RESEARCH ARTICLE

Comparative Efficacy of Kisspeptin and GnRH for Ovulation Synchronization in Cattle

Kalpak B. Kayande[1], Shyam G. Deshmukh[1]*, Chaitanya H. Pawshe[1], Mahesh V. Ingawale[1], Sudhakar P. Awandkar[2], Mahesh V. Bhalke[1]

Abstract

The aim of the study was to investigate the potential of Kisspeptin-10 as an alternative to GnRH in the Ovsynch protocol for improving reproductive performance in postpartum dairy cows. A total of 20 postpartum dairy cows were selected and the animals were equally divided into two groups. In Group T_1, cows were treated with Kisspeptin-10 on Day 0, $PGF_2\alpha$ on Day 7 and Kisspeptin-10 on Day 9. In Group T_2, cows were treated with the conventional Ovsynch protocol consisting of GnRH on Day 0, $PGF_2\alpha$ on Day 7 and GnRH on Day 9. Timed artificial insemination was performed 18-24 h after the final hormonal injection. Estrus attributes, pregnancy rate, and serum FSH and LH concentrations were assessed. Although the percentage of induced estrus response and the onset time for estrus after $PGF_2\alpha$ injection were comparable between groups, kisspeptin-treated cows exhibited a significantly longer duration of estrus. The pregnancy rate was higher in the Kisspeptin-10 treated group than in the GnRH treated group (50.0 vs. 30.0 %). Endocrine evaluation revealed significantly higher FSH levels in GnRH-treated cows, whereas kisspeptin-treated cows showed a significantly higher LH response at 30 min post-injection on Day 9. Overall, incorporation of Kisspeptin-10 into the Ovsynch protocol may serve as an effective alternative to GnRH for ovulation synchronization in postpartum dairy cows.

Key words: Conception rate, Gonadotropins, Kisspeptin-10, Ovsynch protocol, Ovulation synchronization, Postpartum dairy cows.

Ind J Vet Sci and Biotech (2026): 10.48165/ijvsbt.22.3.18

Introduction

Estrus or ovulation synchronization protocols are based mainly on GnRH and/or $PGF_2\alpha$ or their combination. Undoubtedly, the GnRH or its analogues are on the top priority for this purpose (Mondal *et al*., 2018). Ovulation synchronization using GnRH followed by TAI have been reported to be satisfactory in terms of conception rates. Injections of GnRH on days 0 and day 9, $PGF_2\alpha$ on day 7, and timed artificial insemination (TAI) on day 10 make up the Ovsynch regimen (Pursley *et al*., 1995). The treatment is justified by the fact that the first injection of GnRH causes the release of LH, which causes ovulation or luteinization of the dominant follicle on the ovary and the emergence of a new follicular episode within two days after the drug's administration. Seven days following therapy, $PGF_2\alpha$ delivery causes luteolysis, this is followed by a second dose of GnRH injection, which causes LH release and synchronizes the new dominant follicle's ovulation (Bo *et al*., 2002). Ten to thirty percent of cows treated with Ovsynch did not synchronize ovulation in response to final GnRH, despite the drug's overall beneficial effects (Vasconcelos *et al*., 1999; Navanukraw *et al*., 2004). The lowest estrus response in Ovsynch protocol may be caused by a small estrogenic dominant follicle or by variations in the stage of estrus cycle at the start of hormonal treatment (Vasconcelos *et al*., 1999).

In order to improve animal reproductive performance, a new kind of follicle-regulating technique must be developed that can not only synchronize the timing of ovulation, but also enhance follicular development and the quality of the oocyte and corpus luteum (CL) (Bahareldin-Ali *et al*., 2015). Kisspeptin, which is recognized as a key upstream regulator of the hypothalamic-pituitary-gonadal axis, plays a crucial role in regulating the pulsatile secretion of GnRH (Popa *et al*., 2008; Smith *et al.,* 2009; Lehman *et al*., 2010). Kisspeptin mediate various physiological functions like sex differentiation of brain, onset of puberty, hypothalamic GnRH secretion, feedback mechanism of ovarian steroids, ovulation,

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

[2]Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Akola-444104, MAFSU, Maharashtra, India

Corresponding Author: Dr. Shyam Ganesh Deshmukh, Professor, Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Akola-444104, MAFSU, Maharashtra, India. e-mail: shyamdeshmukh@mafsu.ac.in

How to cite this article: Kayande, K. B., Deshmukh, S. G., Pawshe, C. H., Ingawale, M. V., Awandkar, S. P., & Bhalke, M. V. (2026). Comparative Efficacy of Kisspeptin and GnRH for Ovulation Synchronization in Cattle. Ind J Vet Sci and Biotech, 22(3), 94-98.

Source of support: Nil

Conflict of interest: None

Submitted 25/02/2026 **Accepted** 20/03/2026 **Published** 10/05/2026

metabolic control over reproduction, cancer cell metastasis etc (Pinilla *et al*., 2012). Mondal *et al*. (2018) in crossbred dairy heifers and Pottapenjera *et al*. (2018) in buffaloes developed a novel kisspeptin-based ovulation synchronization protocol and reported that the kisspeptin protocol resulted in better follicular growth, earlier initiation of luteolysis, effectively synchronized estrus and ovulation, with a significantly higher ovulation rate and conception rate compared to the conventional Ovsynch protocol, indicating its superiority for ovulation synchronization in bovines. These findings suggest that kisspeptin could be a more potent and efficient alternative to GnRH analogues in synchronization protocols for dairy animals. Keeping the above points in view and limited literature on kisspeptin-based ovulation synchronization in dairy animals, the present study was undertaken to compare the effect of exogenous administration of Kisspeptin-10 and GnRH for estrus synchronization in cattle.

Materials and Methods

The present research work was approved by Institutional Ethical Committee for Veterinary Clinical Research, College of Veterinary and Animal Science, Akola, Maharashtra, India (IEC-VCR approval number HD/VCM/COVAS/201/2025). A total of 20 cows at 50-60 days postpartum were selected for present study. Cows were examined for health disorders and palpated per rectum for ovarian activity before synchronization and randomly divided into two equal groups. In group T1 (modified Ovsynch with Kisspeptin-10 group), cows were treated with Kisspeptin-10 (20 µg/kg, i/v, Viron Enterprises; Mumbai) on day 0, $PGF_2\alpha$ (Cloprostenol, 500 µg, i/m, Pregova,Virbac Animal Health Pvt. Ltd; Mumbai) on day 7 and a second dose of Kisspeptin-10 (20 µg/kg, i/v) on day 9. In group T2 (conventional Ovsynch protocol), cows were treated with GnRH (Buserelin acetate, 10 µg, i/m, Pregulate, Virbac Animal Health Pvt. Ltd; Mumbai) on day 0 followed by $PGF_2\alpha$ (Cloprostenol, 500 µg, i/m) on day 7 and a second dose of GnRH (Buserelin acetate, 10 µg, i/m) on day 9. Timed artificial insemination was carried out using frozen semen 18-24 h after the last hormonal injection in all the cows irrespective of the presence of behavioural signs of estrus. The inseminated cows were examined for pregnancy by per-rectal palpation 60 days after insemination. The estrus attributes such as induced estrus response, time required for onset of estrus and duration of estrus were recorded. The intensity of the estrus was decided by the expression of behavioural symptoms of the estrus and total score method as described by Singh and Kharche (1985).

4 mL of blood was collected aseptically into vacutainer tubes from the jugular vein of each animal on day 9 of synchronization before administration (t1) of GnRH or Kisspeptin-10 and subsequently at 30 min (t2) and 60 min (t3) after treatment. Serum was separated by centrifugation at 1006 x g for 15 min and stored at –20°C until hormonal analysis. FSH concentration was estimated using a commercial bovine FSH ELISA kit (BT-LAB, Cat. No. EA0025BO) and LH concentration was estimated using a bovine LH ELISA kit (BT-LAB, Cat. No. EA0026BO).

Preparation of Stock Solution of Kisspeptin-10

Kisspeptin-10 (Kp-10; Tyr-Asn-Trp-Asn-Ser-Phe-Gly-Leu-Arg-Tyr-NH_2) obtained from Viron Enterprises; Mumbai was used in the study. A stock solution was prepared by dissolving 150 mg of lyophilized peptide in 25 mL sterile distilled water to obtain a concentration of 6 mg/mL. The solution was stored at –20°C until use and diluted with normal saline to obtain a working concentration of 1 mg/mL prior to administration.

The data obtained during the study were analyzed using Web Agri Stats Package (WASP-2) developed by ICAR, Goa.

Results and Discussion

Estrus Attributes and Pregnancy Rate

Estrus attributes and pregnancy rate in modified Ovsynch with Kisspeptin-10 and Conventional Ovsynch are presented in Table 1.

The findings of the present investigation indicated that the efficiency in terms of induced estrus response showed non-significant difference though it was numerically higher in the modified Ovsynch with Kisspeptin-10 protocol as compared to the conventional Ovsynch protocol. The present findings are in agreement with the observations of Pottapenjara *et al*. (2018) and Dixit *et al*. (2023), who reported a non-significant difference in induced estrus response among Kisspeptin-10 and Ovsynch protocol in buffaloes and cattle, respectively. Similarly, Vishalkumar (2019) recorded a comparatively higher estrus response in Kisspeptin-10 treated buffaloes

Table 1: Estrus attributes and pregnancy rate (n=10 each)

Sr. No.	Parameter	Modified Ovsynch with Kisspeptin-10 (T_1)	Conventional Ovsynch Group (T_2)
1	Estrus response rate (%)	90.00	80.00
2	Time required for onset of estrus (h)	47.77± 5.11	53.63 ± 2.40
3	Duration of estrus (h)	26.56* ± 0.81	22.75* ± 0.94
4	Intensity of estrus (%): Intense	22.22	25.00
	Intermediate	55.55	50.00
	Weak	22.22	25.00
5	Conception rate (%)	50.00	30.00

*Values marked differ significantly between groups at p<0.01.

as compared to GnRH treated buffaloes. The numerically higher estrus response observed in the modified Ovsynch with Kisspeptin-10 protocol in the present study might be attributed to the potent stimulatory action of kisspeptin on the hypothalamic-pituitary-gonadal axis. It can also be due to higher concentration of Kisspeptin-10 used against GnRH in two protocols. Kisspeptin is known to act as a key upstream regulator of GnRH neurons, thereby enhancing GnRH release and subsequent secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Popa *et al*., 2008; Smith *et al.,* 2009; Ahmed *et al*., 2009; Lehman *et al.,* 2010) which are essential for follicular maturation and estrus expression.

The cows subjected to the modified Ovsynch with Kisspeptin-10 protocol exhibited a numerically earlier onset of estrus as compared to conventional Ovsynch protocol with non-significant difference. There is a paucity of literature specifically addressing the onset of estrus following Kisspeptin-10 administration in cattle for direct comparison with the present findings. The present finding is in contrast with the observations of Vishalkumar (2019), who reported a significantly delayed onset of estrus in the Kisspeptin-10 treated group as compared to the Ovsynch treated group in buffalo. Kisspeptin has been reported to induce a rapid and synchronized LH release indicating an early activation of the hypothalamic-pituitary-gonadal axis (Kadokawa *et al*., 2008). Studies in cattle and buffaloes have also demonstrated that kisspeptin-based protocols promote earlier follicular wave emergence and dominant follicle growth (Mondal *et al*., 2018; Pottapenjera *et al*., 2018) and increased estrodiol production (Datta *et al*., 2024), which might contribute to earlier onset of estrus. The conventional Ovsynch protocol is highly dependent on the stage of the estrus cycle and follicular status at initiation, and animals possessing smaller or less steroidogenically active dominant follicles might exhibit delayed time to estrus onset (Vasconcelos *et al*., 1999).

In the present study, significantly longer ($p<0.01$) mean duration of estrus in cows treated with the modified Ovsynch with Kisspeptin-10 protocol as compared to those subjected to the conventional Ovsynch protocol concurred with findings of Pottapenjera *et al*. (2018) in Murrah buffaloes and elucidated that the Kisspeptin-10 induced LH release is short-term and lower than GnRH induced LH release which might be the reason of prolonged estrus duration in Kisspeptin-10 group as compared to Ovsynch group. The mean duration of induced estrus (22.75±0.94 h) observed in conventional Ovsynch group was in accordance with Deshmukh *et al*. (2015) and Hirole *et al*. (2018), who reported the duration as 23.80±0.55 and 21.20±0.58 h, respectively, in postpartum dairy cows.

In the present study, the frequencies of intense, intermediate and weak type of estrus between the groups showed non-significant differences, which was in accordance with Dixit *et al*. (2023) in cows treated with Kisspeptin-10 and conventional Ovsynch protocol.

The difference of conception rate was statistically non-significant between the groups but the numerically higher conception rate observed in the modified Ovsynch with Kisspeptin-10 treated group compared to conventional Ovsynch group was in accordance with results of Pottapenjera *et al*. (2018) and Dixit *et al*. (2023) in buffaloes and cattle, respectively, treated with Kisspeptin-10 and GnRH. The higher ovulatory follicle size and higher ovulation rate in kisspeptin group (Mondal *et al.,* 2018) might be attributed to higher pregnancy rate in Kisspeptin-10 treated group than GnRH treated group. It can also be due to higher concentration of Kisspeptin-10 used against GnRH in two protocols.

Effect of Kisspeptin and GnRH on Serum FSH and LH Concentration

The serum FSH and LH concentration at different time interval in modified Ovsynch with Kisspeptin-10 and conventional Ovsynch group were recorded and depicted in Table 2.

From the present findings, it was observed that the mean serum follicle stimulating hormone (FSH) concentration increased progressively from basal (t1) to higher (t3) levels in both modified and conventional Ovsynch treated groups, indicating activation of the hypothalamic–pituitary axis following hormonal intervention. However, the magnitude of increase in FSH concentration following treatment was significantly higher in the GnRH treated group compared to the Kisspeptin-10 treated group, particularly at t2 and t3 intervals. These findings supported the previous observations of Gottsch *et al*. (2004), who reported that kisspeptin administration stimulates FSH release; however, the magnitude of FSH response is generally lower compared to LH and is less pronounced than that induced by direct GnRH

Table 2: Serum FSH and LH concentration at different time interval in cattle under two synchronization groups

Sr. No	Time of sampling on Day 9	FSH concentration (ng/mL)		LH concentration (ng/mL)	
		Kisspeptin-10 Group	Ovsynch Group	Kisspeptin-10 Group	Ovsynch Group
1	Before administration of injection (t1)	$0.341^{a} \pm 0.008$	$0.349^{a} \pm 0.006$	$0.348^{a} \pm 0.008$	$0.370^{a} \pm 0.009$
2	30 min after administration of injection (t2)	$0.504^{*b} \pm 0.009$	$1.057^{*b} \pm 0.02$	$5.689^{*b} \pm 0.338$	$3.938^{*b} \pm 0.165$
3	60 min after administration of injection (t3)	$0.649^{*c} \pm 0.008$	$1.355^{*c} \pm 0.014$	$4.717^{*c} \pm 0.141$	$7.263^{*c} \pm 0.184$

*Values marked differ significantly within the row at $p<0.01$ between groups regarding FSH and LH level. Means bearing different superscripts in column differ significantly ($p<0.01$).

administration. Navarro *et al*. (2005) and Ahmed *et al*. (2009) also demonstrated that FSH secretion following kisspeptin treatment was slower and of lower amplitude compared to LH, emphasizing the preferential stimulatory effect of kisspeptin on LH release. The comparatively moderate rise in FSH concentration observed in the kisspeptin group might be due to the indirect mode of action of kisspeptin, which stimulates gonadotropin release primarily through activation of hypothalamic GnRH neurons rather than acting directly on the pituitary.

The basal LH concentrations recorded at t1 in both modified and conventional Ovsynch groups were low and comparable. It was observed that the mean serum luteinizing hormone (LH) concentration showed a significant increase following treatment in both groups demonstrating the responsiveness of the pituitary gonadotrophs to both Kisspeptin-10 and GnRH administration. However, in the Kisspeptin-10 treated group, LH concentration recorded at t3 was lower than that observed at t2, whereas in the GnRH-treated group, LH concentration continued to rise up to t3. The present findings are in close agreement with Dhillo *et al*. (2007), who reported a robust and rapid increase in LH levels following peripheral administration of kisspeptin, particularly during the preovulatory phase. Similarly, Kadokawa *et al*. (2008) reported that intravenous administration of kisspeptin in cattle induced a rapid LH surge. Supporting these observations, Pottapenjera *et al*. (2018) reported that kisspeptin induced a rapid but transient LH surge occurring within 15-30 min and returning to basal levels within 1-2 h in Murrah buffaloes. The early and pronounced rise in LH concentration observed at t2 in the Kisspeptin-10 group might be attributed to the potent stimulatory effect of kisspeptin on hypothalamic GnRH neurons, resulting in synchronized GnRH release and a rapid LH surge.

Conclusion

It was concluded that incorporation of Kisspeptin-10 in the Ovsynch protocol improves estrus expression, prolongs the duration of estrus, elicits an effective gonadotropic hormonal response and improves pregnancy rate, thereby making it a promising alternative to GnRH for ovulation synchronization in postpartum dairy cows.

Acknowledgement

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

References

Ahmed, A.E., Saito, H., Sawada, T., Yaegashi, T., Yamashita, T., Hirata, T., & Hashizume, T. (2009). Characteristics of the stimulatory effect of kisspeptin-10 on the secretion of luteinizing hormone, follicle-stimulating hormone and growth hormone in prepubertal male and female cattle. *Journal of Reproduction and Development, 55*(6), 650-654.

Bahareldin-Ali, A., Tsigkou, A., Huang, J., & Li, H. (2015). Endocrine and ovarian responses in water buffalo cows immunized against inhibin and subjected to the Ovsynch protocol. *Journal of Integrative Agriculture*, *14*(9), 1827-1837.

Bo, G.A., Baruselli, P.S., Moreno, D., Cutaia, L., Caccia, M., Tríbulo, R., Tríbulo H., & Mapletoft R.J. (2002). The control of follicular wave development for self-appointed embryo transfer programs in cattle. *Theriogenology*, *57*(1), 53-72.

Datta, A., Baruah, A., Bora, A., Dutta, D., Goswami, J., Bhuyan D., & Das, S. (2024). Exploring the impact of exogenous Kisspeptin-10 on ovarian follicular development and estrus behaviour in Lakhimi cows of Assam. *The Indian Journal of Animal Sciences*, *94*(7), 604-609.

Deshmukh, S.G., Bakshi, S.A., Ingawale, M.V., & Chepte, S.D. (2015). Ovulation synchronization for improving fertility in postpartum dairy cows. *The Indian Journal of Veterinary Sciences and Biotechnology*, *10*(4), 9-11.

Dhillo, W.S., Chaudhri, O.B., Thompson, E.L., Murphy, K.G., Patterson, M., Ramachandran R., & Bloom, S.R. (2007). Kisspeptin-54 stimulates gonadotropin release most potently during the preovulatory phase of the menstrual cycle in women. *The Journal of Clinical Endocrinology & Metabolism*, *92*(10), 3958-3966.

Dixit, C.P., Bhuyan, D., Bhuyan, M., Ahmed, K., Baruah, A., Kalitacand, D., & Malik, M. (2023). Ruling out efficacy of hormonal protocols alone or fortified with mineral and bypass fat for treatment of postpartum anoestrus in cattle. *Indian Journal of Animal Research, 57*(12), 1613-1619.

Gottsch, M.L., Cunningham, M.J., Smith, J.T., Popa, S.M., Acohido, B.V., Crowley, W.F., & Steiner, R.A. (2004). A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. *Endocrinology*, *145*(9), 4073-4077.

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 protocols in postpartum dairy cows. *International Journal of Livestock Research, 8*(11), 283-290.

Kadokawa, H., Matsui, M., Hayashi, K., Matsunaga, N., Kawashima, C., Shimizu T. ., & Miyamoto, A. (2008). Peripheral administration of kisspeptin-10 increases plasma concentrations of GH as well as LH in prepubertal Holstein heifers. *Journal of Endocrinology*, *196*(2), 331.

Lehman, M.N., Coolen, L.M., & Goodman, R.L. (2010). Minireview: Kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: A central node in the control of gonadotropin-releasing hormone secretion. *Endocrinology*, *151*(8), 3479-3489.

Mondal, M., Baruah, K.K., Karunakaran, M., Ghosh, M.K., & Dutta, T.K. (2018). Development of a new kisspeptin based method of ovulation synchronization for crossbred dairy heifers. *Journal of Dairy Science and Technology, 4*(3), 12-16.

Navanukraw, C., Redmer, D.A., Reynolds, L.P., Kirsch, J.D., Grazul-Bilska, A.T., & Fricke, P.M. (2004). A Modified presynchronization protocol improves fertility to timed artificial insemination in lactating dairy cows. *Journal of Dairy Science, 87,* 1551-1557.

Navarro, V.M., Castellano, J.M., Fernández-Fernández, R., Tovar, S., Roa, J., Mayen A., & Tena-Sempere, M. (2005). Characterization of the potent luteinizing hormone-releasing activity of KiSS-1 peptide, the natural ligand of GPR54. *Endocrinology*, *146*(1), 156-163.

Pinilla, L., Aguilar, E., Dieguez, C., Millar, R.P., & Tena-Sempere, M. (2012). Kisspeptins and reproduction: Physiological roles and regulatory mechanisms. *Physiological Reviews*, *92*(3), 1235-1316.

Popa, S.M., Clifton, D.K., & Steiner, R.A. (2008). The role of kisspeptins and GPR54 in the neuroendocrine regulation of reproduction. *Annual Reviews of Physiology*, *70*(1), 213-238.

Pottapenjera, V., Rajanala, S.R., Reddy, C., Gangineni, A., Avula, K., Bejjanki S.K., & Velmurugan, S. (2018). Kisspeptin modulates luteinizing hormone release and ovarian follicular dynamics in pre-pubertal and adult Murrah buffaloes. *Frontiers in Veterinary Science, 5,* 149.

Pursley, J.R., Meeand M.O., & Wiltbank, M.C. (1995). Synchronization of ovulation in dairy cows using $PGF_2\alpha$ and GnRH. *Theriogenology, 44,* 915-923.

Singh, M.M., & Kharche, K.G. (1985). Sexual behaviour and reproductive efficiency of crossbred cows. *Livestock Advisor, 10,* 9-13.

Smith, J.T., Li, Q., Pereira A., & Clarke, I.J. (2009). Kisspeptin neurons in the ovine arcuate nucleus and preoptic area are involved in the preovulatory luteinizing hormone surge. *Endocrinology*, *150*(12), 5530-5538.

Vasconcelos, J.L.M., Silcox, R.W., Pursley, J.R., & Wiltbank, M.C. (1999). Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. *Theriogenology*, *52,* 1067-1078.

Vishalkumar, P. (2019). Studies on follicular dynamics in pre-pubertal and adult buffaloes using kisspeptin. *Doctoral Dissertation*. P.V. Narasimha Rao Telangana Veterinary University, Hyderabad, Telangana, India.