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Trials to Increase the Availability of Ovsynch Program Under Field Conditions in Dairy Cows

Jae-Kwan Jeong, In-Soo Choi, Soo-Chan Lee, Hyun-Gu Kang, Tai-Young Hur* and Ill- Hwa Kim[1]

College of Veterinary Medicine, Chungbuk National University, Cheongju 28644, Korea
**National Institute of Animal Science, RDA, Wanju 55365, Korea*

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Abstract : This study investigated whether presynchronization with GnRH 6 days before initiation of the Ovsynch program improved reproductive outcomes in dairy cows. Additionally, postponement of initiation of the Ovsynch program for cows during the metestrus phase by 5 days was investigated to determine if it improved reproductive outcomes. To accomplish this, 941 Holstein dairy cows with unknown estrous cycle were randomly allocated into an Ovsynch group (n = 768; 100 μg gonadorelin [a GnRH analogue], 500 μg of cloprostenol [$PGF_{2\alpha}$ analogue] seven days later, 100 μg gonadorelin 56 h later and timed artificial insemination [AI] 16 h after) and a G6-Ovsynch (n = 173) that received 100 μg GnRH followed by the Ovsynch program 6 days later. Additionally, 272 dairy cows with known estrous cycle (metestrus stage) received the Ovsynch 5 days later (Day 5-Ovsynch group, n = 272). The odds ratio (OR) for pregnancy was analyzed by logistic regression using the LOGISTIC procedure in SAS. The treatment group ($p < 0.001$) and AI season ($p < 0.05$) significantly affected the probability of pregnancy, whereas farm, cow parity, calving to AI interval, and body condition score had no affect ($p > 0.05$). The Day 5-Ovsynch group had a higher probability of pregnancy (OR: 1.71) than the Ovsynch group, while that of the G6-Ovsynch group was intermediate ($p > 0.05$). Cows inseminated during winter had a higher OR (1.39) than those inseminated during spring. Overall, additional GnRH treatment 6 days before the Ovsynch did not improve reproductive outcomes, whereas postponement of the initiation of Ovsynch by 5 days for cows during metestrus improved reproductive outcomes.

***Key words* :** dairy cows, synchronization programs, probability of pregnancy, estrous cycle.

Introduction

Genetic selection for higher milk yield and intensive production in dairy cows has led to a remarkable increase in milk production with a concomitant inverse decrease in reproductive performance (16). Because weak estrus or anestrus leads to lost opportunities for inseminations in dairy herds, especially in high milk-yielders, various reproductive hormone treatments including Ovsynch have been developed to inseminate dairy cows without estrous detection (8,13,21). The Ovsynch program is initiated by injection of GnRH at random stages of the estrous cycle, followed 7 d later by an injection of $PGF_{2\alpha}$. At 48 or 56 h after injection of $PGF_{2\alpha}$, a second injection of GnRH is administered, and cows are inseminated approximately 16 h after GnRH (5,22). Although the Ovsynch program has been used as a powerful reproductive management tool, the stage of the estrous cycle at which the Ovsynch program is initiated affects subsequent pregnancy rates (18,26). Initiation of the Ovsynch program at late luteal phase (Day 13 to 17 of the estrous cycle), metestrous phase (Day 1 to 4 of the estrous cycle), or proestrous phase (Day 18 to 21 of the cycle) may result in premature regression or incomplete regression of the corpus luteum, or failed synchronization of a new follicular wave, leading to lower pregnancy rates (18). Thus, the optimal stage of the estrous cycle at which the Ovsynch program should be initiated should correspond to the early diestrus phase (i.e., between d 5 and 9 of the estrous cycle) (4,26).

GnRH has frequently been used in veterinary practice to promote ovulation of preovulatory follicles in cattle. Thus, presynchronization with GnRH makes it possible to initiate the Ovsynch program in many cows during the early diestrus phase, which is the optimal phase in dairy cows. Our first hypothesis was that presynchronization with GnRH 6 days before initiation of the Ovsynch program would result in ovulation of a follicle inducing a new follicular wave, thus setting a favor phase (Day 5 of the estrous cycle; early luteal phase) for the program. Our second hypothesis was that postponement of initiation of the Ovsynch program by 5 days for cows during metestrus phase would set a suitable phase (Day 5 to 9 of the estrous cycle; early luteal phase) for the Ovsynch program. Therefore, this study was conducted to determine if presynchronization with GnRH 6 days before initiation of the Ovsynch program might improve reproductive outcomes in dairy cows. We also investigated whether postponement of the initiation of the Ovsynch program by 5 days for cows during the metestrus phase improved reproductive outcomes.

[1]Corresponding author.
E-mail : illhwa@cbu.ac.kr

Materials and Methods

Animals and reproductive management

This study was conducted on 24 Holstein dairy farms (A-X) located in Chungcheong Province from January 2015 to May 2016. All cows were fed a total mixed ration and milked twice daily. In addition, cows received regular reproductive health checks every 2 to 4 weeks, including examination of ovarian structures and the uterus *via* transrectal palpation and ultrasonography. The voluntary waiting period from calving to the first artificial insemination (AI) was 40 days. In addition to estrous detection, a herd reproductive management program was employed for cows failing to receive AI within the 50-day postpartum interval. Cows that exhibited estrus naturally were inseminated according to the am-pm rule, whereas those treated with Ovsynch received timed AI (TAI). Pregnancy was diagnosed rectally 40-50 days after AI using both ultrasonography and manual palpation.

Study design

A total of 1,213 dairy cows kept at 24 farms were used for the present study. First, 941 Holstein dairy cows with unknown estrous cycle were randomly allocated into two groups: Ovsynch, which was composed of 100 μg gonadorelin (a GnRH analogue; Godorel, Uni-Biotech, Yesan, Korea), 500 μg of the $PGF_{2\alpha}$ analogue, cloprostenol (Estrumate, MSD Animal Health, Seoul, Korea) 7 days later, 100 μg GnRH 56 h later and TAI 16 h after (Ovsynch group, n = 768); or 100 μg gonadorelin followed by the Ovsynch 6 days later (G6-Ovsynch, n = 173). In addition, 272 dairy cows with a known estrous cycle (metestrus stage) received Ovsynch 5 days later (Day 5-Ovsynch group, n = 272). Evaluation of the metestrus stage of the estrous cycle was based on continuing reproductive histories for the individual cows, including previous inseminations, estrous behaviors, post-estrus breeding, and ultrasonography of the ovaries (detection of the ovulation and corpus hemorrhagicum).

Data collection and statistical analysis

The data collected detailed information describing the farm, cow parity, treatment groups, and body condition score (BCS) of the cow at the time of treatment, as well as the dates of previous calving, insemination and conception. Table 1 lists the independent variables describing the farm, treatment groups, cow parity, BCS, and calving to AI interval of the cow, as well as the TAI season. For statistical analyses, cow parity was categorized as primiparous or multiparous cows, and BCS was grouped as ≤ 2.75 (mean [± SD] 2.72 ± 0.08; ranges of 2.50-2.75) and ≥ 3.0 (3.21 ± 0.24; ranges of 3.00-4.00). Calving to AI interval was categorized as ≤ 125 days (91.5 ± 16.6; ranges of 50-125 days) and > 125 days (212.4 ± 87.4; ranges of 126-488 days), whereas AI season was grouped as spring (March to May), summer (June to August), fall (September to November) and winter (December to February).

The probability of pregnancy was analyzed by logistic regression using the LOGISTIC procedure in SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). The logistic regression model included farm, treatment groups, cow parity, BCS, calving to AI interval, AI season, and the interactions between these variables. Backward stepwise regression was

Table 1. Logistic analysis of independent variables to determine the risk factors for the pregnancy following synchronization programs in dairy cows

Variables	Level	No. cows	
		Pregnancy	Non-pregnancy
Farm	A	16	31
	B	8	7
	C	31	60
	D	17	20
	E	25	36
	F	20	18
	G	61	97
	H	18	25
	I	7	14
	J	22	41
	K	9	14
	L	6	10
	M	20	29
	N	18	19
	O	12	15
	P	14	13
	Q	31	32
	R	8	8
	S	21	33
	T	7	10
	U	32	48
	V	35	56
	W	25	40
	X	32	42
Treatment groups[1]	Ovsynch	288	480
	G6-Ovsynch	72	101
	Day 5-Ovsynch	135	137
Cow parity	Primiparous	165	221
	Multiparous	330	497
BCS	≤ 2.75	139	224
	≥ 3.00	356	494
Calving to AI interval (days)	≤ 125	249	344
	> 125	246	374
Timed AI season	Spring	160	252
	Summer	58	120
	Autumn	104	138
	Winter	173	208

[1]Ovsynch: 100 μg gonadorelin (a GnRH analogue) at random stages of the estrous cycle, 500 μg of the cloprostenol ($PGF_{2\alpha}$ analogue) 7 days later, 100 μg GnRH 56 h later and TAI 16 h after; G6-Ovsynch: 100 μg gonadorelin followed by the Ovsynch program 6 days later; Day 5-Ovsynch: initiation of the Ovsynch program 5 days later for cows with known estrous cycle (metestrus stage).

Table 2. Odds ratio (OR) and variables included in the final logistic regression model for the probability of pregnancy following synchronization programs in dairy cows

Variable	Pregnancy rate at artificial insemination (no. of cows)	OR	95% CI	*p*-value
Treatment groups[1]				
Ovsynch	37.5% (288/768)	Reference		
G6-Ovsynch	41.6% (72/173)	1.23	0.876-1.727	> 0.05
Day 5-Ovsynch	49.6% (135/272)	1.71	1.289-2.266	< 0.001
Timed AI season				< 0.05
Spring	38.8% (160/412)	Reference		
Summer	32.6% (58/178)	0.77	0.530-1.119	> 0.05
Autumn	43.0% (104/242)	1.21	0.873-1.673	> 0.05
Winter	45.4% (173/381)	1.39	1.041-1.849	< 0.05
Farm				> 0.05
Cow parity				> 0.05
BCS				> 0.05
Calving to AI interval (days)				> 0.05

[1]Ovsynch: 100 µg gonadorelin (a GnRH analogue) at random stages of the estrous cycle, 500 µg of the cloprostenol ($PGF_{2\alpha}$ analogue) 7 days later, 100 µg GnRH 56 h later and TAI 16 h after; G6-Ovsynch: 100 µg gonadorelin followed by the Ovsynch program 6 days later; Day 5-Ovsynch: initiation of the Ovsynch program 5 days later for cows with known estrous cycle (metestrus stage).

used in all models, and elimination was performed based on the Wald statistic criterion when $p > 0.11$. The odds ratio (OR) and 95% confidence interval (CI) were computed by logistic regression. Results were presented as proportions and OR with their respective 95% CIs. Differences with $p < 0.05$ were considered significant.

Results

The final model revealed that treatment group ($p < 0.001$) and AI season ($p < 0.05$) significantly affected the probability of pregnancy, whereas farm, cow parity, calving to AI interval, and BCS had no affect ($p > 0.05$, Table 2). The Day 5-Ovsynch group had a higher probability for pregnancy (OR: 1.71) than the Ovsynch group, whereas the G6-Ovsynch group was intermediate ($p > 0.05$). Cows inseminated during winter had a higher OR (1.39) than those inseminated during spring.

Discussion

Logistic analysis revealed that treatment group and AI season affected the probability for pregnancy following synchronization programs in dairy cows. Our data indicated that active induction of ovulation with GnRH (presynchronization) 6 days before the initiation of Ovsynch did not improve reproductive outcomes, but postponement of the initiation of the Ovsynch program for 5 days in cows during the metestrus stage improved reproductive outcomes, indicating the importance of timely Ovsynch treatment.

It has been reported that the stage of the estrous cycle at which the Ovsynch program is initiated affects subsequent pregnancy rates, and that the optimal stage of the estrous cycle corresponds to the early diestrus phase (26). Our first hypothesis was that presynchronization with GnRH makes it possible to initiate the Ovsynch program during the early diestrus phase, which is the optimal phase for initiation of the Ovsynch program. However, an additional GnRH treatment 6 days before the Ovsynch program for cows with unknown estrous cycle did not improve reproductive outcomes in the present study, which is consistent with the results of previous studies (1,5). These results might be a consequence of a greater proportion of cows not responding to the GnRH (25). In contrast, Lopes et al. (14) observed an increase in pregnancy rate of 5% for cows presynchronized with GnRH 7 days before initiation of the Ovsynch program. Moreover, other studies showed that presynchronization with GnRH 7 days before initiation of a resynchronization program 25 or 39 days after AI increased the pregnancy rate compared with control cows (7,10).

Postponement of the initiation of Ovsynch program for cows during the metestrus phase by 5 days resulted in a higher probability of pregnancy compared to the Ovsynch program. We assume that postponement of the initiation of the Ovsynch program for cows during the metestrus phase by 5 days led to setting a suitable phase (Day 5 to 9 of the estrous cycle; early luteal phase) for the Ovsynch program. We obtained detailed data describing the continuing reproductive histories for individual cows, estrous behaviors, postestrus breeding, and ultrasonography of the ovaries, which would helped to decide as metestrus phase for the cows. Overall, our results indicate that intentional induction of ovulation with GnRH (presynchronization) 6 days before the initiation of Ovsynch did not improve reproductive outcomes, but postponement of the initiation of the Ovsynch program by 5 days for cows during the metestrus stage improved reproductive outcomes. Thus, for cows in the metestrus phase, postponement of initiation of the Ovsynch program by approximately 5 days is recommended.

Our data showed that cows that received TAI during winter had a higher OR for pregnancy than those that received TAI during spring and summer, consistent with several previous reports (3,9,24). The negative effects of heat stress on the fertility of dairy cows might be associated with endocrine imbalance, compromised folliculogenesis, ovulation failure, and oocyte damage (2,6,15). Moreover, the negative effects of heat stress might occur *via* immediate decreases in plasma inhibin and consequent increases in FSH, as well as delayed effects caused by the marked increase in FSH secretion and reduction in the number of medium size follicles (23).

In the present study, reproductive outcomes did not vary among experimental farms, which is consistent with the results of a previous study (3). Farms in this study were managed by similar feeding and nutritional management system, as well as intensive reproductive health checks, which might eliminate uneven reproductive outcome among farms. Contrary to the results of our study, there was high variation in reproductive performance among farms in previous publications (24,27), which may be associated with different farm management practices, environments, and facilities. Our finding that there was no association between cow parity and reproductive outcomes was consistent with the results of a previous study in which there was no effect of cow parity on pregnancy rate following Ovsynch (19). Conversely, many previous studies showed lower reproductive outcomes in multiparous cows compared to primiparous cows (20,24). Calving to AI interval and BCS may be related to the status of the involution of reproductive organs and/or negative energy balance. Our data showed no associations between calving to AI interval or BCS and reproductive outcomes, which is inconsistent with previous publications (11,12,17,24). The reason for the discrepancy between our and previous studies is not clear. However, we attempted to exclude extremely thin (BCS less than 2.50) or fat (BCS greater than 4.00) cows, as well as the calving to AI interval (less than 50 days after calving) to avoid low fertility in the present study, which might have contributed to this difference. This is because earlier initiation of synchronization programs would have delayed involution of reproductive organs and/or negative energy balance.

Taken together, although presynchronization with single GnRH 6 days before the Ovsynch program did not improve the reproductive outcomes, postponement of the initiation of Ovsynch for 5 days in cows during metestrus phase improved the probability of pregnancy, indicating the importance of proper timing of the initiation for the Ovsynch program.

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