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Effects of Ovarian Status at the Time of Initiation of the Modified Double-Ovsynch Program on the Reproductive Performance in Dairy Cows

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Abstract This study determined the effect of ovarian status at the beginning of the modified Double-Ovsynch program on reproductive performance in dairy cows. In the study, 1,302 cows were treated with a modified Double-Ovsynch program at 56 days after calving. This program comprises administering gonadotropin-releasing hormones (GnRH), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) 10 days later, GnRH 3 days later, GnRH 7 days later, and GnRH 56 h later, followed by timed artificial insemination (TAI) 16 h later. At the beginning of the program, cows were categorized according to the size of the largest follicle and the presence of a corpus luteum (CL) in the ovaries as follows: 1) small follicle (<5 mm, SF group, n = 100), 2) medium follicle (8-20 mm, MF group, n = 538), and 3) large follicle (≥25 mm, LF group, n = 354) without a CL, or 4) the presence of a CL (CL group, n = 310). The pregnancies per AI after the first TAI were analyzed by logistic regression using the LOGISTIC procedure, and the logistic model included the fixed effects of the herd size, parity, body condition score (BCS) at the first TAI, TAI period, and ovarian status. A larger herd size, higher BCS at the first TAI, and TAI period with no heat stress increased ($p < 0.05$) the probability of pregnancy per AI after the first TAI. However, ovarian status at the beginning of the program did not affect ($p > 0.05$) the pregnancies per AI (ranges of 37.9% to 42.9%). These results show that the modified Double-Ovsynch program can be used effectively while maintaining good fertility regardless of the ovarian status in dairy herds.

Key words dairy cow, Double-Ovsynch, ovarian structure, reproductive performance.

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Introduction

Since the introduction of the Ovsynch program, which consists of an injection of gonadotropin-releasing hormone (GnRH), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) 7 days later, 2nd GnRH 56 h later, followed by timed artificial insemination (TAI) 16 h later (10), various ovulation synchronization programs have been used to improve reproductive performance without observing estrus in dairy cows (2,7). Moreover, studies have also shown that the addition of a GnRH or $PGF_{2\alpha}$-based presynchronization prior to Ovsynch resulted in advanced follicular wave emergence, follicle development, and ovulation for TAI, which flexibly controlled the time of the first TAI after calving and increased the pregnancies per AI after the first TAI (3,9). One of these presynchronization-Ovsynch programs, the Double-Ovsynch program, which consists of an Ovsynch, followed by another Ovsynch 7 days later, is widely used in dairy farms (13,14). At the beginning of this program, the ovarian status of cows can vary greatly depending on the stage of the estrus cycle or the presence of ovarian disease. However, to the best of our knowledge, the effect of ovarian status before initiating the Double-Ovsynch program on subsequent reproductive outcomes is not known to date. Therefore, this study was conducted to investigate the effect of ovarian status, according to the size of the largest follicle and the presence of a corpus luteum (CL) in the ovaries, at the beginning of the modified Double-Ovsynch program on pregnancy outcomes in dairy cows.

Materials and Methods

Animals and reproductive management

This study was conducted on 27 dairy farms in Chungcheong Province, Korea. Each herd contained 40-110 cows. The cows were maintained in loose housing systems and fed total mixed rations. All the cows that had calved were milked twice daily. A total of 1,302 (512 primiparous and 790 multiparous) cows were studied.

All the cows underwent biweekly reproductive health checks by veterinarians on the research team; these included an examination of their ovarian structures (follicle size and CL) and uterus by ultrasonography. The size of the largest follicle and the presence of a CL in the ovaries were recorded. At the same time, the body condition score (BCS) was evaluated on a 5-point scale (with quarter-point divisions) using a visual technique (6). All the experiments were performed with the approval of the Institutional Animal Care and Use Committee of Chungbuk National University, Chungbuk, Korea (CBNUA-1299-19-02).

Study design

All the cows were treated with a modified Double-Ovsynch program at 56 (± 15.9) days for the first TAI after calving. The modified Double-Ovsynch program consists of an injection of 10 μg GnRH analog, buserelin acetate (Gestar; Over, San Vicente, Argentina), 500 μg of a $PGF_{2\alpha}$ analog, cloprostenol sodium (Estrumate; MSD Animal Health, Seoul, Korea) 10 (± 1.7) days later, GnRH 3 days later, GnRH 7 days later, $PGF_{2\alpha}$ 7 days later, GnRH 56 h later, followed by TAI 16 h later. At the beginning of the modified Double-Ovsynch program, cows were categorized into four groups according to the size of the largest follicle (8,11) and the presence of a CL in the ovaries as follows: 1) small follicle (<5 mm, SF group, n = 100), 2) medium follicle (8-20 mm, MF group, n = 538), and 3) large follicle (≥25 mm, LF group, n = 354) without a CL, or 4) the presence of a CL (CL group, n = 310), respectively. Pregnancy diagnosis was performed 30 and 45 days after TAI using ultrasonography with a 7.5 MHz array transducer (Tringa Linear VET Ultrasound Scanner, Esaote Pie Medical, Maastricht, The Netherlands).

Statistical analyses

Results are expressed as mean ± SD. Statistical analyses were performed using the SAS program (version 9.4; SAS Inst., Cary, NC, USA). For the statistical analyses, the cows were categorized as primiparous or multiparous, according to their BCS at the first TAI after calving (≤2.75, 3.0, or ≥3.25), and according to herd size (<60 or ≥60 lactating cows). The TAI period was categorized as having taken place during the months with no heat stress (October to May) or the months with heat stress (June to September).

Dichotomous outcomes were analyzed by logistic regression using the LOGISTIC procedure. For analyses of the factors that influenced the probability of pregnancy per AI 30 and 45 days after the first TAI, the models included herd size, parity, ovarian status (SF, MF, LF, or CL), BCS at the first TAI, TAI period, and the interactions between these variables. Variables with $p > 0.1$ were sequentially removed from all the models by backward elimination based on the Wald statistic criterion. Differences with $p \leq 0.05$ were considered to be statistically significant, and $0.05 < p < 0.1$ was considered to indicate a trend.

Results

The pregnancies per AI at 30 and 45 days after the first TAI were 46.0% (46/100) and 40.0% (40/100) for the SF group, 41.6% (224/538) and 37.9% (204/538) for the MF group,

47.7% (169/354) and 42.9% (152/354) for the LF group, and 44.5% (138/310) and 41.6% (129/310) for the CL group, respectively. Table 1 shows the factors that affected the probabilities of pregnancy per AI at 30 and 45 days after the first TAI following the modified Double-Ovsynch program. A herd size, BCS at the first TAI, and TAI period affected the probability of the pregnancy per AI after the first TAI ($p < 0.05$), whereas ovarian status i.e., the size of the largest follicle and the presence of a CL as well as parity did not affect the reproductive outcome ($p > 0.05$) (Table 1). Cows in a herd of ≥60 lactating cows were more likely ($p < 0.05$) to be pregnant at 30 (odds ratio [OR]: 1.28) and 45 (OR: 1.31) days after the first AI than those in herds of <60 lactating cows. Cows with a BCS of ≥ 3.25 at the first TAI after calving were more likely ($p < 0.0001$) to be pregnant at 30 (OR: 1.96) and 45 (OR: 1.98) days after the first AI than cows with a BCS of ≤2.75, and cows with a BCS of 3.0 at the first TAI tended to be more likely ($p < 0.1$) to be pregnant at 30 (OR: 1.28) and 45 (OR: 1.27) days after the first AI than cows with a BCS of ≤2.75. In addition, cows that were inseminated between June and September were less likely ($p < 0.0001$) to be pregnant 30 (OR: 0.53) and 45 (OR: 0.51) days after the first TAI than those that were inseminated between October and May.

Discussion

This study focused on the effect of ovarian status, according to the size of the largest follicle and the presence of a CL in the ovaries, at the beginning of the modified Double-Ovsynch program, on the pregnancy outcomes in dairy cows. The results showed that a larger herd size, higher BCS at the first TAI, and TAI period with no heat stress increased the pregnancy per AI after the first TAI. However, the status of the ovary at the beginning of the program did not affect the pregnancy per AI (ranges of 37.9 to 42.9%), indicating that the modified Double-Ovsynch program can be used effectively while maintaining good fertility regardless of the ovarian condition in dairy herds.

Our findings on the effect of ovarian status prior to the modified Double-Ovsynch program on the pregnancy per AI after the first TAI show that repeated Ovsynch programs, independent of the phase of the ovarian cycle before the programs, were able to restore the postpartum cyclicity and further create a favorable reproductive environment suitable for the first TAI. Before analyzing the results of the study, it was assumed that the SF or LF groups, due to their follicular size, would have inferior pregnancy outcomes following the modified Double-Ovsynch program compared to other groups, but this assumption was proven incorrect. Therefore, the modified Double-Ovsynch program can be used effec-

Table 1. Factors associated with the probability of pregnancy per AI 30 and 45 days after the first TAI following the modified Double-Ovsynch program* in dairy cows

Variable	Probability of pregnancy per AI 30 days after the first TAI			Probability of pregnancy per AI 45 days after the first TAI		
	Odds ratio	95% CI	p-value	Odds ratio	95% CI	p-value
Herd size						
<60 lactating cows	Reference			Reference		
≥60 lactating cows	1.28	1.023-1.609	0.0314	1.31	1.043-1.650	0.0205
Parity			0.5718			0.6596
Ovarian status†			0.6069			0.7600
BCS at TAI						
≤2.75	Reference			Reference		
3.0	1.28	0.972-1.679	0.0794	1.27	0.960-1.680	0.0946
≥3.25	1.96	1.451-2.650	<0.0001	1.98	1.462-2.691	<0.0001
TAI period						
October to May	Reference			Reference		
June to September	0.53	0.412-0.675	<0.0001	0.51	0.395-0.657	<0.0001

CI, confidence interval; AI, artificial insemination; TAI, timed artificial insemination; BCS, body condition score; GnRH, gonadotropin-releasing hormone; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$.

*Modified Double-Ovsynch program consists of an injection of GnRH, a $PGF_{2\alpha}$ 10 (± 1.7) days later, GnRH 3 days later, GnRH 7 days later, $PGF_{2\alpha}$ 7 days later, GnRH 56 h later, followed by TAI 16 h later. †At the beginning of the modified Double-Ovsynch program, cows were categorized into four groups according to the size of the largest follicle and the presence of a CL in ovaries as follows: 1) small follicle (<5 mm, SF group, n = 100), 2) medium follicle (8-20 mm, MF group, n = 538), and 3) large follicle (≥25 mm, LF group, n = 354) without a CL, or 4) the presence of a CL (CL group, n = 310).

tively while maintaining good fertility regardless of the ovarian status in dairy herds.

A larger herd size, higher BCS at the first TAI, and TAI period with no heat stress improved the probability of pregnancy per AI after the first TAI in the present study. Our finding that cows in larger herds were more likely to be pregnant after the first AI than those in small herds of lactating cows seems to be due to better herd management including compliance in the implementation of reproductive programs and/or barn facilities. The beneficial effect of a higher BCS at the first TAI on reproductive outcome is consistent with earlier studies (4,8) and would be associated with attaining balance faster from a state of severe negative energy balance (NEB) during the early lactation period, resulting in an earlier resumption of cyclicity and improvement in fertility (1,5). Our finding of the adverse effects of AI during the period of heat stress on impairment of fertility is also consistent with several previous studies (12,15) and could be related to changes in follicular growth dynamics, reduced follicular steroidogenesis, and reduction in the preovulatory luteinizing hormone surge as well as severe NEB and lower antioxidant status during this period (12,15).

Conclusions

This study determined the effect of ovarian status (size of the largest follicle and presence of a CL in ovaries) at the beginning of the modified Double-Ovsynch program, along with other potential factors, on pregnancy outcomes in dairy cows. A larger herd size, higher BCS at the first TAI, and TAI period with no heat stress increased the probability of pregnancy per AI. However, ovarian status at the beginning of the program did not affect the pregnancy per AI (ranges of 37.9 to 42.9%). These results indicate that the modified Double-Ovsynch program can be used effectively while maintaining a good fertility regardless of the ovarian status in dairy herds.

Conflicts of Interest

The authors have no conflicting interests.

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