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Early View

Original Research Article

Ovsynch-Based Synchronization Protocols on First-Service Pregnancy Rates in Primiparous Simmental Cows

Key words

dairy cows;
estrus response;
Ovsynch;
$PGF_{2}\alpha$;
pregnancy rate;
synchronization protocols

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Abstract: This study compared the effects of three Ovsynch-based synchronization protocols on first-service pregnancy rates in primiparous Simmental cows under field conditions. *Materials and Methods:* A total of 69 primiparous Simmental cows meeting the predefined inclusion criteria were randomly assigned to three groups (n = 23/group): $PGF_{2}\alpha$ pre-treatment followed by Ovsynch (Group 1), classical Ovsynch (Group 2), and Ovsynch with an additional $PGF_{2}\alpha$ administration on day 8 (Group 3). All animals underwent timed artificial insemination, and pregnancy diagnosis was performed by transrectal ultrasonography 30 days post-insemination. *Results:* Pregnancy rates were 56.5%, 43.5%, and 34.8% for Groups 1, 2, and 3, respectively, with no statistically significant differences among groups ($p > 0.05$). Estrus response following the first hormonal administration was observed in 21.7%, 17.4%, and 4.3% of animals, respectively, also without significant differences ($p > 0.05$). *Conclusion:* No statistically significant differences were observed among the synchronization protocols with respect to pregnancy rates or estrus response ($p > 0.05$). The results should be interpreted in light of the pilot nature of the study.

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Introduction

In mammals, oestrus is a behavioural and physiological reproductive indicator that enables the female to mate close to the time of ovulation. Oestrus is an externally observable and identifiable sign of ovulation and is considered the clinical manifestation of the physiologically internal and directly unobservable process of ovulation (1). However, due to the difficulty in detecting oestrous behaviour after parturition and the development of postpartum anoestrus problems in high-producing dairy cows, performing artificial insemination within an economically appropriate time frame has become more difficult (2). In addition, the intensification of dairy production, together with increased feed intake and genetic selection for higher milk yield, has negatively affected the reproductive physiology of dairy cows. Particularly in high-producing herds, weaker oestrous behaviour and the shorter duration of oestrus signs make oestrus detection more difficult and adversely affect female fertility (3). Therefore, ovulation synchronization methods have been developed to allow timed artificial insemination without the need for observing oestrus signs by synchronising follicular development and corpus luteum regression. In this way, insemination can be performed within the appropriate time frame to achieve the targeted reproductive performance in cows whose time of ovulation can be predetermined. Ovsynch protocols, which enable timed artificial insemination, are based on the sequential and coordinated administration of GnRH and $PGF_{2}\alpha$, allowing cows to be inseminated in a planned manner without the need for oestrus detection (2, 4). Synchronization protocols used for timed artificial insemination are widely applied in dairy cattle farming, and among these, the most commonly preferred method is the 7-day Ovsynch protocol (5). In this protocol, the first administration of gonadotropin-releasing hormone (GnRH) induces ovulation or luteinisation of the dominant follicle present in the ovary, thereby initiating a new follicular wave in a synchronised manner. The prostaglandin F2α ($PGF_{2}\alpha$) injection administered 7 days after the first GnRH stimulates regression of the corpus luteum (CL), leading to a decrease in progesterone concentration and supporting the final maturation of the dominant follicle. The second GnRH injection, administered approximately 48 hours after $PGF_{2}\alpha$ treatment, synchronises ovulation of the dominant follicle. Since ovulation generally occurs approximately 28 hours after the second GnRH administration, timed artificial insemination can be performed approximately 16 hours after this injection (6, 7).

Although the Ovsynch protocol is a widely used and successful method for timed artificial insemination, pregnancy rates may not always reach the desired level due to incomplete luteal regression following a single dose of $PGF_{2}\alpha$, variability in ovulatory response, and insufficient reduction of progesterone concentrations during the insemination period. In particular, young corpora lutea formed in response to the first GnRH administration in the Ovsynch protocol may be more resistant to a single dose of $PGF_{2}\alpha$, resulting in persistently high progesterone concentrations during the insemination period and negatively affecting fertility (8, 9). Accordingly, presynchronization protocols such as G6G and G7G have also been developed for this purpose (10). The main purpose of presynchronization strategies applied before the Ovsynch protocol is to improve the ovulatory response to the first GnRH administration by ensuring that cows begin the protocol at a more appropriate stage of the oestrous cycle (11). Modifications developed to reduce these limitations in the Ovsynch protocol, referred to as fertility programmes, play an important role in improving fertility. Among these strategies, presynchronization practices aimed at increasing the ovulatory response to the first GnRH administration and achieving complete regression of the corpus luteum before the final GnRH administration are particularly prominent. Presynchronization strategies using synthetic progesterone (P4), $PGF_{2}\alpha$, or combinations of $PGF_{2}\alpha$ and GnRH allow cows to begin the Ovsynch protocol at a more suitable stage of dioestrus, thereby improving the response of the dominant follicle to the first GnRH administration (3).

To reduce these limitations, various presynchronization strategies have been developed, including a second $PGF_{2}\alpha$ administration, double-dose $PGF_{2}\alpha$ protocols, and protocols such as Presynch, Double-Ovsynch, and G6G. In particular, adjusting the interval between $PGF_{2}\alpha$ and GnRH administration and increasing the presence of a functional corpus luteum at the beginning of the Ovsynch protocol through additional $PGF_{2}\alpha$ treatments may contribute to more effective luteal regression and improved pregnancy rates (11, 12).

In this study, it was aimed to compare the effects of the classical Ovsynch protocol, the Ovsynch protocol initiated after $PGF_{2}\alpha$ pre-treatment, and modified synchronization protocols including additional $PGF_{2}\alpha$ administration during the Ovsynch protocol on pregnancy rates obtained at first insemination in primiparous Simmental cows under field conditions. In addition, it was aimed to evaluate the oestrus responses observed after the first hormonal administration and to determine the relationship between these responses and pregnancy success. Accordingly, the study also aimed to compare the field applicability of different synchronization approaches under field conditions.

The $PGF_{2}\alpha$ pre-treatment evaluated in the present study was designed to establish an approximately 11-day interval between the initial $PGF_{2}\alpha$ administration and the $PGF_{2}\alpha$ treatment administered on Day 7 of the Ovsynch protocol. This approach was intended to incorporate the potential advantages of two-dose $PGF_{2}\alpha$ presynchronization without delaying initiation of the Ovsynch protocol. Furthermore, it was expected to increase the likelihood that cows would enter the Ovsynch protocol at a more appropriate stage of the estrous cycle without the need for ultrasonographic or rectal examination, thereby reducing labor requirements under routine herd management. Cows exhibiting estrus following the initial $PGF_{2}\alpha$ treatment were inseminated directly and did not proceed to the Ovsynch protocol, whereas cows that did not exhibit estrus subsequently entered the Ovsynch protocol.

Materials and methods

Approval for this study was obtained from the Local Ethics Committee for Animal Experiments of Burdur Mehmet Akif Ersoy University (MAKU-HADYEK) with decision number 1649 dated 18.02.2026. The study was designed in accordance with the ARRIVE 2.0 guidelines. The hormonal treatments used were within the scope of routine veterinary practice, and no invasive procedures that could negatively affect animal welfare were applied.

This study was conducted as a randomised controlled pilot field trial to compare the effects of three different Ovsynch-based synchronization protocols on pregnancy rates at first insemination.

Animal Material

The study was carried out on a total of 69 primiparous Simmental dairy cows, aged between 2 and 4 years, raised under the same housing and feeding conditions on the same farm. Animals were enrolled after meeting the predefined inclusion criteria, including normal uterine involution, absence of abnormal vaginal discharge, a body condition score (BCS) between 3.0 and 3.5, and no evidence of uterine disease based on clinical and transrectal ultrasonographic examination. All animals had completed their first calving. Synchronization programmes were initiated between 60 and 70 days postpartum after all inclusion criteria had been fulfilled. The study was conducted during the spring season, when environmental heat stress was minimal.

In order to ensure an unbiased evaluation of protocol efficacy, only the results of the first artificial insemination were considered, and subsequent inseminations were excluded from the analysis. The oestrus response observed after the first injection was also evaluated, and its relationship with pregnancy rates was analysed.

Inclusion and Exclusion Criteria

On postpartum day 35, all animals underwent vaginal discharge examination and uterine involution assessment by transrectal ultrasonography. Animals with a uterine involution score of 1 and a vaginal discharge score of 0 were included in the study. Animals diagnosed with uterine infection or delayed uterine involution were excluded from the study and treated appropriately.

Body condition scores (BCS) were evaluated according to the method described by Edmonson et al. (1989) (13), and only animals with a BCS between 3.0 and 3.5 were included in the study.

Experimental Design and Groups

Subsequently, cows were randomly allocated into three study groups (n = 23/group). The hormonal treatment schedules for the three synchronization protocols are summarized in Table 1.

Table 1: Hormonal treatment schedule for the synchronization protocols evaluated in the study

Protocol	Day −4	Day 0	Day 7	24 h after first $PGF_2\alpha$	56 h after first $PGF_2\alpha$	72 h after first $PGF_2\alpha$
PG4-Ovsynch	$PGF_2\alpha$	GnRH	$PGF_2\alpha$	–	Second GnRH	TAI
Classical Ovsynch	–	GnRH	$PGF_2\alpha$	–	Second GnRH	TAI
Ovsynch + Additional $PGF_2\alpha$	–	GnRH	$PGF_2\alpha$	Additional $PGF_2\alpha$	Second GnRH	TAI

Abbreviations: $PGF_2\alpha$, prostaglandin $F_2\alpha$; GnRH, gonadotropin-releasing hormone; TAI, timed artificial insemination

In the PG4-Ovsynch group, cows exhibiting estrus following the Day −4 $PGF_2\alpha$ treatment were inseminated and did not proceed to the Ovsynch protocol.

The three experimental groups differed only in the synchronization strategy applied. In the PG4-Ovsynch group, $PGF_2\alpha$ was administered four days before initiation of the classical Ovsynch protocol. In the Ovsynch + Additional $PGF_2\alpha$ group, an additional $PGF_2\alpha$ treatment was administered 24 h after the Day 7 $PGF_2\alpha$ injection, whereas the Classical Ovsynch group received the conventional protocol without any modification.

Within the Ovsynch protocol, GnRH was administered intramuscularly (IM) as 2 mL Ovarelin® (Ceva, Türkiye), containing 100 µg gonadorelin per animal. $PGF_2\alpha$ was administered intramuscularly as 5 mL Enzaprost®-T (Ceva, Türkiye), containing 25 mg dinoprost tromethamine per animal. The timing of hormonal administrations followed the schedules presented in Table 1.

Insemination and Blinding

Timed artificial insemination was performed in all animals by the same experienced veterinarian in order to minimize operator-related variability. Group allocation was determined by an independent technician, and blinding was ensured so that the veterinarian performing insemination and pregnancy diagnosis remained unaware of the group assignment of the animals.

Estrus Detection and Pregnancy Diagnosis

Estrus detection was performed by observing the animals twice daily following hormone administration. Estrus was diagnosed based on behavioral signs, including mounting or standing to be mounted, restlessness, and increased physical activity. Initial observations were made by farm personnel, and cows suspected of being in estrus were subsequently examined by the attending veterinarian. Estrus was confirmed by transrectal examination together with assessment of increased uterine tone and the presence of clear vulvar mucus discharge.

Pregnancy Diagnosis

Pregnancy diagnosis was performed by transrectal ultrasonography 30 days after insemination. Visualization of the embryo was accepted as the criterion for pregnancy. In doubtful cases, the examination was repeated 7 days later. Pregnancy rates obtained at first insemination were evaluated.

Pilot Nature of the Study

This study was designed as a pilot field trial conducted under routine commercial dairy farm management conditions. Hormonal treatments, estrus detection, and artificial insemination were performed according to routine veterinary practice without additional intensive reproductive monitoring. The findings obtained in this study represent the initial phase of a broader reproductive research project, and further confirmatory studies with larger animal populations are planned.

Statistical Analysis

Statistical analysis of the obtained data was performed using jamovi (Version 2.7, The jamovi Project, 2025) (14) and R (Version 4.5, R Core Team, 2025) (15). Pregnancy status at first insemination (pregnant/not pregnant) and oestrus response after the first hormonal administration (oestrus present/absent) were evaluated as categorical variables.

The Chi-square test was used to compare pregnancy rates and oestrus response rates among the groups. Fisher's exact test was applied when the expected cell frequencies were below 5. Data were expressed as number (n) and percentage (%). In addition, binary logistic regression analysis was performed to evaluate the odds of pregnancy among the synchronization groups.

Statistical significance was accepted at $p < 0.05$. Due to the pilot nature of the study, numerical differences and potential clinical relevance were also considered in the interpretation of findings when statistical significance was not achieved. Emphasis was placed on observed trends and effect direction in addition to statistical outcomes.

Results

A total of 69 primiparous Simmental cows (n = 23 per group) were included in the study. Pregnancy rates at first insemination were 56.5% (13/23), 43.5% (10/23), and 34.8% (8/23) in Group 1, Group 2, and Group 3, respectively (Table 2). Numerically, the highest pregnancy rate was observed in Group 1, which received $PGF_2\alpha$ pre-treatment. However, Chi-square analysis revealed no statistically significant difference among the groups ($\chi^2 = 2.23$; df = 2; $p = 0.329$), and Fisher's exact test also confirmed the absence of a significant difference ($p = 0.371$).

Estrus response rates following the first hormonal administration were 21.7% (5/23), 17.4% (4/23), and 4.3% (1/23) in Group 1, Group 2, and Group 3, respectively (Table 2). Similarly, although the highest estrus response was observed in Group 1, no statistically significant difference was found among the groups ($\chi^2 = 3.04$; df = 2; $p = 0.219$; Fisher's exact test $p = 0.314$).

Logistic regression analysis indicated lower odds of pregnancy in Group 2 (OR = 0.59) and Group 3 (OR = 0.41) compared with Group 1; however, these differences were not statistically significant ($p > 0.05$).

Table 2: Pregnancy rates at first insemination and estrus response across synchronization protocols

Group	Pregnancy (%)	Pregnant (n)	Estrus response (%)	Estrus (n)
1-PG4 - Ovsynch	56.5	13/23	21.7	5/23
2-Ovsynch	43.5	10/23	17.4	4/23
3-Ovsynch + $PGF_{2}\alpha$	34.8	8/23	4.3	1/23

Data are presented as number of animals (n) and percentages. Group 1: PG4 - Ovsynch; Group 2: Ovsynch; Group 3: Ovsynch + additional $PGF_{2}\alpha$. No statistically significant differences were detected among groups ($p > 0.05$)

Discussion

In this study, the effects of three different Ovsynch-based synchronization protocols on pregnancy rates at first insemination were compared in primiparous Simmental cows. No statistically significant differences were detected among the groups with respect to either pregnancy rate or estrus response. The absence of statistical significance may be associated with the pilot nature of the study and the relatively limited sample size. Nevertheless, the modified synchronization protocols evaluated in this study were developed to simplify reproductive management and therefore merit further investigation in larger-scale studies.

By initiating the Ovsynch protocol 4 days after $PGF_{2}\alpha$ administration, cows with a corpus luteum (CL) and a rapid response to prostaglandin were able to exhibit oestrus earlier. The possibility of inseminating these animals during natural oestrus allowed them to be removed from the herd before inclusion in the Ovsynch protocol, thereby reducing labour requirements and saving the amount of hormones used. Compared with the classical Ovsynch protocol, the requirement of only an additional 4-day period further increases the applicability of this approach under field conditions. Particularly in large herds, this may provide practical advantages in terms of labour planning and economic efficiency.

In the literature, presynchronization before Ovsynch is achieved in G6G and modified G6G protocols by administering GnRH following $PGF_{2}\alpha$ treatment. In contrast, in the present study, a more practical approach using fewer hormones was evaluated by directly initiating the Ovsynch protocol 4 days after $PGF_{2}\alpha$ administration. In this respect, the study protocol can be considered not only as a modified form of the classical Ovsynch protocol but also as an alternative synchronization strategy to the double-dose $PGF_{2}\alpha$ protocol applied at an 11-day interval.

While the first $PGF_{2}\alpha$ administration aims to induce regression of the existing corpus luteum, the second $PGF_{2}\alpha$ administration within the Ovsynch protocol is performed approximately 11 days later, creating a functional alternative to the classical double-dose $PGF_{2}\alpha$ approach. From this perspective, the Ovsynch protocol provides a synchronization model integrated between two $PGF_{2}\alpha$ administrations. Thus, both the effectiveness of luteal regression is enhanced and the evaluation of animals responding early through natural oestrus becomes possible. This represents an important advantage that may increase both the biological effectiveness of the protocol and its practical value under field conditions.

Wiltbank et al. (9) reported that a second $PGF_{2}\alpha$ administration in primiparous cows did not result in a statistically significant difference in pregnancy rates compared with the classical Ovsynch protocol. Similarly, in the present study, no statistically significant difference was detected among the groups. Barletta et al. (16) also reported that the second $PGF_{2}\alpha$ dose administered on day 8 of the Ovsynch protocol tended to increase pregnancy per insemination, although this increase did not reach statistical significance.

In contrast, Carvalho et al. (11)reported that a second $PGF_{2}\alpha$ dose administered 24 hours after the first $PGF_{2}\alpha$ treatment increased pregnancy rates particularly in resynchronized cows subjected to the second or subsequent timed artificial insemination, whereas this effect was not pronounced in cows undergoing the first timed insemination. Similarly, Santos et al. (4) emphasised that the second $PGF_{2}\alpha$ administration provided more evident benefits, especially in progesterone-supported resynchronization protocols.

Stevenson et al. (17), in their study, compared PG-3-G and Double Ovsynch presynchronization programmes with 5- and 7-day Ovsynch protocols. They reported that pregnancy rates were higher, particularly in the 7-day Ovsynch programme. The researchers stated that a single dose of $PGF_{2}\alpha$ in the 5-day protocol was insufficient for luteolysis, whereas luteolysis was more successfully achieved in the 7-day protocol, which positively affected pregnancy rates. The highest pregnancy rate was obtained in the PG-3-G group within the 7-day programme, while the lowest rate was observed in the group receiving a single dose of $PGF_{2}\alpha$ in the 5-day programme. Similarly, in the present study, the higher oestrus response and pregnancy rate observed in the $PGF_{2}\alpha$ pretreatment group support the positive effect of more effective regression of luteal structures on fertility.

Dirandeh et al. (10) reported that an additional $PGF_{2}\alpha$ injection administered 14 days before the G6G and G7G protocols increased progesterone concentrations, reduced pregnancy loss, and improved pregnancy rates on day 60. The researchers emphasised that more effective control of luteal structures before

Ovsynch contributed positively to fertility. Similarly, in the present study, the higher pregnancy rate observed in the PGF$_2\alpha$ pre-treatment group suggests that PGF$_2\alpha$ administered for presynchronization purposes may positively affect reproductive performance.

Yousuf et al. (18) compared the G6G protocol with a simpler presynchronization protocol in which PGF$_2\alpha$ and GnRH were administered on the same day, 7 days before the initiation of Ovsynch, and reported no statistically significant difference among the groups in terms of pregnancy rates. Although some ovulation and progesterone parameters were higher in the classical G6G group, the researchers stated that the simplified protocol provided acceptable pregnancy rates and could be considered a practical alternative under field conditions. Similarly, in the present study, although higher pregnancy rates and oestrus response were obtained in the PGF$_2\alpha$ pre-treatment group, no statistically significant difference was detected among the groups. This suggests that presynchronization protocols requiring less labour and allowing more practical application may be an effective alternative under field conditions.

Martins et al. (19) reported that a simplified presynchronization protocol, created by administering PGF$_2\alpha$ and GnRH on the same day, 7 days before the initiation of Ovsynch, provided pregnancy rates similar to those obtained with the classical Presynch-10/Ovsynch protocol. The researchers emphasised that this approach, which involved fewer application steps, could be a practical and effective alternative under field conditions. Similarly, in the present study, by directly initiating the Ovsynch protocol 4 days after PGF$_2\alpha$ pre-treatment, the protocol duration was further shortened and acceptable pregnancy rates were achieved without the need for additional GnRH administration. This suggests that shorter protocols using fewer hormones may also be successfully applied under field conditions.

Consentini et al. (20), in their study comparing different presynchronization and Ovsynch-based fertility programmes at the first postpartum timed artificial insemination, reported no statistically significant difference in pregnancy rates between the Double Ovsynch and progesterone- and oestradiol-supported Double E-Synch protocols. However, similar fertility rates were obtained in all protocols, and it was emphasised that appropriately controlled ovarian dynamics were the main determinant of pregnancy success. The researchers stated that protocols with different pharmacological bases could provide similar reproductive performance and that selecting the protocol most suitable for herd management was more important than identifying a single superior synchronization programme. These findings are consistent with the present study, in which no statistically significant difference was detected among the groups, although numerically higher pregnancy rates were observed in the PGF$_2\alpha$ pre-treatment group.

Therefore, in the present study, the evaluation of only first insemination results and the absence of progesterone supplementation may explain why no statistically significant difference was observed among the groups despite the second PGF$_2\alpha$ administration.

In the network meta-analysis conducted by Ambarcıoğlu et al. (21), it was reported that the Presynch + Ovsynch protocol increased pregnancy rates at first insemination compared with the classical Ovsynch protocol. The researchers emphasised that presynchronization practices, in particular, could improve fertility by creating a more suitable ovarian environment at the beginning of the Ovsynch protocol. Similarly, in the present study, the higher pregnancy rate obtained in the PGF$_2\alpha$ pre-treatment group suggests that presynchronization strategies applied before Ovsynch may contribute positively to reproductive performance.

Study Limitations

This study was designed as a pilot field trial, and the relatively limited sample size may have made it difficult to demonstrate statistically significant differences among the synchronization protocols. In addition, serum progesterone concentrations were not measured, and repeated ultrasonographic monitoring of ovarian structures was not performed throughout the synchronization protocols. Therefore, ovulatory responses and luteal dynamics could not be fully characterized. However, because the present study was designed as a field-based pilot trial primarily focusing on first-service pregnancy outcomes, more detailed reproductive monitoring procedures were beyond the scope of the study design.

In addition, only the results of the first insemination were evaluated, while subsequent inseminations and long-term reproductive performance were not included in the analysis. These limitations should be taken into consideration when interpreting the results, and the findings obtained should be confirmed by further studies conducted in larger animal populations and supported by more comprehensive reproductive follow-up.

Conclusion

Although no statistically significant differences were detected among the synchronization protocols with respect to pregnancy rate or estrus response, the findings of this pilot study provide preliminary information regarding the reproductive performance of different Ovsynch-based synchronization protocols. In addition, early identification of animals responding to PGF$_2\alpha$ through natural estrus may provide potential advantages in terms of herd management and hormone use. However, due to the pilot nature of the study and the relatively limited sample size, these findings should be interpreted cautiously and confirmed in larger animal populations.

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Data Availability Statement: The data presented in this study are available from the corresponding author upon reasonable request.

Author Declarations

Ethical approval: Approval for this study was obtained from the Local Ethics Committee for Animal Experiments of Burdur Mehmet Akif Ersoy University (MAKU-HADYEK; Decision No: 1649; Date: 18.02.2026).

Conflicts of interest: The author declares no conflict of interest.

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