Int.J.Curr.Microbiol.App.Sci (2026) *15*(3): 110-115

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 15 Number 3 (2026)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2026.1503.011

Study of Estrus Synchronization Protocols in Sahiwal Heifers Using Kisspeptin (Kp-10) and Gonadotropin Releasing Hormone (GnRH)

K. Vaibhavi[1*], G. Aruna Kumari[1], L. Ramsingh[2], J. R. Radhakrishna[3] and T. Raghunandan[4]

[1]Department of Veterinary Gynaecology and Obstetrics, P.V. Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, India
[2]Department of Veterinary Gynaecology and Obstetrics, Mamnoor, Warangal, India
[3]Department of Veterinary Surgery and Radiology, P.V. Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, India
[4]Livestock Fram Complex, P.V. Narsimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, India

**Corresponding author*

Keywords

Kisspeptin, Sahiwal Heifers, GnRH, Estrus Synchronization, Ovsynch

Article Info

Received:
05 January 2026
Accepted:
26 February 2026
Available Online:
10 March 2026

A B S T R A C T

The present experimental study was conducted to evaluate the efficacy of estrus synchronization protocols in Sahiwal heifers at the Dairy Farm Unit of the Livestock Farm Complex, College of Veterinary Science, Rajendranagar, Hyderabad. The investigation focused on comparison of synchronization protocols based on Kisspeptin and GnRH. Eighteen healthy, non-pregnant Sahiwal heifers with a body condition score ranging from 3.0 to 3.5 were selected for the study and were randomly allotted into three equal groups. Group I, II were subjected to the kisspeptin, GnRH based protocol and Group III served as control group. Parameters such as Estrus response rate, onset, intensity, and duration of estrus were recorded in all the three groups. Among the synchronization protocols evaluated, the kisspeptin based protocol showed superior estrus response and earlier onset of estrus compared to the Ovsynch and control groups, without altering estrus duration.

Introduction

Sahiwal is one of India's most valued indigenous dairy breeds, known for its strong milk yield, heat tolerance, and resistance to diseases, making it highly relevant for sustainable genetic improvement programs (Rehman *et al.,* 2014). Modern dairy systems widely depend on estrus synchronization protocols, particularly those incorporating Timed Artificial Insemination (TAI), to improve breeding efficiency and reduce reliance on visual heat detection. Conventional programs commonly use GnRH and $PGF_2\alpha$ to regulate ovulation (Karen and

Darwish, 2010). Recently, Kisspeptin, a neuropeptide encoded by the KiSS1 gene, has gained attention as a potent upstream regulator of the Hypothalamic-Pituitary–Gonadal axis, capable of stimulating GnRH release (De Tassigny, 2010) and inducing a preovulatory LH surge, often with greater efficiency than traditional gonadotropin treatments (Caraty *et al.,* 2007; Lehman *et al.,* 2010). Although GnRH and hCG are routinely used as ovulation-inducing agents, limited information is available on the application of kisspeptin in estrus synchronization of Sahiwal heifers. Therefore, the present study was designed with an objective to evaluate the efficacy of kisspeptin as a substitute for GnRH in the Ovsynch protocol for estrus synchronization.

Materials and Methods

The study was conducted in Eighteen healthy, non-pregnant Sahiwal heifers with a body condition score ranging from 3.0 to 3.5 at the Dairy Farm Unit of the Livestock Farm Complex, College of Veterinary Science, Rajendranagar, Hyderabad. They were randomly allotted into three equal groups to study different estrus synchronization protocols using Kisspeptin (Kp-10) and Gonadotropin Releasing Hormone (GnRH). Kisspeptin, Gonadotropin Releasing Hormone (GnRH) and Prostaglandin F2α were the hormones used for estrus synchronization of heifers in the experimental study.

All the experiments were approved by the Institutional Animal Ethics Committee with number - (IAEC)11/31/C.V.Sc,HYD.IAEC of College of Veterinary Science, Rajendranagar, Hyderabad.

Kisspeptin (Kp10–Tyr-Asn-Trp-Asn-Ser-Phe-Gly-Leu-Arg-Phe-NH2) synthesized at GL Biochem (Shanghai) Ltd, was prepared by reconstituting 1.2 mg of Kisspeptin in 6 ml of distilled water to yield a concentration of 0.2mg/ml according to the recommendation of (Mondal *et al.,* 2015).

Buserelin Acetate (GnRH analouge)-Gynatrop was procured from Alembic Pharmaceuticals Ltd. Bharti Life Sciences, Boisar, Palghar, India. Cloprostenol sodium (Synthetic PGF2α)-Vetmate was procured from Vetcare, Bengaluru, Karnataka, India.

Eighteen heifers were randomly allocated into three groups (n = 6 each). The Group I-Kisspeptin (KPK) group received 0.2 mg kisspeptin intravenously on Days 0 and 9, along with 2 ml cloprostenol sodium (250 µg/ml) intramuscularly on Day 7. The Group II-GnRH (Ovsynch; GPG) group was administered 2.5 ml buserelin acetate (4 µg/ml) intramuscularly on Days 0 and 9, and 2 ml cloprostenol sodium intramuscularly on Day 7. The control group received 10 ml normal saline intravenously on Days 0, 7, and 9. Parameters such as Estrus response rate, onset, intensity, and duration were recorded. Animals were monitored for estrus twice daily, in the morning and evening, following $PGF_2\alpha$ administration in the treated groups and from the initiation of the protocol for ten days in the control group.

Estrus response rate is the proportion of females that exhibit standing estrus (or are brought into estrus) within a predefined period following a synchronization protocol as described by Salman (2022) and Lamb (2010). It was calculated as the percentage of heifers showing estrus out of the total treated animals. Estrus detection was based on behavioral and physical signs, including mounting activity, standing to be mounted, restlessness, vulvar swelling with clear mucus discharge, frequent urination, and reduced feed intake, supported by physical examination.

Onset of estrus was defined as the interval (hours) from $PGF_2\alpha$ administration to the appearance of the first observable estrus signs (Salman, 2022; Tippenhauer *et al.,* 2021). In the control group, onset was calculated from the day of selection to the detection of natural estrus within a 10-day observation period.

Estrus intensity was assessed based on the degree of behavioral and physical signs, including restlessness, vulvar swelling and hyperemia with clear mucus discharge, tail elevation, frequent urination, and standing to be mounted (Madureira *et al.,* 2019; Hafez & Hafez, 2000). To quantify estrus a three-point estrus intensity score card (Yusuf, 1990-Table 1) was used.

Duration of estrus was defined as the time interval (hours) from the first observed estrus sign to the cessation of behavioral and physical heat signs (Tippenhauer *et al.,* 2021). The data was analyzed by Chi square test for estrus response rate and one way ANOVA for onset, intensity and duration of estrus.

Results and Discussion

Estrus Response Rate-Estrus response was observed in

Four, out of Six heifers (66.67 percent) in the kisspeptin treated group and in Three out of Six heifers (50.00 percent) in the ovsynch group, while the control group showed estrus in Two out of Six heifers (33.34 percent). Statistical analysis indicated that the differences in estrus response among the Kisspeptin, Ovsynch, and control groups were not significant (P > 0.05) as shown in the Table 2.

Estrus Response Rate: The estrus response observed with the Kisspeptin (Kp10) protocol in the present study (66.67%; 4/6) is consistent with the findings of Yashaswini (2019) in Deccani ewes under the KPK protocol and was also comparable with Pottapenjera *et al.,* (2018) in Murrah buffaloes (83.33%; 5/6).The estrus response rate observed with the Ovsynch (GPG) protocol (50%,3/6) follows similar trends that are reported by Ghuman (2014), Kandiel *et al.,* (2012), and Deshmukh *et al.,* (2009).

Onset Of Estrus - The mean onset of estrus was 60.0 ± 2.05 hours in the kisspeptin group, 66.83 ± 0.67 hours in the Ovsynch group, and 102.0 ± 12.50 hours in the control group. Statistical comparison revealed no significant difference (P>0.05), between kisspeptin and Ovsynch group. But both the treatment groups were statistically significant (P<0.05), with control group as shown in the Table 3.

Onset of Estrus: The onset of estrus in present study with Kisspeptin is 60.0 ± 2.05h. Similar trends of onset of estrus with KPK protocol was reported by Pottapenjera *et al.,* (2018) in buffaloes.

Intensity of Estrus- In the Kisspeptin group, 75% of responding heifers exhibited moderate estrus and 25% showed intense estrus, with no weak expression observed. The Ovsynch group showed 66.67% moderate and 33.33% intense estrus. In contrast, the control group displayed weaker expression, with 50% moderate and 50% weak estrus, and no intense cases recorded. Overall, estrus intensity was seen as 11.11 percent weak, 66.66 percent moderate, and 22.22 percent intense (Table 4).

Duration Of Estrus-The mean duration of estrus observed in the present study was 22.88 ± 0.98 hours in the Kisspeptin group, 19.33 ± 0.67 hours in the Ovsynch group, and 18.75 ± 0.75 hours in the Control group, with no significant difference among the groups (Table 5).

The mean onset of estrus in Ovsynch (GPG) treatment similarly aligns with the data reported by Senthilkumar and Chandrahasan (2015), Buhecha *et al.,* (2015) and Wankar *et al.,* (2017).

Intensity of Estrus: In the present study, the Kisspeptin group showed 75% moderate and 25% intense estrus, which aligns with Dixit *et al.,* (2024) reporting 75% moderate estrus in postpartum anoestrus cattle. Similarly, the Ovsynch group exhibited 66.67% moderate and 33.33% intense estrus, comparable with findings of Ahmed *et al.,* (2016a), Senthilkumar and Chandrahasan (2015) and Dixit *et al.,* (2024), who also reported predominance of moderate estrus expression. Overall, estrus intensity in the present study was mainly moderate (66.66%), this may be attributed to the weaker and less obvious behavioural expression of estrus typically observed in Bos *indicus* breeds.

Duration Of Estrus: The estrus duration in the Kisspeptin group is comparable to reports by Pottapenjera *et al.,* (2018) and Gustafsson *et al.,* (1986).

Similarly, the duration recorded in the Ovsynch group aligns with findings of Dixit *et al.,* (2023), Shahid *et al.,* (2019), Pottapenjera *et al.,* (2018), Yashaswini (2019) in Deccani ewes. These findings suggest that the estrus duration observed across treatments falls within the physiological range reported in earlier studies.

Table.1 Estus intensity score card for determining intensity of estrus (Yusuf, 1990)

Score	Mucus discharge	Vulva condition (swollen/wet/red)	Appetite	Mounting/Stand to be mounted,restlessness
1	+	+	+	-
2	++	++	+	++
3	+++	+++	+++	+++

Table.2 Estrus response rate in treatment group of heifers

S.No.	Treatment group	No. of animals treated	No. of animals that exhibited estrus	Percentage
01	Kisspeptin	06	04	66.67
02	Ovsynch	06	03	50.00
03	Control	06	02	33.34

Table.3 Onset of the estrus after synchronization

S.No.	Treatment group	No. of animals treated	No.of animals that exhibited estrus	Mean onset of the estrus (hrs) (Mean ± SE)
01	Kisspeptin	06	04	60.0 ± 2.05^{a}
02	Ovsynch	06	03	66.83 ± 0.67^{a}
03	Control	06	02	102.0 ± 12.50^{b}

^{a,b} superscript means with different superscripts in a column differ significantly (P<0.05).

Table.4 Showing the intensity of estrus in synchronized group of heifers.

S. No	Name of the Treatment group	No. of cows exhibited estrus / No. of cows treated	Estrus Intensity Score Card (Yusuf, 1990)					
			1point		2 points		3 points	
			Weak		Moderate		Strong	
			No.	% of weak estrus in cows that exhibited estrus	No.	% of moderate estrus in cows that exhibited estrus	No.	% of strong estrus in cows that exhibited estrus
1	**Kisspeptin**	4/6	-	-	3	75	1	25
2	**Ovsynch**	3/6	-	-	2	66.67	1	33.33
3	**Control**	2/6	1	50	1	50	-	-
Total		9/18	1		6		2	
Overall Percent				11.11		66.67		22.22

Table.5 Duration of estrus (hrs) in synchronized groups of heifers.

S. No	Name of the Group	No of cows that exhibited estrus	Mean duration of estrus (hrs) (Mean ± SE)
1	**Kisspeptin**	4/6	22.88 ± 0.98^{a}
2	**Ovsynch**	3/6	19.33 ± 0.67^{a}
3	**Control**	2/6	18.75 ± 0.75^{a}

There was no significant difference in duration of estrus among all three groups (P>0.05).

In conclusion, the present findings indicate that the Kisspeptin protocol achieved the highest estrus response rate compared with Ovsynch and control groups. Both Kisspeptin and Ovsynch advanced the onset of estrus and promoted clear behavioral expression in most animals, while the duration of estrus remained

comparable across groups. These results demonstrate that the protocols effectively induced timely and well-expressed estrus without altering its length. Kisspeptin, when used as a substitute for GnRH, yielded improved outcomes over the control group. However, further studies are required to confirm and strengthen evidence regarding its efficacy in estrus synchronization programs**.**

Author Contributions

K. Vaibhavi: Investigation, formal analysis, writing—original draft. G. Aruna Kumari: Validation, methodology, writing—reviewing. L. Ramsingh:—Formal analysis, writing—review and editing. J. R. Radhakrishna: Investigation, writing—reviewing. T. Raghunandan: Resources, investigation writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Ahmed N, Kathiresan D, Ahmed F A, Lalrintluanga K, Mayengbam P and Gali J M. 2016a. Pattern of induced estrus and conception rate following Ovsynch and Ovsynch-based GnRH treatments initiated on day 6 of estrous cycle in repeat breeding crossbred cows. Veterinary World 9(4): 342–345. https://doi.org/10.14202/vetworld.2016.342-345

Buhecha K V, Dhami A J, Hadiya K K, Patel M D, Parmar S C and 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.

Caraty A, Smith J T, Lomet D, Ben Said S, Morrissey A, Cognie J, Doughton B, Baril G, Briant C and Clarke I J. 2007. Kisspeptin synchronizes preovulatory surges in cyclical ewes and induces ovulation in seasonally anestrous ewes. Endocrinology 148: 5258–5267. https://doi.org/10.1210/en.2007-0554

De Tassigny X D A and Colledge W H. 2010. The role of kisspeptin signaling in reproduction. Physiology 25: 207–217. https://doi.org/10.1152/physiol.00014.2010

Deshmukh Y D, Markandya N M, Patil M G, Khandalikar S C and Lakde M B. 2009. Studies on induction of estrus with reference to progesterone profile in Red Kandhari cows under Ovsynch protocol. Proceedings of ISSAR National Symposium, Namakkal, India, p. 275.

Dixit C P, Bhuyan D, Bhuyan M, Ahmed K, Baruah A, Kalita D, Haloi S and 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. https://doi.org/10.18805/IJAR.B-4956

Dixit C P, Haloi S, Meena D, Bhuyan D, Bhuyan M, Baruah A, Kalita D, Ahmed K, Yadav S K and Yadava C L. 2024. Evaluation of kisspeptin and Ovsynch protocol in the treatment of postpartum anoestrus cattle with respect to intensity and ovulation rate. International Journal of Advanced Biochemistry Research 8(1S): 567–572.

Ghuman S, Honparkhe M and Singh J. 2014. Comparison of Ovsynch and progesterone-based protocol for induction of synchronized ovulation and conception rate in subestrous buffalo during low-breeding season. Iranian Journal of Veterinary Research 15(4): 375–378.

Gustafsson H, Larsson K, Kindahl H and Madej A. 1986. Endocrine changes and behaviour during oestrus in heifers. Animal Reproduction Science 10(4): 261–273. https://doi.org/10.1016/0378-4320(86)90004-7

Hafez B and Hafez E S E. 2000. Reproductive behavior. In: Reproduction in Farm Animals. 7th ed. Wiley, Chapter 19.

Kandiel M M M, Bassuoni L A, Sosa G A, Abou Ahmed M M and Ghallab A M R. 2012. Comparative efficacy of Ovsynch and Heatsynch protocols in

Egyptian buffalo heifers. Theriogenology Insight 2(3): 173–183.

Karen A M and Darwish S A. 2010. Efficacy of Ovsynch protocol in cyclic and acyclic Egyptian buffaloes during summer. Animal Reproduction Science 119(1–2): 17–23. https://doi.org/10.1016/j.anireprosci.2009.11.003

Lamb G C. 2010. Estrus synchronization protocols for cows. In: Applied Reproductive Strategies in Beef Cattle. San Antonio, Texas.

Lehman M N, Coolen L M and Goodman R L. 2010. Minireviews: Kisspeptin/Neurokinin B/Dynorphin (KNDy) cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. Endocrinology 151: 3479–3489. https://doi.org/10.1210/en.2010-0022

Madureira A M L, Silper B F, Burnett T A, Polsky L B, Cruppe L H, Veira D M, Vasconcelos J L M and Cerri R L A. 2019. Estrus intensity following estradiol–progesterone based timed AI and fertility in dairy cows. Journal of Dairy Science 102(8): 7299–7310. https://doi.org/10.3168/jds.2018-15663

Mondal M, Baruah K K, Karunakaran M, Ghosh M K and Dutta T K. 2015. Development of a kisspeptin-based method of ovulation synchronization for crossbred dairy heifers. Research and Reviews: Journal of Dairy Science and Technology 4(3): 12–16.

Pottapenjera V, Srinivasa R, Rajanala C R, Gangineni A, Avula K, Sriravali S K and Velmurugan S K S. 2018. Kisspeptin modulation of LH release and follicular dynamics in Murrah buffaloes. Frontiers in Veterinary Science 5: 149. https://doi.org/10.3389/fvets.2018.00149

Rehman Z, Khan M S and Mirza M A. 2014. Factors affecting performance of Sahiwal cattle. Journal of Animal and Plant Sciences 24(1).

Salman M. 2022. Estrus synchronization of recipient cows for transfer of in vitro produced Sahiwal embryos. Master's thesis, P. V. Narsimha Rao Telangana Veterinary University, Telangana, India.

Senthilkumar K and Chandrahasan C. 2015. Estrus behaviour following $PGF_{2}\alpha$ with GnRH and hCG in dairy cattle. International Journal of Science, Environment and Technology 4(1): 243–247.

Shahid B K, Andrabi M I, Razzaq S M H and Khan M N. 2019. Estrus duration and expression in indigenous cattle. Journal of Animal and Plant Sciences 29(3): 645–649.

Tippenhauer C M, Plenio J L, Madureira A M L, Cerri R L A, Heuwieser W and Borchardt S. 2021. Factors affecting estrous expression and fertility in dairy cows. Journal of Dairy Science 104(12): 12984–12998. https://doi.org/10.3168/jds.2021-20503

Wankar M S, Ingawale M V, Hajare S W and Ingole R S. 2017. Efficacy of clomiphene citrate and Ovsynch on fertility in buffalo heifers. International Journal of Veterinary Sciences and Animal Husbandry 2(5): 18–20.

Yashaswini K. 2019. Role of kisspeptin as a substitute for GnRH in Ovsynch protocol in ewes. Master's thesis, P. V. Narsimha Rao Telangana Veterinary University, Hyderabad, India.

Yusuf T L. 1990. Pengaruh PGF terhadap aktivitas estrus dan superovulasi dalam rangkaian transfer embrio pada sapi Fries Holand, Bali dan Peranakan Ongole. Doctoral dissertation, Institut Pertanian Bogor, Bogor, Indonesia.

How to cite this article:

Vaibhavi K., Aruna Kumari G., Ramsingh L., Radhakrishna J. R. and Raghunandan T. 2026. Study of Estrus Synchronization Protocols in Sahiwal Heifers Using Kisspeptin (Kp-10) and Gonadotropin Releasing Hormone (GnRH). *Int.J.Curr.Microbiol.App.Sci.* 15(3): 110-115. **doi:** https://doi.org/10.20546/ijcmas.2026.1503.011