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# Effect of resynchronization programs according to the ovarian status on pregnancy outcomes in dairy cows

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## Abstract

This study evaluated the effect of resynchronization programs on pregnancies in dairy cows. Of 1,342 cows confirmed not pregnant after their first artificial insemination (AI), those with a corpus luteum (CL) were resynchronized using Ovsynch or PG-GnRH-Ovsynch and those without a CL were resynchronized using GnRH-Ovsynch or modified Double-Ovsynch. There were no differences ( $p > 0.05$ ) in the pregnancies per AI either between the Ovsynch (31.3%) and PG-GnRH-Ovsynch (34.0%) or between the GnRH-Ovsynch (38.7%) and modified Double-Ovsynch (39.5%). In conclusion, Ovsynch and GnRH-Ovsynch programs could be preferred to resynchronize cows with and without a CL, respectively, from the perspective of reducing costs and labor.

**Keywords:** resynchronization; Ovsynch; presynchronization; dairy cow; reproductive performance

Increase in the herd size and continuous the milk yield makes it difficult to observe estrus in dairy cows. This is one of the main reasons for a decrease in reproductive performance [1]. This has necessitated widespread adoption of hormonal synchronization protocols that allow for timed artificial insemination (AI). When a cow does not become pregnant after AI, immediate implementing a resynchronization program at the time of the nonpregnancy diagnosis may shorten the interval to the next insemination in fields [2,3]. The Ovsynch program, which consists of an injection of gonadotropin-releasing hormone (GnRH), prostaglandin PGF<sub>2α</sub> (PG) 7 days later, 2nd GnRH 56 hours later, followed by timed AI (TAI) 16 hours later, is one of the most widely used programs for resynchronization in dairy cows [4,5]. It has been reported that cows with a corpus luteum (CL) before the initiation of Ovsynch had a higher pregnancy per AI than cows without a CL [6]. Furthermore, initiation of Ovsynch during early diestrus (5 to 9 days of the estrus cycle) has been reported to increase the pregnancies per AI [7]. Thus, various reproductive programs such as Ovsynch, modified Ovsynch, or Double-Ovsynch, which consists of an Ovsynch, followed 7 days later by another Ovsynch, have been used for resynchronization [8–10]. However, cows that did not become pregnant after AI may not be in the same phase of the estrus cycle and could be in the follicular or luteal phase. The primary aim of this study was to select more effective resynchronization programs depending on the presence or absence of a CL in the ovaries of non-pregnant cows, which would lead to an improvement in reproductive performance. Therefore, this study compared the

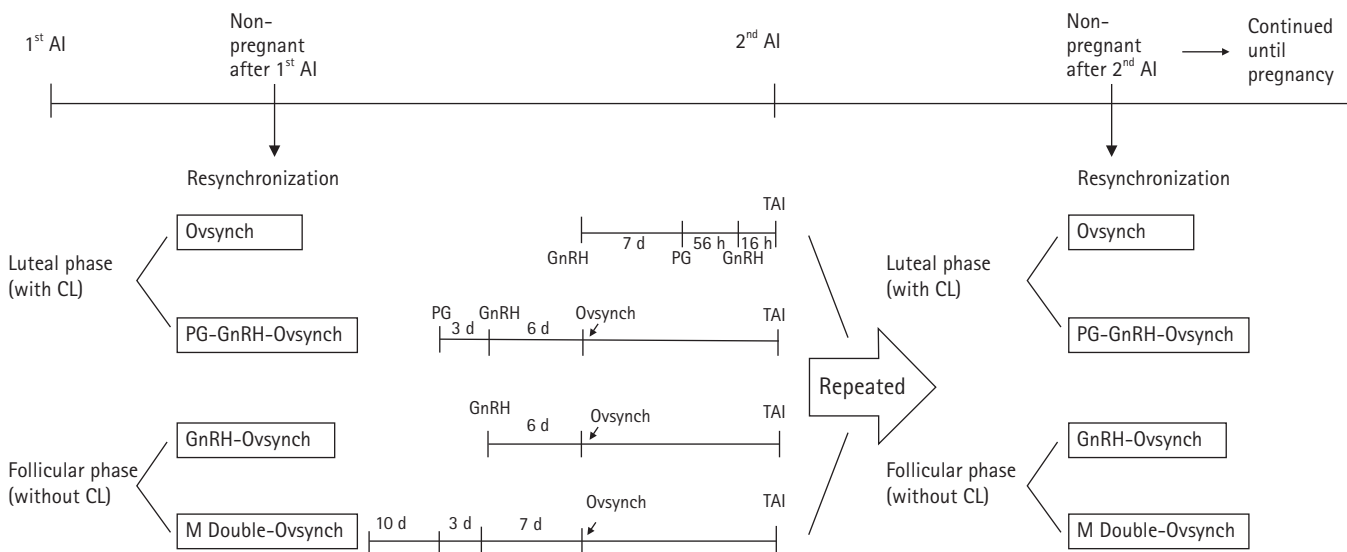
pregnancy outcomes following the Ovsynch or PG-GnRH-Ovsynch programs to resynchronize the cows with a CL, and GnRH-Ovsynch or Double-Ovsynch programs to resynchronize the cows without a CL.

The study was conducted on 20 dairy farms in Chungcheong Province. Each farm had between 40 and 250 cows, which were maintained in loose housing systems, fed total mixed rations, and milked twice daily. All cows in the participating farms underwent reproductive health checks every 2 weeks. These included an examination of their ovarian structures (follicle and CL) and uterus by transrectal palpation and ultrasonography. The voluntary waiting period from calving to the first AI was 50 days.

Of 1,342 dairy cows confirmed to be not pregnant by ultrasonography 30 days after the first AI after calving, cows with a CL were resynchronized as follows: (1) Ovsynch, which consisted of a 10 µg of a GnRH analog, buserelin acetate (Gestar; Over, Argentina), followed by 500 µg of a PG analog, cloprostenol sodium (Estrumate; MSD Animal Health, Korea) after 7 days, a second injection of GnRH 56 hours later, and TAI 16 hours later (Ovsynch, n = 632) or (2) An injection of PG, GnRH 3 days later, followed by Ovsynch 6 days later (PG-GnRH-Ovsynch, n = 191). Cows without a CL were resynchronized as follows: (3) An injection of GnRH, followed by Ovsynch 6 days later (GnRH-Ovsynch, n = 256) or (4) An injection of GnRH, PG 10 days later, GnRH 3 days later, followed by Ovsynch 7 days later (modified Double-Ovsynch, n = 263).

10 days later, GnRH 3 days later, followed by Ovsynch 7 days later (modified Double-Ovsynch, n = 263). Fig. 1 shows a schematic diagram showing repeated resynchronizations, in which 4 resynchronization programs were implemented according to the ovarian status in dairy cows. Pregnancy was evaluated 30 and 45 days after the AI. Cows confirmed as not being pregnant by ultrasonography were resynchronized using the resynchronization programs and this was continued until the cows became pregnant or were culled. The pregnancies per AI following repeated resynchronizations between the Ovsynch, PG-GnRH-Ovsynch, GnRH-Ovsynch, and Double-Ovsynch groups were compared using the chi-square test. A *p*-value < 0.05 was considered statistically significant.

The 1,342 cows included in this study had a mean parity of  $2.1 \pm 1.3$  ( $\pm$  standard deviation). The mean interval between the initiation of each reproductive program and TAI was 10 ( $\pm 0.9$ ), 19 ( $\pm 0.9$ ), 16 ( $\pm 1.0$ ), and 30 ( $\pm 1.3$ ) days in the Ovsynch, PG-GnRH-Ovsynch, GnRH-Ovsynch, and Double-Ovsynch groups, respectively. The pregnancies per AI after the second, third, fourth, or  $\geq$  fifth inseminations were 35.1% (236/673), 34.9% (119/341), 36.2% (59/163), and 31.5% (52/165), respectively. Pregnancies per AI following the Ovsynch, PG-GnRH-Ovsynch, GnRH-Ovsynch, or Double-Ovsynch were 31.3%, 34.0%, 38.7%, and 39.5%, respectively. Therefore, the pregnancies per AI in the GnRH-Ovsynch and Double-Ovsynch groups



**Fig. 1.** A schematic diagram showing repeated resynchronizations, in which 4 resynchronization programs were implemented according to the ovarian status of dairy cows. AI, artificial insemination; CL, corpus luteum; d, day; GnRH, gonadotropin-releasing hormone; PG, prostaglandin  $F_{2\alpha}$ ; M Double-Ovsynch, modified Double-Ovsynch. Resynchronization: (1) Ovsynch, which consists of a GnRH injection, followed 7 days later by PG, a second injection of GnRH 56 hours later, and timed AI (TAI) 72 hours later (Ovsynch, n = 632); (2) an injection of PG, GnRH 3 days later, followed by Ovsynch 6 days later (PG-GnRH-Ovsynch, n = 191); (3) an injection of GnRH, followed by Ovsynch 6 days later (GnRH6-Ovsynch, n = 256); or (4) an injection of GnRH, PG 10 days later, GnRH 3 days later, followed by Ovsynch 7 days later (modified Double-Ovsynch, n = 263).

were higher ( $p < 0.05$ ) than those in the Ovsynch group (Fig. 2). However, there were no differences ( $p > 0.05$ ) in the pregnancies per AI either between the Ovsynch and PG-GnRH-Ovsynch or between the GnRH-Ovsynch and modified Double-Ovsynch (Fig. 2).

Our findings regarding the higher pregnancies per AI in the GnRH-Ovsynch and modified Double-Ovsynch groups compared to the Ovsynch group are consistent with a previous study [9], and this could be due to increased synchronization of cows during the Ovsynch program before TAI by way of the presynchronization with a single GnRH or an Ovsynch. However, unlike our findings, earlier studies found no beneficial effect of the presynchronization with GnRH before Ovsynch or CO-Synch, which consists of an injection of GnRH, PG 7 days later, followed by 2nd GnRH and concurrent TAI 72 hours later, on the pregnancies per AI in resynchronized dairy cows [8,11,12]. The reason for the difference between our study and others is not known, but it could be mainly because the cows were randomly grouped without checking for the presence of a CL in their ovaries in the earlier studies [8,11,12]. In cows with a CL, we found no benefit of the presynchronization with PG and GnRH before Ovsynch compared to the Ovsynch group that received no presynchronization. We expected that presynchronization with PG and GnRH before Ovsynch would improve the pregnancies per AI compared to only Ovsynch through more systematic follicular wave emergence, follicular development, and ovulation. However, our findings were not in sync with our expectations. Contrary to our findings, a previous study found that a presynchronization with PG and GnRH led to a greater ovulatory and luteolytic response to the first GnRH and PG of Ovsynch, resulting in a tendency toward a higher

pregnancy per AI [13]. This discrepancy between studies highlights the need for further research to identify the reasons for the same. In addition, the development of a better resynchronization program that can improve the fertility in cows with a CL might be required and could be the subject of future study.

In conclusion, our results show that the addition of presynchronization with a single GnRH or an Ovsynch before the Ovsynch for resynchronization in cows without a CL improves the pregnancy per AI compared to the only Ovsynch program in cows with a CL. However, we found no differences in the pregnancies per AI either between the Ovsynch and PG-GnRH-Ovsynch programs in cows with a CL or between the GnRH-Ovsynch and modified Double-Ovsynch programs in cows without a CL. Therefore, it is recommended that the Ovsynch and GnRH-Ovsynch programs could be preferred to resynchronize cows with and without a CL, respectively, from the perspective of reducing medication costs and saving labor.

## ORCID

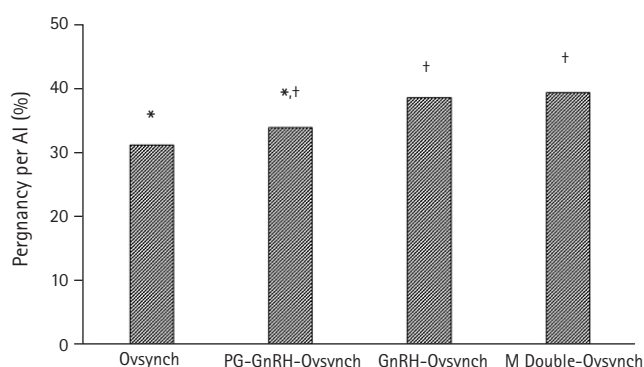
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**Fig. 2.** Comparison of the pregnancies per artificial insemination (AI) following the Ovsynch, PG-GnRH-Ovsynch, GnRH-Ovsynch, and modified Double-Ovsynch groups. M Double-Ovsynch, modified Double-Ovsynch. \*†Columns with different superscripts are significantly different ( $p < 0.05$ ).

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