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Comparative Efficacy of Ovsynch and Presynch-Ovsynch Protocols on Estrous Expression and Pregnancy Outcome in Holstein Friesian Cattle

Atthar Uddin

Department of Theriogenology, Faculty of Veterinary Sciences, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Sindh, Pakistan.

Qudratullah Kalwar

Department of Theriogenology, Faculty of Veterinary Sciences, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Sindh, Pakistan.

Email: qudratullahkalwar@gmail.com

Muhammad Mohsen Rahimoon

Department of Theriogenology, Faculty of Veterinary Sciences, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Sindh, Pakistan

Rashid Ali Korejo

Department of Animal Nutrition, Faculty of Animal Production and Technology, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Sindh, Pakistan

Abdul Saboor Brohi

Department of Theriogenology, Faculty of Veterinary Sciences, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Sindh, Pakistan

Shakeel Ahmed

Department of Dairy Technology, Faculty of Animal Production and Technology, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Sindh, Pakistan.

Rafique Ahmed

Department of Theriogenology, Faculty of Veterinary Sciences, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Sindh, Pakistan.

Manthar Ali

Department of Theriogenology, Faculty of Veterinary Sciences, Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Sindh, Pakistan

Haseeb Ahmed

Department of Veterinary Medicine, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tando Jam, Sindh, Pakistan.

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Corresponding Author:

Qudratullah Kalwar

Email: qudratullahkalwar@gmail.com

ABSTRACT

Efficient reproductive management is essential for improving fertility and maintaining productivity in dairy cattle, as delayed conception can prolong the calving interval and reduce reproductive efficiency. The present study evaluated and compared the effectiveness of Ovsynch and Presynch-Ovsynch protocols in Holstein Friesian cattle, with particular emphasis on estrous response, onset and duration of estrus, estrous intensity, total estrous score, and pregnancy outcome. Fourty healthy Holstein Friesian cattle were randomly divided into two equal groups of 20 animals. Group A received the Ovsynch protocol, whereas Group B was subjected to the Presynch-Ovsynch protocol. Estrous behavior was monitored through visual observation, and the onset, duration, intensity, and total estrous score were recorded. Pregnancy was subsequently confirmed using the established reproductive diagnostic procedure. Detectable estrus was observed in 60% of cows in the Ovsynch group and 80% in the Presynch-Ovsynch group. The mean onset of estrus was 50.40 ± 0.75 h in Ovsynch-treated cows compared with 63.04 ± 1.21 h in Presynch-Ovsynch-treated cows, while the corresponding estrous duration was 16.10 ± 1.13 h and 21.81 ± 0.53 h, respectively. Strong estrus was expressed by 45% of Ovsynch cows compared with 70% of Presynch-Ovsynch cows, whereas weak estrus was observed in 35% and 10%, respectively. The total estrous score was considerably higher in the Presynch-Ovsynch group (132.3 ± 15.123) than in the Ovsynch group (66.2 ± 10.870). Pregnancy was confirmed in 45% of cows receiving Ovsynch and 70% of those receiving Presynch-Ovsynch. The Presynch-Ovsynch protocol resulted in stronger and more sustained estrous expression, a higher total estrous score, and numerically improved estrous response and pregnancy rate compared with Ovsynch. It was concluded that Presynch-Ovsynch may provide a more effective reproductive management approach for enhancing estrous expression and potentially improving fertility in Holstein Friesian cattle.

Introduction

Reproductive efficiency is a key determinant of biological and economic performance in dairy cattle. Delayed conception increases days open and extends calving intervals, while inconsistent estrus expression can reduce the effectiveness of conventional breeding systems. (Priyanto et al., 2026). Timed artificial insemination (TAI) programs overcome some of these limitations by coordinating ovulation and allowing insemination at a predetermined time, reducing dependence on accurate visual estrus detection (Ayantoye et al., 2025). Ovsynch is a widely adopted TAI protocol involving sequential administration of GnRH and PGF $_{2\alpha}$ to synchronize follicular development, luteal regression, and ovulation. Although effective, its success can vary according to the ovarian and cyclic status of cows at protocol initiation (Vazquez & R. 2022). Presynchronization was therefore introduced to improve the proportion of animals entering Ovsynch at a physiologically favorable stage of the estrous cycle (Hassanein et al., 2024). Evidence from controlled studies has shown improved pregnancy per AI following presynchronization, with Navanukraw et al. (2004) reporting 49.6% pregnancy per AI following Presynch compared with 37.3% following Ovsynch alone. Meta-analytic evidence further indicates that reproductive responses vary with protocol design and cow characteristics (Borchardt et al., 2017; Wiltbank et al., 2017). Estrous expression is another useful indicator of reproductive response. Although TAI does not require visible estrus, the presence and intensity of behavioral signs can provide valuable information about reproductive activity and may be associated with subsequent fertility (Borchardt et al., 2021; Bretzinger et al., 2023). However, estrous expression varies considerably among cows and can be influenced by health, metabolic status, parity, days postpartum, and environmental conditions such as heat stress (Tippenhauer et al., 2021). Therefore, behavioral estrus should be considered alongside pregnancy outcome when evaluating synchronization programs. This issue is particularly relevant under South Asian dairy production systems, where climatic and management conditions may differ from those in temperate dairy regions. Recent research from Sindh, Pakistan, has demonstrated measurable reproductive responses to Ovsynch in Holstein Friesian and crossbred cattle, emphasizing the importance of evaluating synchronization strategies under local production conditions (Shah et al., 2024). Against this background, the present study compared Ovsynch and Presynch-Ovsynch protocols in Holstein Friesian cattle maintained under commercial farm conditions. The study focused on detectable estrous response, estrous intensity, and pregnancy outcome. It was hypothesized that presynchronization before Ovsynch would improve the expression of estrus and pregnancy rate compared with Ovsynch alone.

Materials and Methods

Study location and animals

The study was conducted at Dairy Land Farm Pvt. Ltd., Dhabeji, Sindh, Pakistan, using Holstein Friesian dairy cattle maintained under commercial farm-management conditions. Forty cattle were randomly allocated to two treatment groups of 20 animals each. Group A received Ovsynch and Group B received Presynch-Ovsynch.

Synchronization protocols

Animals in the Ovsynch group received the conventional sequence of GnRH, PGF $_{2\alpha}$, a second GnRH treatment, and timed artificial insemination according to the predetermined study schedule. The protocol was based on the established Ovsynch concept of synchronized ovulation and TAI (Pursley et al., 1995). Animals in the Presynch-Ovsynch group first received the presynchronization sequence with PGF $_{2\alpha}$ before entering the Ovsynch program. Presynchronization was intended to improve reproductive synchronization before the breeding sequence, consistent with the biological rationale and evidence for Presynch-Ovsynch (Borchardt et al., 2017).

Estrus detection and behavioral assessment

Estrous behavior was assessed by direct visual observation. Observable signs included characteristic standing/mounting behavior, increased activity or restlessness, and other recognizable signs of estrus. Estrous response was expressed as the number and percentage of cows in which estrus was detected. Visual assessment was used as the field-based behavioral measure in this study.

Onset, duration, intensity and total estrous score

Onset of estrus and estrous duration were recorded in hours as documented in the study dataset. Estrous intensity was categorized as strong, moderate, or weak according to the study scoring system. Total estrous score was analyzed as the composite score generated by the study's estrous scoring procedure. The present manuscript reports these values exactly as recorded in the experimental data and does not reconstruct or alter the underlying scoring formula.

Artificial insemination and pregnancy diagnosis

Artificial insemination was performed according to the predetermined timing of the respective synchronization protocols. Pregnancy was subsequently confirmed using the reproductive diagnostic procedure established for the study, including reproductive examination and ultrasonographic assessment where applicable. Pregnancy rate was calculated as the number of pregnant cows divided by the number of inseminated cows multiplied by 100.

Statistical analysis

Categorical outcomes were expressed as numbers and percentages. Continuous estrous variables were expressed as Mean $\pm$ SE. Differences were interpreted at $P < 0.05$. The reported P-values and LSD values were retained from the study data. Because the treatment groups contained only 20 cows each, nonsignificant findings are interpreted cautiously and numerical differences are not described as treatment effects unless supported by statistical significance.

Results

Estrous response

In the Ovsynch group, 12 of 20 cows (60%) exhibited observable estrous behavior, whereas 8 cows (40%) did not show detectable estrus. In comparison, 16 of 20 cows (80%) in the Presynch-Ovsynch group exhibited observable estrus, while 4 cows (20%) did not Show Observable estrous .

Table 1: Estrous Response among Ovasynch and Presynch-ovasynch

Treatment	Total animals (n)	Estrus detected (n)	Estrous response (%)	No detectable estrus (n, %)
Ovsynch	20	12	60 %	8 (40)
Presynch-Ovsynch	20	16	80 %	4 (20)

Onset and duration of estrus

Mean onset of estrus was 50.40 ± 0.75 h in the Ovsynch group and 63.04 ± 1.21 h in the Presynch-Ovsynch group, indicating a 12.64-h later mean onset after Presynch-Ovsynch. Estrous duration was 16.10 ± 1.13 h and 21.81 ± 0.53 h, respectively, corresponding to a 5.71-h longer observed estrous period in the Presynch-Ovsynch group.

Table 2: Effect of Ovsynch and Presynch-Ovsynch on onset and duration of estrus

Parameter	Ovsynch (n=20), Mean $\pm$ SE	Presynch-Ovsynch (n=20), Mean $\pm$ SE	P-value	LSD
Onset of estrus (h)	50.40 ± 0.75^b	63.04 ± 1.21^a	0.045	2.32
Estrous duration (h)	16.10 ± 1.13^b	21.81 ± 0.53^a	0.045*	2.32*

Estrous intensity

Estrous intensity shifted toward stronger expression following Presynch-Ovsynch. In the Ovsynch group, 9/20 cows (45%) exhibited strong estrus, 4/20 (20%) moderate estrus, and 7/20 (35%) weak estrus. In the Presynch-Ovsynch group, 14/20 cows (70%) exhibited strong estrus, 4/20 (20%) moderate estrus, and only 2/20 (10%) weak estrus.

Table 3: Distribution of estrous intensity following Ovsynch and Presynch-Ovsynch

Treatment	Strong	Moderate	Weak
Ovsynch	9/20 (45%)	4/20 (20%)	7/20 (35%)
Presynch-Ovsynch	14/20 (70%)	4/20 (20%)	2/20 (10%)

Total estrous score

Total estrous score was significantly higher following Presynch-Ovsynch. The Ovsynch mean was 66.2 ± 10.870 , compared with 132.3 ± 15.123 in the Presynch-Ovsynch group. The absolute difference was 66.1 score units, with the Presynch-Ovsynch mean approximately twice that of Ovsynch.

Table 4: Comparison of total estrous score between Ovsynch and Presynch-Ovsynch.

Parameter	Ovsynch Mean $\pm$ SE	Presynch-Ovsynch Mean $\pm$ SE	LSD	P-value
Total estrous score	66.2 ± 10.870^b	132.3 ± 15.123^a	37.703	0.001

Pregnancy outcome

Pregnancy was confirmed in 9 of 20 Ovsynch cows (45%) and 14 of 20 Presynch-Ovsynch cows (70%). The reported P-value was 0.1098; therefore, the pregnancy difference was not statistically significant at $P < 0.05$.

Table 5: Effect of Ovsynch and Presynch-Ovsynch on pregnancy rate

Treatment	Total animals (n)	Pregnant (n)	Pregnancy (%)	Not pregnant (n, %)
Ovsynch	20	9	45	11 (55)
Presynch-Ovsynch	20	14	70	6 (30)

Discussion

The present study showed a generally favorable numerical reproductive pattern following Presynch-Ovsynch compared with Ovsynch. Detectable estrus increased from 60% to 80%, strong estrous expression from 45% to 70%, while weak estrus declined from 35% to 10%. Pregnancy rate also increased from 45% to 70%. However, overall estrous response ($P = 0.301$) and pregnancy rate ($P = 0.1098$) did not differ significantly between protocols. Therefore, these findings should be interpreted as a favorable numerical response rather than definitive evidence of superior fertility (Borchardt et al., 2017; Fricke et al., 2022). The greater estrous response observed with Presynch-Ovsynch is biologically consistent with its purpose of reducing variation in ovarian status before the breeding Ovsynch sequence. Presynchronization may increase the likelihood that cows enter Ovsynch at a more suitable stage of the estrous cycle, potentially improving follicular and luteal synchronization (Galvão et al., 2007; Borchardt et al., 2017). A notable finding was the shift in estrous intensity. Presynch-Ovsynch produced more cows with strong estrus and fewer cows with weak estrus. Mean estrous duration was also longer with Presynch-Ovsynch (21.81 ± 0.53 h) than with Ovsynch (16.10 ± 1.13 h), while mean onset of estrus differed by 12.64 h (63.04 ± 1.21 vs. 50.40 ± 0.75 h; $P = 0.045$). These differences indicate that presynchronization may influence the observable pattern of estrous behavior. Because stronger and longer estrous expression may be easier to recognize under field conditions, this response could have practical value in farms relying partly on visual observation (Borchardt et al., 2021; Tippenhauer et al., 2021). The total estrous score provided further support for this pattern. The mean score increased from 66.2 ± 10.870 in Ovsynch to 132.3 ± 15.123 in Presynch-Ovsynch, with a significant difference ($P = 0.001$; $LSD = 37.703$). The agreement between the composite score and the categorical distribution strengthens the consistency of the behavioral findings. Nevertheless, estrous intensity should not be regarded as a direct substitute for fertility because conception also depends on ovulation, oocyte competence, embryo development, uterine health, luteal function, metabolic status, and environmental conditions (Borchardt et al., 2021). Pregnancy rate followed the same numerical direction, with five more pregnancies in Presynch-Ovsynch than Ovsynch. This pattern agrees with evidence that presynchronization can improve reproductive outcomes in dairy cattle, although responses vary among herds, parity groups, management systems, and synchronization strategies (Borchardt et al., 2017). The 70% pregnancy rate observed in the present Presynch-Ovsynch group should therefore not be generalized because only 20 cows were included per treatment. Accordingly, the appropriate conclusion is that Presynch-Ovsynch produced a numerically higher pregnancy rate, rather than a significantly improved pregnancy rate. Similar caution is warranted for overall estrous response. Several factors may have contributed to variation in reproductive responses, including parity, body condition, postpartum interval, health status, season, and heat stress. These factors can influence ovarian function, estrous expression, and fertility independently of synchronization treatment (Wang et al., 2023). This is particularly relevant under commercial dairy conditions in Sindh, where environmental and management conditions may differ from those of temperate dairy systems. Regional evidence from Sindh has also demonstrated measurable reproductive responses following Ovsynch in Holstein Friesian and crossbred cattle, supporting the importance of locally generated evidence (Shah et al., 2024). The present study has some limitations. The sample size of 20 cows per treatment restricts statistical power, particularly for categorical outcomes such as estrous response and pregnancy. In addition, visual estrous scoring may be affected by observation frequency and observer consistency. Larger studies incorporating parity, body condition, days postpartum, health status, and season, together with automated activity monitoring, would provide more robust estimates of treatment effects (Borchardt et al., 2021; Tippenhauer et al., 2021). Presynch-Ovsynch produced a significantly stronger behavioral estrous profile, characterized by greater total estrous score, longer estrous duration, more strong estrus, and less weak estrus. Pregnancy rate was also numerically higher, although the difference was not statistically significant. These findings suggest potential reproductive and management benefits of presynchronization, but larger trials under diverse Pakistani commercial dairy conditions are needed to confirm whether the observed behavioral advantage consistently translates into improved fertility.

Conclusion

The study demonstrated that Presynch-Ovsynch was more effective than Ovsynch in improving the overall estrous response of postpartum Holstein Friesian cattle. Presynchronization resulted in a more favorable estrous onset, prolonged estrous duration, and stronger and more consistent estrous expression, accompanied by an improved pregnancy outcome. These findings indicate

that incorporating presynchronization into the Ovsynch program can enhance the predictability and quality of estrous expression and may contribute to improved reproductive performance under commercial dairy conditions. These findings confirm that the Presynch-Ovsynch offers most reliable and efficient approach in terms of optimizing Estrous Response, estrous intensity, estrous duration and Fertility rate in Holstein Friesian cattle under farm condition.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

To be completed according to the actual contributions of the authors.

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