postpartum intervals to first service (≤ 100 DIM; early). Select Synchrony = GnRH-PGF_{2α} (detection of estrus + AI); 2 × PGF = PGF_{2α}-PGF_{2α} (detection of estrus + AI). US = ultrasound examination; B = blood sample; TAI = timed AI.

randomly to 3 treatments based on BCS and lactation number at the time of enrollment (Figure 1B). Control cows ($n = 20$) received no treatment, but were AI using frozen-thawed semen after daily detection of estrus. Select Synchrony cows ($n = 42$) were administered 8 μ g of buserelin (GnRH analog; Receptal) followed in 7 d with 500 μ g of PGF_{2α} analog (Estrumate, Schering-Plough Animal Health). Cows were monitored closely for signs of behavioral estrus for 5 d and inseminated using frozen-thawed semen. Select Synchrony cows not detected in estrus received the same protocol 14 d later until inseminated. The third treatment (Ovsynch, $n = 32$) consisted of 2 injections of 8 μ g of buserelin (Receptal, Intervet International) 9 d apart plus 1 injection of 500 μ g of PGF_{2α} analog (Estrumate, Schering-Plough Animal Health) given 48 h before the second injection of GnRH. Cows received a timed AI using frozen-thawed semen 16 to 20 h after the second injection of GnRH. The first GnRH injection was administered randomly

without regard to stage of the estrous cycle or anovulation.

Blood Collection

Blood samples were collected via coccygeal venipuncture before each hormonal injection or at corresponding times from control cows (Figure 1). Blood samples were stored at 4°C for 12 h until serum was harvested after centrifugation ($1,935 \times g$ for 20 min). Sera samples were stored at -20°C until assayed. Concentrations of serum metabolites and tri-iodothyronine (T₃) were measured in the first blood sample collected from each cow. Measurements of serum progesterone concentration were carried out in all blood samples.

Biochemical Analysis of Serum Metabolites and Hormonal Assays

Serum total protein, albumin, urea, glucose, total lipids, Na, K, and P were determined colorimetrically us-

ing a visible light spectrophotometer (Pharmacia, LKB Biochrom, Cambridge, UK). All assays were carried out using commercial kits according to the manufacturers' guidelines. Serum globulin was calculated by subtracting serum albumin concentrations from serum total protein concentrations.

An ELISA was used to measure serum concentrations of progesterone (Calbiotech Inc., Spring Valley, CA). The lower limit of detection of progesterone was 0.3 ng/mL. Intra- and inter-assay CV were 7.1 and 2.4%, respectively. Progesterone concentrations <1 ng/mL in the first blood sample was used to classify early-inseminated cows ≤ 100 DIM into anovular or ovular (≥ 1 ng/mL) status. Concentrations of T3 in serum were measured using solid-phase enzyme immunoassay kits (Diatech Diagnostic Inc., Boston, MA). The lower limit of T3 detection (95% B/Bo, where B = mean optical density of each pair of wells and Bo = mean optical density of "0" standard wells) was 0.16 ng/mL and intra- and interassay CV were 6.4 and 5.1%, respectively.

Reproductive Outcomes

Pregnancy was diagnosed by transrectal ultrasonography 29 to 30 d after AI and later confirmed by transrectal palpation of the uterus and its contents 40 to 50 d after AI by the herd veterinary practitioner. Cows that returned to estrus were reinseminated accordingly.

Reproductive outcomes included DIM at first service, conception rate to first AI, cumulative pregnancy rate (**PR**), number of services per conception, and days open. Days to first service was the difference between calving and the first AI date; **CR/AI** = proportion of cows pregnant divided by the number of cows inseminated; **PR** = proportion of cows pregnant divided by the number of cows enrolled in the study; and days open = interval between calving and confirmed pregnancy.

Statistical Analyses

Data for serum total protein, albumin, globulin, urea, glucose, total lipids, Na, K, P, and T3 were analyzed in separate linear models (procedure GLM; SAS Inst. Inc., Cary, NC) with ovarian status (anovular vs. ovular) and DIM (≤ 100 vs. >100) as main effects. Other variables (BCS, lactation number, and milk yield) and treatment by lactation number interactions were included in the model. To determine relationships among metabolites and conception status, pregnancy status was included in subsequent models.

Proportion of cows >100 DIM displaying estrus after $\text{PGF}_{2\alpha}$ treatment was analyzed by using χ^2 (SAS Inst. Inc.) and the Fisher exact option with lactation num-

ber (first- vs. multiple-lactation cows), BCS (<2.5 vs. ≥ 2.5), and daily milk yield (<25 vs. ≥ 25 kg), and their interaction with treatment included in the model.

Multivariate logistical regression models (procedure LOGISTIC, SAS Inst. Inc.) were developed to analyze the effects of hormone treatments (Select Synch and $2 \times \text{PGF}_{2\alpha}$ in cows >100 DIM and control, Select Synch, and Ovsynch in cows ≤ 100 DIM), lactation (first- vs. multiple-lactation cows), BCS (<2.5 vs. ≥ 2.5), and daily milk yield (<25 vs. ≥ 25 kg) on categorical outcome variables (CR/AI and overall PR). All multivariate logistical regression models were constructed using a backward selection procedure with treatment retained as a fixed effect in each model. A Wald statistic criterion of $P < 0.15$ was set for including variables in the model. Odds ratios (**OR**) and 95% CI were calculated for significant and nonsignificant factors remaining in the final models. Results presented are percentages and proportions with P -values for main effects derived from the multivariate logistical regression analysis.

Days in milk at first service, number of services per conception, and days open were analyzed by ANOVA (procedure GLM; SAS Inst. Inc.) with Select Synch and $2 \times \text{PGF}_{2\alpha}$ treatments (cows >100 DIM) or control, Select Synch, and Ovsynch treatments (cows ≤ 100 DIM) plus BCS, lactation number, daily milk yield, and interactions with treatments included in the model as described previously.

Progesterone concentrations in the second blood sample were analyzed by ANOVA (procedure GLM; SAS Inst. Inc.) with Select Synch and $2 \times \text{PGF}_{2\alpha}$ (cows >100 DIM) and control, Select Synch, and Ovsynch (cows ≤ 100 DIM) treatments, plus BCS, lactation number, and daily milk yield, and their interactions with treatment included in the model.

Differences among least squares means were detected by the method of least-significant difference only when F -tests were significant ($P \leq 0.05$) in the ANOVA.

RESULTS

Blood Biochemical Traits

Serum concentrations of total protein, albumin, globulin, urea, T3, total lipids, glucose, Na, K, and P in cows before hormone-based breeding treatments are illustrated in Figure 2 based on their ovulatory status and stage of lactation.

Total Protein. Early anovular cows (≤ 100 DIM) had greater ($P < 0.01$) serum total protein concentrations (11.6 ± 0.9 g/dL) compared with late (>100 DIM) anovular and ovular cows and early ovular cows (8.7 ± 0.5 , 7.6 ± 0.3 , and 8.5 ± 0.3 g/dL, respectively).

First-lactation cows had greater ($P < 0.05$) serum total protein (9.5 ± 0.5) than multiple lactation cows (8.4 ± 0.4 g/dL).

Albumin. Late anovular cows had lesser ($P < 0.01$) serum albumin concentrations (3.6 ± 0.3 g/dL) than early anovular (5.0 ± 0.4 g/dL) and early ovular (4.6 ± 0.2 g/dL) cows. Serum globulin concentrations were greater ($P < 0.01$) in early anovular cows (6.6 ± 0.8 g/dL) than in early ovular (3.9 ± 0.3 g/dL) cows and late anovular (5.1 ± 0.6 g/dL) and late ovular (3.5 ± 0.2 g/dL) cows.

Urea. Late (anovular and ovular) cows had greater ($P < 0.01$) serum urea concentrations (43.5 ± 2.5 and 39.2 ± 3.2 mg/dL) than early (anovular and ovular) cows (28.5 ± 1.8 and 31.7 ± 1.1 mg/dL). Late cows with low (<2.5 , $n = 28$) BCS had greater ($P < 0.05$) serum urea concentration (44.6 ± 2.2 mg/dL) than late cows with higher (≥ 2.5 , $n = 12$) BCS (34.0 ± 3.6 mg/dL) and early cows either with low (<2.5) BCS (31.0 ± 1.1 mg/dL) or higher (≥ 2.5) BCS (31.4 ± 1.9 mg/dL). Cows that conceived to first AI had lower ($P < 0.01$) serum urea concentrations (29.4 ± 2.8 mg/dL) than those failed to conceive to first AI (37.9 ± 1.4 mg/dL).

T3. Early anovular cows had lower ($P < 0.05$) serum T3 concentrations (2.5 ± 0.2 ng/mL) than early ovular (4.2 ± 0.3 ng/mL) and late anovular (4.1 ± 0.3) and late ovular (4.0 ± 0.4 ng/mL) cows.

Total Lipid. Late ovular cows had lower ($P < 0.05$) serum total lipid concentration (738 ± 46 mg/dL) than late anovular (897 ± 36 mg/dL) and early ovular (987 ± 35 mg/dL) cows, but did not differ from early anovular (849 ± 43) cows.

Potassium. Late anovular cows had greater ($P < 0.01$) serum K concentrations (7.5 ± 0.1 mmol/L) than late ovular (5.6 ± 0.2 mmol/L) cows. Serum K concentration in early anovular (6.4 ± 0.5 mmol/L) cows did not differ from that in early ovular cows (6.6 ± 0.3 mmol/L), but remained different from that in late ovular cows (5.6 ± 0.2 mmol/L). First-lactation cows had greater ($P < 0.05$) serum K concentrations (7.0 ± 0.1) than older cows (6.4 ± 0.2 mmol/L). In addition, cows that conceived at first AI service had greater ($P < 0.05$) serum K concentrations than those cows that failed to conceive (7.0 ± 0.3 vs. 6.4 ± 0.2 mmol/L).

Other Metabolites. No differences were detected in serum glucose, Na, or P concentrations among cycling statuses and stages of lactation.

Reproductive Outcomes

Serum Progesterone. Ovarian scans revealed that 55% (21/40) of late cows (>100 DIM) were anovular (no detectable CL), but had 3.5 ± 0.5 follicles ≥ 10 mm. Average diameter of the largest follicle was 12.5 ± 1.2

mm. The remaining 45% (19/40) of late cows (>100 DIM) were ovular (had detectable luteal structures; 1.3 ± 0.2 CL/cow with a diameter of 19.4 ± 2.1 mm). Anovular cows had lower ($P < 0.05$) serum concentrations of progesterone (1.7 ± 0.1 ng/mL) at the time of the GnRH injection than ovular cows (4.9 ± 0.5 ng/mL). Ovular cows had a greater ($P < 0.01$) estrus detection rate (79%; 15/19) than anovular cows (24%; 5/21) after PGF_{2 α} .

Before the GnRH injection (Select Synch or Ovsynch), all early cows (≤ 100 DIM) had similar serum concentrations of progesterone. At the time of PGF_{2 α} injection (7 d after first GnRH injection), cows treated with Ovsynch and Select Synch had greater ($P < 0.01$) progesterone (4.2 ± 0.5 ng/mL) than controls (2.8 ± 0.5 ng/mL).

Fertility Traits. All late-AI cows had low CR/AI whether they were anovular (Select Synch) or ovular ($2 \times$ PGF_{2 α} , Table 1). Further, among early cows inseminated after detected estrus (Select Synch) or by appointment, CR/AI did not differ (Table 1). Fewer ($P = 0.049$; OR = 0.34, 95% CI = 0.11 to 0.99) early inseminated cows with BCS <2.5 conceived (21%; 11/54) than cows having BCS ≥ 2.5 (35%; 14/40). In addition, more ($P = 0.023$; OR = 4.13; 95% CI = 1.22 to 14.01; 37.5%, 12/32) early inseminated, first-lactation cows conceived at first AI compared with multiple-lactation cows (21.3%, 13/61).

Late inseminated ovular ($2 \times$ PGF_{2 α}) and anovular (Select Synch) cows had similar cumulative PR at the end of the 120-d breeding period (Table 2). In contrast, PR in early inseminated cows after observed estrus (Select Synch and control) did not differ, whereas Ovsynch-treated cows had greater PR than controls inseminated after observed estrus.

Early and late inseminated cows had similar cumulative PR (Table 2). Early cows having milk yield <25 kg (21.1 ± 0.5 kg) tended ($P = 0.08$; OR = 0.28, 95% CI = 0.07 to 1.17) to have lower PR than cows producing ≥ 25 kg (32.3 ± 0.5 kg) of milk.

No difference in days to first service was detected between Select Synch (anovular) and $2 \times$ PGF_{2 α} (ovular) cows after treatment (Table 3). Late cows with low milk yield (<25 kg/d) had more ($P < 0.01$) days to first service (276 ± 19 d) than cows with greater milk yield (≥ 25 kg/d; 198 ± 23 d). Early control cows inseminated at observed estrus had more ($P < 0.01$) days to first service than cows that received the Select Synch protocol (Table 3). Ovsynch cows had the fewest ($P < 0.01$) days to first service.

Number of services per conception did not differ for any of the comparisons examined (Table 3). Days open did not differ in late cows, but early cows that received the Ovsynch protocol had fewer ($P < 0.01$) days open

Figure 2. Serum concentrations of total protein, albumin, globulin, urea, tri-iodothyronine (T3), total lipids, glucose, Na, K, and P in early (≤ 100 DIM; median = 64) and late (> 100 DIM; median = 193) lactating dairy cows depending on ovular (solid bars) or anovular (open bars) status before treatment with hormone-based protocols. ^{a-c}Mean concentrations within measure having different letters differ ($P \leq 0.05$).

Table 1. Conception rates in late and early postpartum dairy cows after synchronization of ovulation or estrus with GnRH and PGF-based programs

DIM	Treatment	Conception rate, % (no./no.)	Odds ratio ¹	95% CI	P-value
Late ²	2 × PGF _{2α}	21.0 (4/19)	Referent		
	Select Synch	9.5 (2/21)	0.33	0.05–2.26	0.259
Early ³	Control	25.0 (5/20)	Referent		
	Select Synch	26.2 (11/42)	1.47	0.38–5.59	0.576
	Ovsynch	29.0 (9/31)	1.72	0.42–7.04	0.452
Overall					
Late		15.0 (6/40)	Referent		
Early		27.9 (25/93)	2.08	0.78–5.56	0.143

¹Odds ratio is function of estimated probability of pregnancy. Ratios exceeding 1 indicate increased probability of pregnancy, whereas ratios less than 1 indicate decreased probability of pregnancy.

²Late = cows not detected in estrus during first 100 DIM; 2 × PGF_{2α} = corpus luteum was present (ovular) and cows were treated with PGF_{2α} 12 d apart. Select Synch = No CL present (anovular) and cows received GnRH injection followed with PGF_{2α} injection 7 d later.

³Early = cows assigned to breeding clusters before 100 DIM. Control = detection of estrus + AI; Select Synch = GnRH injection 7 d before PGF_{2α}; Ovsynch = GnRH injection 7 d before and 48 h after PGF_{2α}, with timed AI administered 16 to 20 h after the second GnRH injection.

than controls and Select Synch cows. Early cows with higher BCS (≥ 2.5) had fewer ($P < 0.05$) days open (183 ± 19 d) than cows having low BCS (< 2.5 ; 237 ± 18 d).

DISCUSSION

Altered serum concentrations of total protein, globulin, and albumin and increased blood urea (observed in all cows > 100 DIM) may indicate some liver impairment that is associated with prolonged anovulation and low fertility. An excessive intake of protein may cause ruminal alkalosis and increased rumen ammonia, resulting in energy deficits and disorders of hepatocytes (Sommer, 1975).

Excess degradable (Elrod and Butler, 1993) or undegradable protein (Elrod et al., 1993) contributed to decreased uterine pH during the luteal phase, which might be involved in the observed reduction in fertility. Dairy cows are commonly fed diets containing large amounts of CP to maximize milk production (Rhoads et al., 2006). As a consequence of increased CP in their diets, lactating dairy cows have increased plasma urea nitrogen associated with decreased fertility (Ferguson, 1991; Butler et al., 1996).

In the present study, multiple-lactation anestrous cows (milk yield = 27 kg/d) or those with low BCS had greater blood urea concentrations compared with first-lactation cows (milk yield = 21 kg/d) and cows with high BCS (data not shown). Increased blood urea

Table 2. Total pregnancy rates in late and early postpartum cyclic dairy cows after synchronization with GnRH and PGF-based programs

DIM	Treatment	Cumulative pregnancy rate, % (no./no.)	Odds ratio ¹	95% CI	P-value
Late ²	2 × PGF _{2α}	73.6 (14/19)	Referent		
	Select Synch	76.2 (16/21)	1.16	0.23–5.81	0.857
Early ³	Control	70.0 (14/20)	Referent		
	Select Synch	85.7 (36/42)	3.19	0.81–12.53	0.096
	Ovsynch	93.5 (29/31)	7.54	1.22–46.49	0.029
Overall					
Late		75.0 (30/40)	Referent		
Early		84.9 (79/93)	1.88	0.75–4.69	0.175

¹Odds ratio is function of estimated probability of pregnancy. Ratios exceeding 1 indicate increased probability of pregnancy, whereas ratios less than 1 indicate decreased probability of pregnancy.

²Late = cows not detected in estrus during first 100 DIM; 2 × PGF_{2α} = corpus luteum (CL) was present (ovular) and cows were treated with PGF_{2α} 12 d apart. Select Synch = no CL present (anovular) and cows received GnRH injection followed with PGF_{2α} injection 7 d later.

³Early = cows assigned to breeding clusters before 100 DIM. Control = detection of estrus + AI; Select Synch = GnRH injection 7 d before PGF_{2α}; Ovsynch = GnRH injection 7 d before and 48 h after PGF_{2α}, with timed AI administered 16 to 20 h after the second GnRH injection.

Table 3. Days in milk at first service, services per conception, and days open in late (>100 DIM) and early (≤ 100 DIM) postpartum dairy cows after synchronization of estrus or ovulation with hormone-based programs

DIM	Treatment	n	Days to first service	Services per conception	Days open
Late ¹	2 $\times$ PGF _{2α}	19	277 $\pm$ 25	2.5 $\pm$ 0.4	345 $\pm$ 25
	Select Synch	21	239 $\pm$ 21	2.6 $\pm$ 0.4	341 $\pm$ 26
Early ²	Control	20	149 $\pm$ 16 ^a	2.6 $\pm$ 0.5	258 $\pm$ 29 ^a
	Select Synch	42	117 $\pm$ 7 ^b	3.4 $\pm$ 0.4	233 $\pm$ 19 ^a
	Ovsynch	31	84 $\pm$ 10 ^c	2.0 $\pm$ 0.5	161 $\pm$ 20 ^b
Overall					
Late		40	257 $\pm$ 16 ^d	2.6 $\pm$ 0.4	343 $\pm$ 18 ^c
Early		93	112 $\pm$ 6 ^c	3.0 $\pm$ 0.5	213 $\pm$ 13 ^d

^{a,b}Means within DIM group having different superscript letters differ ($P < 0.01$).

^{c,d}Overall means within column having different superscript letters differ ($P < 0.01$).

¹Late = cows not detected in estrus during first 100 DIM; 2 $\times$ PGF_{2 α} = corpus luteum (CL) was present (ovular) and cows were treated with PGF_{2 α} 12 d apart. Select Synch = no CL present (anovular) and cows received GnRH injection followed with PGF_{2 α} injection 7 d later.

²Early = cows assigned to breeding clusters before 100 DIM. Control = detection of estrus + AI; Select Synch = GnRH injection 7 d before PGF_{2 α} ; Ovsynch = GnRH injection 7 d before and 48 h after PGF_{2 α} , with timed AI administered 16 to 20 h after the second GnRH injection.

concentrations in anestrus cows of low body condition (<2.5) might have contributed to the increased days to first AI and more days open compared with early inseminated cows.

Consistent with our observations of decreased serum albumin in late anovular cows, albumin concentration was significantly decreased in cows with moderate and severe fatty liver compared with controls and cows having mild fatty livers (Sevinc et al., 2003).

A significant relationship between increasing concentrations of T3 or thyroid hormone and fertility provides evidence that thyroid hormones are metabolic signals that trigger the onset of ovarian cycles (Reist et al., 2003). Thyroid hormones have stimulatory effects on ovarian function in cattle acting at the level of granulosa and theca cells (Spicer et al., 2001). The finding of reduced serum T3 concentrations in anovular cows ≤ 100 DIM in the current study supports these studies.

Proportions of late ovular (45%) and anovular (55%) cows >100 DIM were similar in the current herd and these cows were classified as anestrus based on the absence of detected estrus (Rhodes et al., 2003). A short period of elevated progesterone concentration during the postpartum period resulting from endogenous or exogenous sources is important for subsequent expression of estrus and normal luteal function (McDougall et al., 1992). Therefore, the percentage of anovular cows displaying estrus after PGF_{2 α} treatment was low compared with their ovular herd mates that had a detectable CL from a previous ovulation and were exposed to endogenous progesterone.

Smaller follicles found in anovular cows produced meager CL after GnRH treatment and resulted in reduced progesterone concentrations. Follicles <10 mm

resulted in reduced ovulation risk to GnRH (Sterry et al., 2009). The low response to GnRH treatment might be explained on the basis that follicles cannot be classified as either normal or atretic by an ultrasound examination. Further, the average diameter of the largest follicle was 12.5 mm in the current study, which is less than the size of normal ovulatory follicles (Vasconcelos et al., 1999). Based on follicular diameter, these cows would have fit the first category of anovulatory follicles (deviation to ovular size of 9 to 14 mm in diameter) as classified by Wiltbank et al. (2002).

In both low- and high-yielding dairy cows, an economic benefit is associated with reducing the number of cows with extended intervals from calving to conception (Esslemont et al., 2001). Traditionally, treatment of cows that have not been detected in estrus but have a detectable CL has focused on the use of PGF_{2 α} (Rhodes et al., 2003). Within 6 d of treatment with PGF_{2 α} , 55% of cows with an ultrasonographically detectable CL were detected in estrus (Smith et al., 1998). When a CL was detected following ovarian palpation, estrous-response rates within 6 d of treatment averaged 52% and ranged from 36 to 68%, with the majority of cows displaying estrus within 4 d of treatment (Plunkett et al., 1984; Whittier et al., 1989). These results demonstrate that the estrous response to PGF_{2 α} treatment in this class of cows (anestrus with detectable CL) is not great compared with that observed in cows that resumed earlier estrous cycles, where 63 to 88% of cows were detected in estrus within 7 d of PGF_{2 α} treatment (Xu et al., 1997).

Conception rate following GnRH + PGF_{2 α} treatment and estrus detection + AI in the current study (9.5%) was comparable with that in anovular cows following

Ovsynch (9%; Gümen et al., 2003) or comparable with other reports from 24 to 37% in anovular cows after Ovsynch (Fricke and Wiltbank, 1999; Bartolome et al., 2000). An explanation for reduced CR/AI in anovular cows was the reportedly greater rate of post-AI premature luteal regression (23 vs. 6%) in anovular compared with ovular cows (Gümen et al., 2003).

Extended days open may have occurred because of impaired metabolic functions that resulted in increased serum urea and decreased albumin concentration in cows >100 DIM. Ovular cows had fewer days open (120 d) than anovular cows (150 d; Gümen et al., 2003). Ultrasound scanning started earlier (47 to 60 DIM) in the previous study compared with ≥ 100 DIM in the current study. In addition, DIM at first breeding was 256 d in our study, resulting in increased days open beyond what was reported earlier (Gümen et al., 2003).

Ovsynch treatment reduced days to first AI (54 vs. 83 d) compared with controls (Pursley et al., 1997). In the same study, days to second and third AI were also reduced. Conception rates to first AI were similar for control and Ovsynch, although Ovsynch cows were inseminated 19 d sooner. Our results support these previous reports where our cows inseminated after detected estrus (controls and Select Synch) and inseminated at fixed TAI (Ovsynch) had similar CR/AI. Our cumulative PR at the end of breeding period supports previous results (Pursley et al., 1997) where our Ovsynch and Select Synch cows had greater PR than controls. The cumulative PR in the present study was greater than that in other studies (Mialot et al., 1999; McDougall, 2001), where PR in Ovsynch cows were 36 and 58%, respectively. Disadvantages of AI after detected estrus (control and Select Synch) were the lower estrus detection rates compared with AI submission rates (100%) after Ovsynch and that the incidence of ovulation may be less after the PGF_{2 α} injection compared with the second GnRH injection given 48 h after the PGF_{2 α} injection in the Ovsynch program.

CONCLUSIONS

Extended postpartum intervals were associated with management omission of detected estrus in ovular cows and biochemical differences, including elevated concentrations of blood urea and reduced concentrations of albumin. Furthermore, all anovular cows regardless of stage of lactation had greater serum globulin than their ovular herd mates. Delayed synchronization treatment and reduced detection of estrus contributed to extended postpartum intervals to first AI and increased days open. More attention should be addressed to diet composition (degradable and undegradable protein), DMI, and BCS during the transition period. In addition,

prolonged anestrus may be circumvented before AI by applying GnRH + PGF-based treatments that induce ovulation and produce suitable pregnancy rates. In early postpartum cows, TAI produced comparable CR/AI to first AI and superior PR, fewer DIM at first AI, and fewer days open than AI after observed estrus. Reduced fertility at first AI was associated with lower serum K, low BCS, and elevated serum urea concentration.

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