SHORT COMMUNICATION

Progesterone Levels and Estrus Symptoms in Modified Ovsynch Protocols in Khillar Cows

Mayank Diwakar Barde[1], Maheshkumar Vitthalarao Ingawale[1]*, Shyam Ganesh Deshmukh[1], Chaitanya Hemednrakumar Pawshe[1], Ranjit Suresh Ingole[2], Sudhakar Prahlad Awandkar[3]

Abstract

The progesterone levels and estrus signs were studied in Ovsynch, double PG Ovsynch and increasing dose of PG in Ovsynch synchronization protocols in multiparous lactating Khillar cows (6 each) under field conditions. The tumefaction of vulval lips and cervical mucus discharge were most prominent estrus behaviour signs exhibited by 88 % cows in all the three groups. The mean serum progesterone levels on the day of estrus were 0.95 ± 0.77, 0.82 ± 0.04 and 0.90 ± 0.03 ng/mL in Ovsynch, double PG Ovsynch and increasing dose of PG in Ovsynch protocols, respectively, which did not differ statistically. The pregnancy rate observed was also non-significantly higher in double PG Ovsynch synchronized cows than other two groups (50% vs 33.33%). The serum progesterone during estrus in double PG Ovsynch synchronization protocol was lower indicating better estrus quality with recorded higher pregnancy rate than Ovsynch and increasing dose PG protocol.

Key words: Estrus, Khillar, Ovsynch, Progesterone, Synchronization.

Ind J Vet Sci and Biotech (2026): 10.48165/ijvsbt.22.1.41

Introduction

Khillar is a draught cow breed primarily used for draught and bull racing purposes in Maharashtra and the population was 967.2 thousand as per 20th Livestock Census, 2019. The synchronization of estrus is effective method for optimization of reproductive efficiency and effective use of sex-sorted semen (Hirole *et al.,* 2018). Ovsynch is most adopted estrus synchronization and fixed time insemination protocol in field conditions. The Ovsynch program consists of injection of GnRH, PGF2α, and GnRH on day 0, 7, and 9, respectively, and cows are inseminated 16 to 25 h after the second injection of GnRH. There is synchronization of follicle maturation with regression of the corpus luteum before the GnRH induced ovulation and timed insemination. Ovsynch has some shortcomings, where up to 40% of animals do not respond to this protocol, either due to insufficient synchronization of follicular waves, or failed luteolysis (Martins and Pursley, 2016). In the first case, in cows whose dominant follicles do not respond to the first GnRH, the reason is probably a follicular age of 3-4 days, when there is a possibility of their submission to atresia before PGF2α administration. The second problem encountered in Ovsynch is a failed response of CL to PGF2α application. The lack of luteolytic effect results in suppressed growth of the second follicular wave, thus disabling synchronization of ovulation and FTAI (Nowicki *et al*., 2019). The new CL, induced by the first GnRH treatment in Ovsynch, is inappropriately regressed with the single treatment with PGF2α. Lack of complete regression of the CL to the PGF2α treatment has been observed in 10-25% of the cows treated with Ovsynch and small elevations in circulating P4 near AI, due to inadequate CL regression, have reduced fertility (Wiltbank and Pursley, 2014).

To overcome the problem with inadequate CL regression during the timed AI protocols, two general strategies have been used. One strategy is an increased dose of PGF2α during either the 5 day or 7 day Ovsynch protocols (Giordano *et al*., 2013). A second strategy is to give a second dose of PGF2α on the subsequent day after the first PGF2α treatment of a

[1]Department of Animal Reproduction, Gynecology & Obstetrics, College of Veterinary & Animal Sciences, Akola-444104, Maharashtra Animal & Fishery Sciences University (MAFSU), Maharashtra, India

[2]Departments of Veterinary Pathology, College of Veterinary & Animal Sciences, Akola-444104, MAFSU, Nagpur, Maharashtra, India

[3]Departments of Veterinary Microbiology, College of Veterinary & Animal Sciences, Akola-444104, MAFSU, Maharashtra, India

Corresponding Author: Dr. M.V. Ingawale, Department of Animal Reproduction, Gynecology & Obstetrics College of Veterinary & Animal Sciences, Akola-444 104, MAFSU, India. e-mail: maheshingawale@mafsu.ac.in

How to cite this article: Barde, M. D., Ingawale, M. V., Deshmukh, S. G., Pawshe, C. H., Ingole, R. S., & Awadhkar, S. P. (2026). Progesterone Levels and Estrus Symptoms in Modified Ovsynch Protocols in Khillar Cows. Ind J Vet Sci and Biotech, 22(1), 200-203.

Source of support: Nil

Conflict of interest: None

Submitted 16/10/2025 **Accepted** 18/11/2025 **Published** 10/01/2026

5d or 7d Ovsynch protocol. In the 7-d protocol, increasing the dose of cloprostenol from 500 to 750 µg increased CL regression and tended to increase fertility (Giordano *et al.,* 2013). The estrus signs exhibited by the cows and the levels of progesterone during estrus in synchronized cows have positive correlation on pregnancy rate. Considering the above proposition, this research work was implemented on progesterone levels and estrus signs in modified Ovsynch protocols in Khillar cows.

Materials and Method

For the present study, total eighteen Khillar, 60-days postpartum with normal genitalia, free from clinical or subclinical infection were randomly selected from villages in Shirol teshil, Dist: Kolhapur, Maharashtra, India. Paddy straw, sorghum straw, groundnut straw and local grass amongst the dry roughages, and sugarcane tops and maize amongst the green forages were being fed to the animals. The two kg concentrate was provided in the morning to the animals along with chelated mineral mixture supplementation @ 50 g/day/animal. The selected multiparous cows were initially given pre-synchronization treatment like injection Ivermectin @ 1 mL per 50 kg body weight subcutaneously and injection vitamin AD3E & H, 5 mL i/m.

These cows were divided into three groups containing six cows in each group (n=6). The cows from the group I were synchronized with Ovsynch synchronization protocol (Inj. Buserlin acetate 10 µg i/m on day 0, Inj. Cloprostenol sodium 500 µg on day 7 and Inj. Buserlin acetate 10 µg i/m on day 9), cows from the group II were synchronized with double PGF2α Ovsynch synchronization protocol (PGF2α Inj. Cloprostenol sodium 500 µg given twice on day 7 and 8), and the cows from the group III were synchronized with increasing dose of $PGF_2α$ (Inj. Buserlin acetate 10 µg i/m on day 0, Inj. Cloprostenol sodium 750 µg on day 7 and Inj. Buserlin acetate 10 µg i/m on day 9). The cows in all three groups were inseminated with male sex-sorted semen (purchased from BAIF, Urli Kanchan, Dist: Pune) 16-20 h after last GnRH injection. All the cows were observed for the presence or absence of visible estrus signs like cervical mucus discharge, vulvar swelling (tumefaction), bellowing and frequent urination after PGF2α injection by visual observations in morning and evening till insemination.

The 10 mL blood samples from all the cows of three groups were collected on the day of estrus in heparinised vials from jugular vein. The collected blood samples were centrifuged and serum was separated, which was stored at –20°C until analyzed. The serum progesterone concentrations were measured through PROG-EASIA (Catalogue No: E-EL-0154) kit manufactured by Elabscience Biotechnology Co., Ltd, USA. The pregnancy diagnosis was carried out two months after insemination, and pregnancy rate was recorded. The data obtained was analyzed using Web Agri Stat Package (WASP-2, ICAR, Goa).

Results and Discussion

All the 18 cows (100%) in Ovsynch, double PG Ovsynch and increasing dose of PG in Ovsynch synchronization protocols responded to treatment and exhibited estrus. All the cows exhibited either one or more behavioural estrus signs like cervical mucus discharge, vulvar swelling (tumefaction), bellowing and frequent urination. In Group-I, the tumefaction of vulval lips, cervical mucus discharge, and frequent micturition were shown by 100 % (6/6) cows, while bellowing was exhibited by 50 % (3/6) cows. In the Group-II, the tumefaction of vulval lips, cervical mucus discharge, and frequent micturition were shown by 83.33 % (5/6) cows, while bellowing was exhibited by 66.66 % (4/6) cows. In the Group-III, the tumefaction of vulval lips and cervical mucus discharge was exhibited by 83.33% (5/6) cows, while frequent micturition and bellowing was exhibited by 66.66 % (4/6) cows (Table 1). The tumefaction of vulval lips and cervical mucus discharge were most prominent estrus behaviour signs exhibited by 88 % cows in all the three groups, while bellowing was the exhibited by 61.11 % cows, which was least exhibited estrus sign.

The results of present study in regard to estrus signs concurred with Singh (2018), who recorded estrus signs in 75 % Sahiwal cows following Ovsynch protocol, out of which 70 % (14/20) showed CVM discharge, 30% (6/20) showed vulvar swelling, 10 % (2/20) showed bellowing and 20 % (4/20) showed frequent micturition, which are lower than those observed in present study. Shinde *et al.* (2025) observed congestion of vaginal mucus membrane in 70 % (14/20), vaginal discharge in 60 % (12/20), and the tumefaction of vulval lips in 55 % (11/20) Gir cows synchronized with Ovsynch protocol. Contrary to the present study, Mattoni *et al.* (1988) observed vaginal mucus discharge in 64% of estrus periods in Zebu cattle with spontaneous estrus.

Serum Progesterone Levels

The mean serum progesterone levels recorded on the day of estrus in Ovsynch, double PG Ovsynch and increasing dose of

Table 1: Estrus signs (%) exhibited in Ovsynch, double PG Ovsynch and increasing dose of PG in Ovsynch protocols in Khillar cows

Groups	Estrus signs exhibited by cows			
	Tumefaction of vulval lips	Vaginal discharge	Frequent micturition	Bellowing
Ovsynch	6 (100%)	6 (100%)	6 (100%)	3 (50.00%)
Double PG Ovsynch	5 (83.33%)	5 (83.33%)	5 (83.33%)	4 (66.66%)
Increasing dose of PG in Ovsynch	5 (83.33%)	5 (83.33%)	4 (66.66%)	4 (66.66%)

Table 2: Serum progesterone concentration and pregnancy rate Ovsynch, double PG Ovsynch and increasing dose of PG in Ovsynch protocols in cows

Groups	Ovsynch	Double PG Ovsynch	Increasing Dose PG	Overall
No. of cows inseminated	6	6	6	18
Mean serum progesterone (ng/mL)	0.95 ± 0.77^a	0.82 ± 0.04^a	0.90 ± 0.03^a	0.90 ± 0.02
Pregnancy rate (%)	2/6 (33.33)[a]	3/6 (50.00)[a]	2/6 (33.33)[a]	7/18 (38.88)

Means with common superscript within the rows do not differ significantly from each other ($p<0.05$).

PG in Ovsynch protocols did not differ between the groups, though the values were lower in modified Ovsynch protocols (Table 2). The higher pregnancy rate was observed in double PG Ovsynch synchronized cows than other two groups (50% vs 33.33%), which was also non-significant at $p<0.05$.

The results with regard to serum progesterone during the estrus in Ovsynch treated cows concurred with Deshmukh *et al.* (2010), who observed 1.10 ng/mL in Red Kandhari cows, while Buhecha *et al.* (2015) recorded it as 1.33 ng/mL in normal cyclic cows. The slightly lower progesterone levels were recorded than the current values by Naikoo *et al.* (2012) in anestrus Kankrej (0.50 ng/mL); Parmar *et al.* (2015) in cyclic cows (0.70 ng/mL) and Manokaran *et al.* (2023) in Indigenous Kangayam cows (0.85 ng/mL).

Wiltbank *et al*. (2015) reported 0.4 and 0.2 ng/mL progesterone in Ovsynch, and double PG Ovsynch groups, while Carvalho *et al.* (2015) observed these as 0.35 and 0.15 in ng/mL, respectively, which are lower than the results obtained in present study. Barletta *et al.* (2018) recorded 0.3, 0.18 and 0.28 ng/mL serum P4 in Ovsynch, double PG Ovsynch and increasing dose PG in Ovsynch protocols, which are also lower than the results observed in present study. The variation in serum progesterone levels during estrus may be due to variation in type of estrus (induced or spontaneous), method of serum estimation, breed, cyclic and non-cyclic stage and protocol and dose of PG in estrus synchronization protocol. In the present study, the serum progesterone level during estrus in double PG group was observed lower than Ovsynch and increasing dose PG Ovsynch groups. The pregnancy rate observed in double PG group was also higher than Ovsynch and increasing dose PG Ovsynch groups indicating correlation with lower progesterone during estrus and higher pregnancy rate (Wiltbank *et al.,* 2015; Tippenhauer *et al.,* 2021; Borchardt *et al.,* 2021).

The findings indicate that double PG Ovsynch synchronization protocol is better than routine Ovsynch and increasing dose PG Ovsynch protocol as revealed by the lower serum progesterone and better estrus quality with higher pregnancy rate in this group of Khillar cows.

Acknowledgement

The authors are thankful to Associate Dean, College of Veterinary & Animal Sciences, Akola for providing necessary facilities and financial support for the research work.

References

20th Livestock Census (2019). Department of Animal Husbandry and Dairying, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India. Retrieved from http://dadf.gov.in/sites/default/filess/ Provisional%20Key%20Results%20of%2020th%20Livestock%20 Census.pdf.

Barletta, R.V., Carvalho, P.D., Santos, V.G., Melo, L.D.F., Consentini, C.E., Netto, A.S., & Fricke, P.M. (2018). Effect of dose and timing of prostaglandin $F_2\alpha$ treatments during a Resynch protocol on luteal regression and fertility to timed artificial insemination in lactating Holstein cows. *Journal of Dairy Science, 101*(2), 1730-1736.

Borchardt, S., Tippenhauer, C.M., Fricke, P.M., & Heuwieser, W. (2021). Economic impact of adding a second prostaglandin $F_2\alpha$ treatment during an Ovsynch protocol using a meta-analytical assessment and a stochastic simulation model. *Journal of Dairy Science, 104* (11), 12153-12163.

Buhecha, K.V., Dhami, A.J., Hadiya, K.K., Patel, M.D., Parmar, S.C., & Killedar, A. (2015). Influence of different estrus synchronization protocols on fertility and plasma progesterone in anoestrus crossbred cows. *Indian Journal of Animal Reproduction, 36*(2), 1-5.

Carvalho, P.D., Fuenzalida, M.J., Ricci, A., Souza, A.H., Barletta, R.V., Wiltbank, M.C., & Fricke, P.M. (2015). Modifications to Ovsynch improve fertility during resynchronization: Evaluation of presynchronization with gonadotropin-releasing hormone 6 d before initiation of Ovsynch and addition of a second prostaglandin F2α treatment. *Journal of Dairy Science, 98*(12), 8741-8752.

Deshmukh, Y.D., Markandeya, N.M., Chaudhari, R.J., & Deshmukh, V.V. (2010). Progesterone profile in two different protocols of estrus synchronization in postpartum Red Kandhari cows. *Indian Journal of Animal Reproduction, 31*(2), 37-39.

Giordano, J.O., Wiltbank, M.C., Fricke, P.M., Bas, S., Pawlisch, R., Guenther, J.N., & Nascimento, A.B. (2013). Effect of increasing GnRH and PGF2α dose during Double-Ovsynch on ovulatory response, luteal regression, and fertility of lactating dairy cows. *Theriogenology, 80*(7), 773-783.

Hirole, P.D., Deshmukh, S.G., Ingawale, M.V., Kuralkar, S.V., Thorat, M.G., Ratnaparkhi, A.R., & Godbole, P.V. (2018). Comparative efficacy of two different synchronization protocol in postpartum dairy cows. *International Journal of Livestock Research, 8*(11), 283-290.

Manokaran, S., Selvaraju, M., Geetha, T., Palanisamy, M., Devipriya, K., & Periyannan, M. (2023). Comparative study on effect of different estrus synchronization protocols on the pattern of estrus, conception rate and serum hormonal profile in indigenous Kangayam cows. *Indian Journal of Animal Research, 57*(2), 172-177.

Martins, J.P.N., & Pursley, J.R. (2016). Fertility programs for lactating dairy cows, their physiological basis, and the factors that are critical for their success. *Animal Reproduction, 13,* 283-289.

Mattoni, M., Mukasa-mugerwa, E., Cecchini, G., & Sovani, S. (1988). The reproductive performance of east African zebu cattle in Ethopia. *Theriogenology, 30*(5), 961-971.

Naikoo, M., Dhami, A.J., & Ramakrishnan, A. (2012). Effect of estrus synchronization on plasma progesterone profile and fertility response in postpartum suckled anestrous Kankrej cows. *Indian Journal of Animal Research, 50*(4), 460-465.

Nowicki, A., Baranski, W., Tobolski, D., Zdunczyk, S., & Janowski, T., (2019). Second prostaglandin F2α treatment during Ovsynch protocol does not improve fertility outcomes in dairy cows. *Polish Journal of Veterinary Sciences, 22*(1), 157-161.

Parmar, C.P., Patel, D.M., Hadiya, K.K., Dhami, A.J., Buhecha, K.V., & Sarvaiya, N.P., (2015). Fertility and plasma progesterone profile in repeat breeding cows and buffaloes in Ovsynch and mid-cycle PGF2α treatment protocols. *Indian Journal of Animal Reproduction, 36*(2), 29-32.

Shinde, P.M., Ingawale, M.V., Pawshe, C.H., Waghmare, S.P., Hajare, S.W., & Ingole, R.S. (2025). Effect of sex sorted semen in synchronized estrus on pregnancy rate and female ratio in Gir cows. *Indian Journal of Animal Research, 59*(3), 372-376.

Singh, A. (2018). Evaluation of estrus synchronization protocols in Sahiwal cows. *Doctoral Dissertation*. Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India.

Tippenhauer, C.M., Steinmetz, I., Heuwieser, W., Fricke, P.M., Lauber, M.R., Cabrera, E.M., & Borchardt, S. (2021). Effect of dose and timing of prostaglandin F_2α treatments during a 7-d Ovsynch protocol on progesterone concentration at the end of the protocol and pregnancy outcomes in lactating Holstein cows. *Theriogenology, 162,* 49-58.

Wiltbank, M.C., & Pursley, J.R. (2014). The cow as an induced ovulator: Timed AI after synchronization of ovulation. *Theriogenology, 81*(1), 170-185.

Wiltbank, M.C., Baez, G.M., Cocharne, F., Barletta, R.V., Trayford, C.R., & Joseph, R.T. (2015). Effect of second treatment with PGF_2α during Ovysnch protocol on luteolysis and pregnancy in dairy cows. *Journal of Dairy Science, 98*(12), 8644-8654.