Induction of successive follicular waves by gonadotropin-releasing hormone and prostaglandin $F_{2\alpha}$ to improve fertility of high-producing cows during the summer and autumn

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ABSTRACT

Reduced conception rate during the hot summer and subsequent autumn is a well-documented phenomenon. Evaporative cooling systems greatly increase milk production but only slightly improve reproductive performance; hence, additional approaches to improving fertility during the hot season are required. The purpose of the present study was to examine whether the combination of an efficient cooling system and hormonal manipulation (GnRH + PGF_{2α}) might improve fertility during the summer and autumn. The experiment was conducted from July to December in 2 commercial herds in Israel and included 382 healthy Holstein cows. Cows (50 to 60 d in milk) were hormonally treated to induce 3 consecutive 9-d follicular waves, with GnRH administration followed by PGF_{2α} injection 7 d later. Both control (n = 187) and treated (n = 195) cows were inseminated following estrus, and pregnancy was determined by palpation 45 d post-insemination. Data revealed an interaction between treatment and primiparous cows, reflected by a 16% increase in conception rate [odds ratio (OR) 2.32, 95% confidence interval (CI): 0.96–5.61] and 14% increase in pregnancy rate at 120 d in milk (OR 3.16, 95% CI: 0.93–10.47). Interaction between treatment and high body condition score was reflected by a 14% increase in pregnancy rate at 90 d in milk (OR 3.02, 95% CI: 1.14–7.96). About 60% of the treated cows expressed estrus at the expected time (normal response within 5 d following the third PGF_{2α} injection); the remaining 40% that manifested estrus later (late response) had higher milk yield and lower body condition score. Additional analyses indicated that treatment interacted with normal response to raise conception rates and pregnancy rates of primiparous cows and cows with high body condition score. On the other hand, treatment by late-response interaction lowered conception rate during the summer. Implementa-

tion of such hormonal treatment in combination with an efficient cooling system may improve reproductive performance of dairy cows during the summer and subsequent autumn.

Key words: fertility, gonadotropin-releasing hormone, hot season, prostaglandin $F_{2\alpha}$

INTRODUCTION

Fertility of high-lactating cows during the hot season is greatly reduced because of the deleterious effects of heat stress on reproduction. For instance, data from the Israeli Herd Book (ICBA, 2006–2008) indicate that first AI conception rate (CR) for cows is 38.7% in the winter (January to March), decreases to 22.0% during the summer (July to September), and slightly increases to 28.9% in the autumn (October to December). Evaporative cooling systems, which are based on intervals of wetting and ventilation, greatly increase milk production but only slightly improve CR (Berman and Wolfenson, 1992; De Rensis and Scaramuzzi, 2003). Additional approaches to improving fertility during the summer and autumn are required.

The effects of heat stress on follicular growth and function have been well documented: they are characterized by reduced size of the first- and second-wave dominant follicles (Badinga et al., 1993; Wilson et al., 1998), impaired growth of medium-sized follicles (6–9 mm), and attenuation of dominance (Badinga et al., 1993; Wolfenson et al., 1995; Roth et al., 2000). In addition, several studies indicate that not only the follicles but also their enclosed oocytes are highly susceptible to thermal stress and that a period of 2 to 3 estrous cycles is required to resume normal fertility during the fall (Roth et al., 2001a). Accordingly, it has been shown that heat stress effects are transient, as reflected by the spontaneous improvement of fertility throughout autumn and early winter (Zeron et al., 2001).

Alterations in follicular steroidogenesis and circulating hormone concentrations indicate that the hormonal milieu is also affected in cows exposed to heat stress. Decreased estradiol and androstenedione production

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was recorded in granulosa and thecal cells obtained from follicles during the summer and fall (Wolfenson et al., 1997) and 3 to 4 wk after acute heat stress (Roth et al., 2001b). Estradiol concentration in follicular fluid aspirated from cows was relatively low in late summer and increased throughout autumn (Roth et al., 2004). A reduction in steroid production during the hot season leads to reduced estradiol concentrations in the plasma (Wolfenson et al., 1995, 1997), which in turn may affect the preovulatory LH surge. This was demonstrated by the low amplitude of the GnRH-induced LH surge in heat-stressed cows expressing low estradiol concentration (Badinga et al., 1993; Gilad et al., 1993).

Our previous findings indicate that heat stress also affects the ovarian pool of small, approximately 0.5 to 1.0 mm in diameter, antral follicles, resulting in carry-over effects on follicular function (Roth et al., 2001a). Therefore, mechanical and hormonal strategies to enhance the removal of impaired follicles and the reappearance of healthy follicles have been suggested (for review, see Roth, 2008). An FSH-induced increase in the number of follicles emerging within follicular waves resulted in earlier ovarian recovery following summer thermal stress. This was reflected by higher morphological grade-1 oocytes and increased cleavage rate in oocytes aspirated from FSH-treated cows in the autumn (Roth et al., 2002). Induction of several consecutive follicular waves by GnRH and PGF_{2α} resulted in the development of healthy preovulatory follicles in heat-stressed cows relative to normothermal cows (Guzeloglu et al., 2001). Similarly, induction of 6 successive 9-d follicular waves with GnRH and PGF_{2α} through the autumn increased follicular volume and steroid concentration of previously heat-stressed preovulatory follicles (Roth et al., 2004). Nevertheless, although the above approach appears to improve ovarian function, its possible beneficial effect on fertility of dairy cows has not been examined.

The objective of the present study was to examine whether induction of short successive follicular waves by GnRH and PGF_{2α} improves dairy cow fertility during the summer and autumn. As cooling is an essential management practice to alleviate the deleterious effects of thermal stress on reproduction, the study was carried out in dairy farms equipped with efficient cooling systems. Data were analyzed in relation to potential risk factors associated with reduced fertility, including BCS, parity, reproductive disorders, milk yield, and SCC.

MATERIALS AND METHODS

Animals and Data Collection

The study was conducted for 2 consecutive years in 2 commercial dairy herds located in the coastal plain

of Israel, in accordance with the guidelines of the ethics committee of the Hebrew University. Holstein dairy cows that calved between May and September were included in the study. Cows were housed in open sheds and had access to open yards, were milked 3 times a day, and were fed a TMR containing 1.7 NE_L Mcal/kg of DM, 16.5% protein, and 32% NDF. All animals were subjected to similar routine veterinary health management and were diagnosed and treated for periparturient diseases on d 5 to 12 postpartum. Retained placenta was defined as the presence of fetal membranes 24 h after calving. Metritis was diagnosed according to the character of vaginal discharge and by rectal palpation of uterus tone and consistency. Cows with retained placenta or metritis were defined as having a reproductive disorder. Body condition score was evaluated by the veterinarian on a 5-point scale (Wildman et al., 1982) after parturition and at peak lactation; that is, 50 to 60 DIM. Estrous behavior was recorded by a computerized pedometric system (SAE, Afikim, Israel) and by visual observation. Milk production was recorded monthly and SCC were analyzed by the Central Laboratory for Milk Recording of the Israeli Cattle Breeding Association. The second to fourth milking records postpartum, coinciding with the experimental period, were used for data analysis.

Experimental Protocol

Cows at 50 to 60 DIM were examined by the herd veterinarian and randomly assigned to control or treatment groups. Treated cows were injected (i.m.) with synthetic GnRH analog (200 µg of gonadorelin, Gonabreed, Parnell Laboratories, Alexandria, Australia) and PGF_{2α} analog (500 µg of cloprostenol, Estroplan, Parnell Laboratories) 7 d later. A second cycle of injections was begun with GnRH 2 d later. This GnRH-PGF_{2α} administration was repeated 3 times to induce 3 successive 9-d follicular waves. Estrus was detected following the third PGF_{2α} administration (i.e., 25 d after treatment initiation). Control cows were inseminated following manifestation of spontaneous estrus; AI was carried out by a professional technician from local AI services. Pregnancy diagnosis was performed by rectal palpation at 40 to 50 d post-AI. A schematic illustration of the experimental protocol is presented in Figure 1.

Temperature Measurements

Records of environmental temperature and relative humidity were taken from stations located within 20 km of the farms. To evaluate the effectiveness of cooling management and determine whether cows maintained normothermia, rectal temperatures of random cows (10

Figure 1. Schematized treatment protocol. Cows 50 to 60 DIM were assigned to control or treatment groups. Treated cows were administered GnRH by intramuscular injection (day = 0) followed by PGF_{2α} on d 7 and a second GnRH injection on d 9. The procedure enabled the induction of 3 successive follicular waves. Cows were inseminated at estrus and pregnancy diagnosis was performed 40 to 50 d post-insemination.

to 15 cows per measurement) were measured throughout the day using a digital thermometer (Toshiba, Tokyo, Japan). A portable device (model number 45158, Extech Instruments Corp., Waltham, MA) was used to record air temperature and relative humidity on the farm.

Statistical Analysis

Days open, days to first AI, number of calving, BCS, and milk yield were analyzed by one-way ANOVA using JMP version 7.0 (SAS Institute Inc., Cary, NC). Data are presented as means $\pm$ SEM. Multiple logistic regression (SAS 9.1) was used to model the success of conception (CR; number of pregnancies resulting from first AI divided by number of inseminated cows) and pregnancy rate (PR; number of pregnancies at 90 and 120 DIM, PR 90 and PR 120, respectively, divided by total number of cows). The following variables were considered by use of a stepwise procedure: farm, year, parity (primiparous vs. multiparous), high BCS at calving (>2.7), high BCS at initiation of treatment (50 to 60 DIM; >2.0), large decrease in BCS between calving and treatment initiation (>0.7), high milk yield (>40 kg/d), summer AI (July to September), reproductive disorders, high SCC ($>150,000$ cells/mL), and their interactions with treatment. The model for CR also included the interval between calving and AI. Variables included in the final model were those passing the entry criteria set at $P < 0.15$. The same variables were used to model the success of response to treatment with regard to timing of estrus. Treated cows that were

detected in estrus within 5 d from the third PGF_{2α} injection were defined as normal-responding cows (designated treatment-normal); cows detected in estrus more than 5 d after the end of treatment were defined as late-responding cows (treatment-late). Variables were considered, as above, by use of a forward stepwise procedure with entry criteria set at $P < 0.15$.

When taking into account the latter division criterion (i.e., treatment-normal and treatment-late), the variables included in the final model for analysis of CR were experimental group, parity, reproductive disorders, SCC, BCS, and season, and their interactions with treatment groups. The variables included in the final model for analysis of PR 90 were experimental group, parity, and BCS, and interactions of treatment group with BCS. The variables included in the final model for analysis of PR 120 were experimental group, parity, reproductive disorders, SCC, and change in BCS, and interactions with treatment groups.

Using time to conception as the outcome variable, survival analysis was performed to compare the effects of treatment. Cows contributed to the analysis for a maximum of 150 d, and observations were excluded when a cow was culled or excluded from breeding.

RESULTS

A total of 246 multiparous cows and 155 primiparous cows were included in the study. Of these, 18 cows (7 controls and 11 treated cows) were culled before pregnancy diagnosis and were excluded from the analysis. An additional cow was excluded from analysis due to

Table 1. Hormonal treatment (GnRH and PGF_{2α}) for the induction of successive follicular waves and its effect on first-AI conception rate (CR) during summer and autumn

Item	Actual CR, % (no./no.)	Adjusted odds ratio	95% CI	P-value
Treatment	37 (72/195)	1.17	(0.60, 2.26)	0.646
Control	32 (60/187)	Referent		
Parity				
Primiparous	45 (68/151)	1.47	(0.79, 2.74)	0.230
Multiparous	28 (64/231)	Referent		
Primiparous × treatment	53 (40/75)	2.32	(0.96, 5.61)	0.063
Primiparous × control	37 (28/76)	Referent		
Reproductive disorders				
Diagnosed	27 (25/94)	0.56	(0.32, 0.96)	0.035
Healthy	37 (107/288)	Referent		
SCC ¹				
High	29 (43/149)	0.97	(0.50, 1.86)	0.923
Low	38 (89/233)	Referent		
High × treatment	28 (22/79)	0.45	(0.18, 1.15)	0.097
High × control	30 (21/70)	Referent		

¹High SCC was defined as >150,000 cells/mL in one of the monthly milking records (second to fourth).

incomplete hormone injections. Overall, 187 controls and 195 treatment cows were included in the analysis.

The average calving number did not differ between control and treatment cows (2.2 ± 0.1 and 2.3 ± 0.1 , respectively) nor did the average milk yield (43.3 ± 0.6 and 43.8 ± 0.6 kg/d, respectively). Average BCS at calving and peak lactation did not differ between experimental groups (2.6 ± 0.04 and 2.0 ± 0.03 vs. 2.6 ± 0.04 and 2.1 ± 0.04 for control and treatment cows, respectively). The proportion of cows with reproductive disorders did not differ between control and treatment cows (26 and 23%, respectively).

Environment Temperature and Body Temperature

The mean minimal and maximal air temperature and relative humidity were 20.7 and 30.8°C, 51 and 91%, respectively during the summer (July to September) and 12.4 and 23.6°C, 45 and 90%, respectively during the autumn (October to December). The average rectal temperature measurements for farms A and B, determined in representative groups of cows on a representative summer day, were 39.3 ± 0.1 and 38.8 ± 0.1 °C, respectively. Air temperature and relative humidity on the day of rectal temperature measurement were as follows: farm A, 30.3 ± 0.8 °C and 70.1 ± 1.8 %; farm B, 28.5 ± 0.4 °C and 65.6 ± 1.4 %.

Reproductive Parameters

Overall, CR was 32 and 37% for control and treatment cows, respectively (not significant). Among multiparous cows, no difference was noted in CR (29 and 27% for control and treatment, respectively). The CR for cows with low SCC (<150,000 cells/mL) was 33 and 43% for control and treatment cows, respectively; for

cows with high milk production, CR was 30% and did not differ between control and treated cows; for treated cows with low milk production, CR was numerically higher (by 15 percentage units) than in their control counterparts (51 and 36%, respectively).

Logistic regression to model CR is presented in Table 1. Data revealed a tendency to interaction between treatment and primiparous cows [odds ratio (OR) 2.32, 95% CI: 0.96–5.61; $P = 0.063$], reflected by a 16 percentage unit increase in CR of treated primiparous cows relative to control primiparous cows. Reproductive disorders lowered the CR by 12 percentage units compared with that of healthy cows (OR 0.56, 95% CI: 0.32–0.96; $P = 0.035$); however, an interaction between reproductive disorders and treatment was not detected. A tendency to interaction between treatment and SCC (OR 0.45, 95% CI: 0.18–1.15; $P = 0.097$) was reflected by a 10 percentage unit increase in CR for treated versus control cows with low SCC.

Logistic regression to model PR 90 and PR 120 is presented in Table 2. High milk production (>40 kg/d) decreased the probability of PR 90 (OR 0.54, 95% CI: 0.33–0.87; $P = 0.011$), and reproductive disorders decreased the probability of PR 120 (OR 0.46, 95% CI: 0.28–0.77; $P = 0.003$); however, interactions with treatment were not detected. Treatment increased the probability of cows with high BCS at 50 to 60 DIM being pregnant (OR 3.02, 95% CI: 1.14–7.96; $P = 0.026$), as reflected by a 14 percentage unit increase in PR 90. Similarly, treatment tended to increase the probability of primiparous cows being pregnant (OR 3.16, 95% CI: 0.93–10.47; $P = 0.066$), as reflected by a 14 percentage unit increase in PR 120.

Days open for pregnant cows at 90 DIM did not differ between control and treatment groups (76.3 ± 1.1 and 77.2 ± 0.4 , respectively). The variance of the treated

Table 2. Hormonal treatment (GnRH and PGF_{2α}) for the induction of successive follicular waves and its effect on pregnancy rate (PR) at 90 and 120 DIM during summer and autumn

Item	Actual PR, % (no./no.)	Adjusted odds ratio	95% CI	P-value
Pregnancy rate at 90 DIM				
Treatment	26 (51/195)	0.70	(0.34, 1.43)	0.329
Control	28 (52/187)	Referent		
SCC ¹				
High	23 (34/149)	0.89	(0.46, 1.75)	0.743
Low	30 (69/233)	Referent		
High × treatment	19 (15/79)	0.60	(0.23, 1.59)	0.303
High × control	27 (19/70)	Referent		
Milk yield ²				
High	21 (52/242)	0.54	(0.33, 0.87)	0.011
Low	36 (51/140)	Referent		
BCS ³				
High	33 (46/140)	0.81	(0.41, 1.62)	0.551
Low	23 (55/236)	Referent		
High × treatment	39 (31/79)	3.02	(1.14, 7.96)	0.026
High × control	25 (15/61)	Referent		
Pregnancy rate at 120 DIM				
Treatment	49 (95/193)	0.62	(0.18, 2.16)	0.449
Control	52 (98/187)	Referent		
Parity				
Primiparous	58 (88/151)	0.67	(0.27, 1.71)	0.404
Multiparous	46 (105/229)	Referent		
Primiparous × treatment	65 (49/75)	3.16	(0.93, 10.74)	0.066
Primiparous × control	51 (39/76)	Referent		
Reproductive disorders				
Diagnosed	39 (36/93)	0.46	(0.28, 0.77)	0.003
Healthy	55 (157/287)	Referent		
Milk yield ²				
High	46 (111/240)	0.64	(0.25, 1.64)	0.353
Low	58 (82/140)	Referent		
High × treatment	41 (53/128)	0.87	(0.25, 3.04)	0.830
High × control	52 (58/112)	Referent		

¹High SCC was defined as >150,000 cells/mL in one of the monthly milking records (second to fourth).

²High milk yield was defined as average >40 kg/d (milking records second to fourth).

³BCS >2 at initiation of treatment was defined as high.

group was lower than that of the controls (Bartlett test; $P < 0.001$). Similarly, days open for pregnant cows at 120 DIM did not differ between control and treatment groups (88.9 ± 1.6 and 91.1 ± 1.6 , respectively).

Among the treated cows, about 60% were defined as treatment-normal (detected in estrus within 5 d after the last PGF_{2α} injection) and 40% were defined as treatment-late. Days to first AI did not differ between control and treatment-normal groups (80.4 ± 1.0 and 78.6 ± 1.2 , respectively) but were lower ($P < 0.001$) relative to that of treatment-late cows (101.6 ± 1.5). An additional analysis was performed upon dividing the treated cows into treatment-normal and treatment-late groups. Interactions between treatment and variables resulting with P -value <0.10 in the final model for analyses of CR are presented in Table 3. A tendency to positive interaction between treatment-late and primiparous cows (OR 3.57, 95% CI: 0.93–13.70; $P = 0.064$), and between treatment-normal and high BCS (OR 2.76, 95% CI: 0.96–7.95; $P = 0.060$), was noted,

as reflected by a 13 to 14 percentage unit increase in CR compared with control counterparts. A tendency to negative interaction between treatment-late and summer season was noted (OR 0.20, 95% CI: 0.04–1.11; $P = 0.066$), as reflected by a 15 percentage unit decrease in CR compared with summer control cows.

Interactions between treatment-normal or treatment-late groups and variables resulting in P -values <0.10 in the final model for analysis of PR are presented in Table 4. Logistic regressions revealed that treatment-late cows had much lower PR 90 than controls (OR 0.11, 95% CI: 0.02–0.46; $P = 0.003$). A positive interaction between treatment-normal and high BCS was noted (OR 3.00, 95% CI: 1.06–8.45; $P = 0.039$), as reflected by a 32 percentage unit increase in PR 90 compared with control high-BCS cows. Logistic regression to model PR 120 revealed a positive interaction between treatment-normal and primiparous cows (OR 5.72, 95% CI: 1.96–16.71; $P = 0.001$); PR 120 was higher by 27 percentage units compared with control counterparts.

Table 3. First-AI conception rate (CR) for treatment-normal response and treatment-late response cows during summer and autumn¹

Item	Actual CR, % (no./no.)	Adjusted odds ratio	95% CI	P-value
Treatment-normal	41 (48/117)	0.86	(0.34, 2.18)	0.752
Treatment-late	31 (24/78)	1.12	(0.39, 3.19)	0.835
Control	32 (60/187)	Referent		
Parity				
Primiparous × treatment-normal	55 (27/49)	1.79	(0.61, 5.29)	0.291
Primiparous × treatment-late	50 (13/26)	3.57	(0.93, 13.70)	0.064
Primiparous × control	37 (28/76)	Referent		
SCC ²				
High × treatment-normal	28 (13/46)	0.30	(0.10, 0.91)	0.033
High × treatment-late	27 (9/33)	0.60	(0.16, 2.20)	0.437
High × control	30 (21/70)	Referent		
BCS ³				
High × treatment-normal	57 (30/53)	2.76	(0.96, 7.95)	0.060
High × treatment-late	23 (6/26)	0.75	(0.17, 3.25)	0.699
High × control	33 (20/61)	Referent		
Season of AI ⁴				
Summer × treatment-normal	46 (21/46)	1.72	(0.57, 5.12)	0.334
Summer × treatment-late	13 (3/24)	0.20	(0.04, 1.11)	0.066
Summer × control	28 (19/69)	Referent		

¹Cows were treated with GnRH and PGF_{2α} to induce successive follicular waves. Cows detected in estrus and inseminated within 5 d after the third PGF_{2α} treatment were defined as treatment-normal response.

²High SCC was defined as >150,000 cells/mL in one of the monthly milking records (second to fourth).

³BCS >2 at initiation of treatment was defined as high.

⁴Summer months (July–September).

An attempt was made to find predisposing factors that might be associated with normal or late response to the hormonal treatment. Logistic regressions to model cows' responses to treatment revealed that of all examined variables, only high milk production (>40 kg/d) entered into the model (OR 0.532; 95% CI: 0.284–0.999; $P = 0.049$). A linear relationship was found between BCS at initiation of treatment and the proportion of normal-responding cows ($R^2 = 0.87$). Hence, when BCS was included in the logistic regressions model as a continuous variable, the OR for a normal response tended to increase by 2.029 for each increase in BCS unit at treatment initiation (95% CI: 0.965–4.267; $P = 0.062$). Although not significant, high milk production also entered the latter model with an OR of 0.619 (95% CI: 0.323–1.188; $P = 0.1493$).

Survival analysis curves are shown in Figure 2. The PR at 80 DIM for the treatment-normal cows was much higher than that for controls (40 vs. 18%); the log-rank test to compare time to conception showed a significant difference between the control and treatment-late cows ($P = 0.03$) and between treatment-normal and treatment-late cows ($P = 0.01$).

DISCUSSION

Thermal stress-induced alterations in the ovarian pool of follicles and their enclosed oocytes have been

suggested as a major cause for reduced dairy cow fertility through the summer and subsequent autumn (Roth, 2008). Here, we report that combining hormonal treatment with intensive cooling management has the potential to improve reproductive performance of high-lactating cows during the hot seasons. In particular, administration of hormonal treatment (GnRH and PGF_{2α}) to induce successive follicular waves had a substantial effect on the fertility of subpopulations of cows, mainly primiparous cows and cows with high BCS.

The most prominent result of the current study was the improved fertility in primiparous cows during the summer and subsequent autumn by hormonal administration. This finding is highly relevant because about one-third of the cow population in modern dairy herds comprises primiparous cows. These cows exhibit higher CR than multiparous cows year-round (Chebel et al., 2004), possibly because of their lower milk yield (Lucy, 2001). In the current study, the milk production of primiparous cows was 25% lower relative to multiparous cows, which might improve maintenance of normothermia and consequently lead to a better response to the hormonal treatment. This assumption is also supported by the 15% increase in PR at 90 DIM of low-yielding cows compared with cows with high milk production.

Postpartum resumption of cyclicity has been shown to be lower in primiparous than in multiparous cows

Table 4. Pregnancy rate (PR) at 90 and 120 DIM for treatment-normal response and treatment-late response cows during summer and autumn¹

Item	Actual PR, % (no./no.)	Adjusted odds ratio	95% CI	P-value
Pregnancy rate 90 DIM				
Treatment-normal	41 (48/117)	1.07	(0.54, 2.12)	0.839
Treatment-late	3 (3/78)	0.11	(0.02, 0.46)	0.003
Control	28 (52/187)	Referent		
BCS ²				
High × treatment-normal	57 (30/53)	3.00	(1.06, 8.54)	0.039
High × treatment-late	4 (1/26)	1.02	(0.08, 13.08)	0.989
High × control	25 (15/61)	Referent		
Pregnancy rate 120 DIM				
Treatment-normal	58 (67/115)	0.86	(0.36, 2.03)	0.722
Treatment-late	36 (28/78)	0.49	(0.17, 1.37)	0.171
Control	52 (98/187)	Referent		
Parity				
Primiparous × treatment-normal	78 (38/49)	5.72	(1.96, 16.71)	0.001
Primiparous × treatment-late	42 (11/26)	1.37	(0.42, 4.50)	0.608
Primiparous × control	51 (39/76)	Referent		
SCC ³				
High × treatment-normal	50 (22/44)	0.40	(0.14, 1.16)	0.093
High × treatment-late	42 (14/33)	1.67	(0.53, 5.25)	0.377
High × control	50 (35/70)	Referent		

¹Cows were treated with GnRH and PGF_{2α} to induce successive follicular waves. Cows detected in estrus and inseminated within 5 d after the third PGF_{2α} treatment were defined as treatment-normal response.

²BCS >2 at initiation of treatment was defined as high.

³High SCC was defined as >150,000 cells/mL in one of the monthly milking records (second to fourth).

(Chebel et al., 2006). Therefore, one might suggest that the hormonal treatment used in the current study facilitates resumption of cyclicity, which consequently improves primiparous fertility to a greater extent than

that in multiparous cows. In this regard, improved conception of primiparous cows under normothermic conditions after administration of the “double-Ovsynch” protocol has been related to GnRH-induced cyclicity

Figure 2. Kaplan-Meier survival analysis. The proportion of pregnant cows and days open for control, treatment-normal response, and treatment-late response cows. Control versus treatment-late cows and treatment-normal versus treatment-late cows differed significantly as determined by log-rank test ($P = 0.03$ and 0.01 , respectively).

during presynchronization (Souza et al., 2008). Nevertheless, in the present study, the proportion of cyclic cows at 50 DIM (determined by pedometric system) did not differ between primiparous and multiparous cows and was even numerically higher in the former (64 and 53%, respectively). Thus, it is not likely that the observed beneficial effect of the hormonal treatment noted in primiparous cows is related to resumption of cyclicity. It is more likely to be a result of induced successive follicular waves and, presumably, a better response of primiparous cows to GnRH administration. Supporting this assumption, Kaim et al. (2003) reported that GnRH administration at the onset of estrus results in a more pronounced improvement of CR in primiparous cows relative to multiparous cows. A more permissive response to GnRH administration in the current study might have enabled efficient removal of follicles that were damaged upon heat exposure. Amstalden et al. (2004) showed that administration of mammalian GnRH increases plasma FSH concentrations, in addition to the increase in plasma LH level. In our previous studies, FSH administration increased the number of emerging follicles (Roth et al., 2002; Friedman et al., 2010) and improved oocyte quality, as determined by an increased proportion of grade-1 oocytes and of oocytes that were fertilized and cleaved (Roth et al., 2002). Although not examined here, GnRH administration might increase endogenous FSH secretion, which in turn would enhance the number of impaired follicles emerging and being removed within an induced follicular wave.

Another prominent result in the current study was the improved fertility of treated cows with high BCS at initiation of the hormonal treatment. Similarly, Ovsynch protocols that are based on GnRH-PGF_{2α}-GnRH administration have been shown to result in improved fertility under normothermic conditions in cows with high BCS (Burke et al., 1996; Stevenson et al., 1999; Moreira et al., 2000). High BCS through lactation is associated with earlier recovery of ovarian function postpartum, earlier return to cyclicity, and higher first-AI CR (Roche et al., 2009). Conversely, low BCS is negatively associated with renewed cyclicity around 50 DIM (Chebel et al., 2006), presumably due to reduced sensitivity to LH, low estradiol concentration, and ovulation failure (Diskin et al., 2003). It is possible that in the current study, a higher proportion of cows with low BCS did not respond to the treatment, whereas cows with high BCS responded well to GnRH-PGF_{2α} administration and therefore showed marked improvement in their reproductive performance. Taken together, the findings suggest that low BCS is a predisposing parameter to identifying potential cows that will not benefit from the suggested treatment.

Efficient response to GnRH administration might also involve threshold plasma progesterone levels. High BCS at initiation of the Ovsynch protocol was associated with higher concentrations of plasma progesterone at the onset of treatment and improved reproductive performance (Burke et al., 1996; Moreira et al., 2000). In the current study, low BCS was associated with both low CR and PR and a high proportion of late-responding cows; thus, it is reasonable that progestin-based synchronization for cows with low BCS before hormonal administration might lead to a better response to treatment. Presynchronization of noncyclic dairy cows using a controlled intravaginal drug-releasing (CIDR) device containing progesterone resulted in an increased proportion of cows returning to cycle (Chebel et al., 2006). In addition, administering progestin-based synchronization in noncyclic suckled beef cows enhanced ovulation events (Lamb et al., 2010).

It should be noted that in the current study, other factors such as reproductive disorders also affected fertility without any interaction with the examined hormonal treatment. Moreover, recent studies have shown that clinical and subclinical mastitis impair ovarian function (Lavon et al., 2010). Although data from the present study do not distinguish between clinical and subclinical mastitis, SCC interacted with hormonal treatment. Nevertheless, this interaction is not clear-cut and requires further investigation.

About 60% of the treated cows expressed estrus at the expected time, whereas the rest of the cows exhibited delayed estrus. In comparison, Stevenson et al. (1999) reported that about 68 to 82% of cows expressed estrus at the expected time following induction of a single follicular wave by GnRH-PGF_{2α} administration. Differences between the latter and current study might be related to different experimental protocols (i.e., induction of one vs. multiple follicular waves) or reduced synchronization throughout successive hormonal injections. One could suggest that the late response to treatment is a result of heat-detection failure. However, this possibility is less likely because cows were housed in open sheds and estrous behavior was recorded by using a computerized pedometric system and by visual observation. On the other hand, late response to the treatment was associated in the present study with high milk production and low BCS at initiation of treatment. Both are tightly related to negative energy balance (Butler, 2000), a main factor in determining successful conception in cows inseminated postpartum (Opsomer et al., 2000), which is often associated with ovarian dysfunction and anestrus (Lucy et al., 1992; Jolly et al., 1995; Kendrick et al., 1999). The latter cannot be ruled out because pedometric data indicated that at treatment initiation, the proportion of noncyclic cows in the

normal-responding group (37%) was lower than that in the late-responding group (51%). In addition, high milk production is associated with increased blood flow in the liver, increased steroid metabolism, and alterations in the endocrine milieu (Wiltbank et al., 2000). Previous studies have documented that lactating cows have decreased plasma concentrations of progesterone and estradiol relative to nonlactating cows (De la Sota et al., 1993) or heifers (Wolfenson et al., 2004). Thus, it is possible that elevated clearance of steroid hormones (i.e., progesterone and estradiol) from the circulation (Sartori et al., 2002) or alternatively, increased PGF_{2α} uptake by the liver (Schuster, 1998) moderated, in the current study, the effect of hormone administration in high lactating cows.

In summary, administration of hormonal treatment (GnRH and PGF_{2α}) to induce successive follicular waves improved fertility of dairy cows during the summer and autumn. The hormonal treatment was beneficial for primiparous cows and for cows with high BCS at initiation of treatment. Reproductive management that is based on hormonal treatment in subpopulations of cows known to benefit from it may improve efficiency of treatment and reduce expenses.

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