RESEARCH ARTICLE

Profile of LH Release in Response to Intravenous Administration of Kisspeptin-10 in Postpartum Anoestrus Buffaloes

Abhijeet Fernandes[1], Nishant Kumar*[1], Rubina Kumari Baithalu[1], Selvaraj Ragulraj[2], Selladurai Praveen[2], Tushar Kumar Mohanty[1]

Abstract

The main objective of this study was to evaluate the effect of intravenous (IV) administration of different doses of kisspeptin (Kp) on the pattern of luteinizing hormone (LH) release in postpartum anoestrus buffaloes. Postpartum buffaloes weighing 510-520 kg (n=24; aged 4-6 years) were enrolled in this study. Non-cyclicity (anovulatory status) was confirmed by the absence of corpus luteum (CL) as determined by ultrasound scanning of the ovaries and low circulating P_4 concentrations (<1.0 ng/ml) evaluated at 10-day intervals (on day -10 and day 0). Buffaloes were randomly assigned to one of four treatments: Kp @ 10 µg/kg body weight (Kp10, T_1), 15 µg/kg (Kp15, T_2), 20 µg/kg (Kp20, T_3), or Normal saline solution (equivalent volume of kisspeptin solution, control), all administered by IV injection. A dose-dependent response was observed during induced LH release ($p<0.05$) in the treated animals. Nevertheless, the highest LH concentration was obtained in the Kp15 dose (2.4±0.03 ng/mL). In all Kp treatment groups, LH concentration rose 20 min post-kisspeptin administration, peaked at 40 min, and declined thereafter. The early onset of the LH surge (in a dose-dependent manner) after Kp treatment, together with its short duration, suggests that the effects of Kp were likely due to pituitary action. Finally, kisspeptin at 15 µg/kg body weight was the optimized dose for postpartum anoestrus buffaloes as it elicits the highest LH peak, and this dose can be used to formulate new therapeutic strategies for treating sub-fertile buffaloes.

Key words: Anoestrus, Buffalo, Kisspeptin, LH.

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Introduction

In the modern era, Kisspeptin (Kp) has been an enthralling topic in reproductive physiology and has attracted attention as a central regulator of GnRH release in many mammalian species, including rodents, ruminants, dromedary camels, and primates (Naniwa *et al.,* 2013; Ainani *et al.,* 2020). Kisspeptin is an endogenous neuropeptide, a primary translation product, synthesized primarily by the Kiss1 gene in the hypothalamus majorly in the pre-optic and arcuate nucleus (Popa *et al.,* 2008) encrypted by the KISS1 gene in humans and Kiss1 in non-human species (Gottsch *et al.,* 2009). The initial product of the Kiss1 gene is a 145-amino-acid peptide, a preprohormone which, after proteolytic processing, produces shorter fragments of 54 (Kp-54/metastin or kiss 1), 14 (Kp-14), 13 (Kp-13) or 10 (Kp-10) amino acids, which are all biologically dynamic peptides (Wahab *et al.,* 2013). All by-products can effectively activate their cognate receptor 'Kiss1R/GPR-54, a 396 amino acid receptor that belongs to the Rhodopsin family / GPCR class A (Roseweir and Millar, 2009), forming the kisspeptinergic system (KPS) that stimulates the release of gonadotropin-releasing hormone (GnRH). Kp is now best known as a multifunctional peptide because of its role in cancer, the cardiovascular system, and reproduction (Mead *et al.,* 2007).

Centrally, kisspeptin and its receptor are expressed in the preoptic area (POA)/ anteroventral periventricular nucleus (AVPV) region in rodents, the second is the arcuate nucleus (ARC) (Wahab *et al.,* 2013; Mishra *et al.,* 2019) and peripherally in the pituitary gland, liver, intestine, pancreas, placenta, ovary (preovulatory follicle, granulosa and theca cells), and corpus luteum of rodents, primates, and buffaloes (Bhattacharya and Babwah, 2015; Rajin *et al.,* 2022). Exogenous administration of kisspeptin (dose-dependent manner) both centrally and peripherally induces gonadotropin secretion (mainly LH) and also has an effect on ovarian follicular growth, maturation, ovulation and estrus induction in rat (Greives *et al.,* 2007),

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monkey (Plant and Witchel, 2006), sheep (Caraty *et al.,* 2007; Sébert *et al.,* 2010), pig (Lents *et al.,* 2008), cows (Naniwa *et al.,* 2013; Mondal *et al.,* 2018; Leonardi *et al.,* 2022), buffaloes (Pottapenjera *et al.,* 2018; Chaikhun *et al.,* 2019), mare (Decourt *et al.,* 2014), goat (Tanaka *et al.,* 2012), and primates (Plant and Witchel, 2006), humans (Jayasena *et al.,* 2014).

Postpartum anoestrus is one of the major causes of poor reproductive performance in buffaloes, which limits the lifetime productivity of animals. The key cause is insufficient gonadotropic reinforcement from the hypothalamic-pituitary axis. It has been shown that the anterior pituitary in acyclic animals is rich in gonadotropins as compared to cyclic buffaloes; however, anoestrus buffaloes have an insufficient pulsatile release of LH to endure the final stages of follicular growth and, as a result, estrus activity and ovulation do not occur, limiting the reproductive capacity (Das and Khan, 2010; de Carvalho *et al.,* 2016). GnRH or Progesterone-based protocols (alone or in combination) are being used to induce ovulation via an estrus synchronisation protocol in sub-fertile buffaloes. However, the conception rate of the synchronisation protocol is only 50-55%. Kisspeptin directly acts on hypothalamic neurons to release GnRH and gonadotropins. Therefore, it can be used to formulate new therapeutic strategies aimed at increasing conception rates and reducing fertility disorders. This study was aimed to standardise the optimal dose of KP to induce LH release when administered intravenously in postpartum anoestrus Murrah buffaloes.

Materials and Methods

The present study was conducted in October and November after obtaining approval from the Institute's Animal Ethics Committee (IAEC Number 44-IAEC-19-11) at the Livestock Research Centre of ICAR-National Dairy Research Institute, Karnal, Haryana, India, situated at 250 m above mean sea level, at latitude 29.43°N & longitude 77.2°E.

Selection and Grouping of Animals

Twenty-four healthy multiparous postpartum anoestrus Murrah buffaloes were selected. The non-cyclic stage of all postpartum buffaloes was confirmed by AI records of LRC. It was further confirmed by per rectal palpation of the reproductive tract, ultrasound examination to ascertain follicular activity and presence of any luteal structure in the ovaries and measuring circulating serum progesterone at every 10-day intervals by sandwich ELISA Kit. All animals were housed in a well-ventilated shed in the livestock research centre and were quarantined for 1 month and acclimatised for the experimental shed. Proper cleanliness and healthy surroundings were ensured throughout the experimental period. All the animals were fed a ration consisting of a concentrate mixture and roughage fodder as per ICAR (2013) feeding standards. The mean body weights of the selected animals were in the range of 510-520 kg, and their age was between 4-6 years.

The selected animals were randomly divided into 4 groups of 6 animals each, with three different kisspeptin dosages and one control group. The buffaloes in Groups I, II, and III were administered IV kisspeptin at 10, 15, and 20 µg/kg body weight, respectively, and the fourth control group received an equal volume of normal saline only. On the day of the administration of drugs, the buffaloes were fitted with intravenous catheters in the jugular vein, enabling sequential blood sampling.

Blood samples were collected from all animals at the start of the experiment and then at 20-min intervals (0, 20, 40, 60, 80, 100, and 120 min; 0 indicates blood sampling before injecting kisspeptin). About 5 mL of blood was collected in sterile K_2EDTA vacutainer tubes starting in the morning (around 9 AM), posing minimal disturbance to the animal during collection. Collected samples were kept in an ice box and immediately brought to the laboratory, and centrifuged at 200 g for 20 min. Plasma was separated, stored in 2-mL vials at -20 °C until estimation of luteinizing hormone (LH).

Preparation and Administration of Kisspeptin Injection

Bovine kisspeptin (Tyr-Asn-Trp-Asn-Ser-Phe-Gly-Leu-Arg-Tyr-NH2), a decapeptide, was commercially synthesized (GL Biochem Ltd). Stock solutions were prepared at a concentration of 5 mg/mL in distilled water, and aliquots were frozen at -20°C until use. Stocks were diluted in normal saline to a working solution of 2.5 mL volume and administered intravenously.

Estimation of Luteinizing Hormone (LH) Levels by Sandwich ELISA

Plasma luteinizing hormone (LH) was quantified by "Bovine specific ELISA kits" (Bovine Progesterone ELISA KIT, Bioassay Technology Laboratory, China) according to the manufacturer's protocols. The optical density (OD) was measured by an ELISA reader (Multiskan Go, Thermo Scientific, Finland). The minimum detectable limit of bovine luteinizing hormone (LH) was 0.051 ng/mL, with a detection range of 0.1 ng/mL - 40 ng/mL. The intra- and inter-assay CVs were 8% and 10%, respectively.

Statistical Analysis

All data were presented as mean ± standard error of mean (SEM) and were, analyzed by repeated-measures two-way ANOVA (mixed model) followed by Dunnett's or Sidak's multiple-comparison tests using GraphPad Prism (Version 8.4.2, GraphPad Software Inc.) statistical software. Hypothesis testing was performed at a 5% level of significance, followed by Fisher's multiple comparison test using SAS software, version 9.1 of the SAS system for Windows, copyright © (2011), SAS Institute Inc., CARY, NC, USA.

Results and Discussion

The LH concentrations (ng/mL) recorded at 20-min intervals from 0 to 120 min following treatment with different doses of kisspeptin are presented in Table 1.

During the pre-treatment period (0 min), similar LH concentrations were observed across all groups (Table 1). LH concentration was at a basal level in the control group in the entire post-treatment period of 120 min. However, in the treatment groups, there was a significant difference ($p<0.05$) in LH levels at 20-, 40-, and 60-min post-treatment. The maximum LH peak was observed at 40 min, but was of lower amplitude ($p<0.05$). LH level then further decreased and reached the basal level at 80 min post-treatment in all treatment groups and remained at the basal level till 2 h (Table 1). Although a significantly higher LH level was observed at 40 min in all treatment groups, treatment group 2, in which 15 µg/kg body weight of kisspeptin (Kp-10) was injected, showed the maximum peak level of LH (2.15±0.02 ng/mL).

To the best of our knowledge, this is the first report on the effect of the IV injection of KP on the LH release response in postpartum anoestrus buffaloes, and this study provides novel insights into the reproductive physiology of anoestrus buffaloes. Furthermore, the LH response was dose-dependent, confirming the initial hypothesis.

Macedo *et al.* (2019) also reported dose-dependently increased plasma LH concentration 3.54±0.54 ng/mL and 6.44±0.54 ng/mL by using KP @ 10 mg/kg in *Bos indicus* (Zebu) and *Bos taurus* at 20-26 min and ending at 82-98 min, respectively. This pattern of immediate LH release followed by a decline has been observed previously in studies of kisspeptin administration by Matsui *et al.* (2004). In another study, Macedo *et al.* (2021) reported that estrogen priming before kisspeptin (4 mg murine KISS1-10) administration in ovariectomized Nellore cattle resulted in increased amplitude and duration of LH release in treated animals (with a peak between 20-40 min). Pottapenjera *et al.* (2018) found a peak level of LH concentration (17.4 ng/mL) within 30 min of injection in pre-pubertal Murrah buffaloes; however, the doses used by them were 5, 10, and 20 µg KP/kg body weight. The observed variation in LH levels across studies may be due to kisspeptin's short half-life. This dose-dependent release and short peak of LH after Kisspeptin were also reported in different studies. Kadokawa *et al.* (2008) found a peak level of LH (5.0±0.9 ng/mL) at 27 min by administration of 1 mg KP-10 in a pre-pubertal HF heifer. Redmond *et al.* (2011) reported a peak LH concentration of 4.5±0.2 ng/mL within 15 min after administration of 20 µg/kg Kp in pre-pubertal ewes. Leonardi *et al.* (2018) reported a peak concentration of LH (1.24±0.4 ng/mL) at 15 min after IV administration of 1 mg KP-10 in Herford cross heifers during the luteal phase. Naniwa *et al.* (2013) also showed that IV administration of full-length KP (KP-53) releases more LH in the dose of 2 nmol/kg than 0.2 nmol/kg in adult Japanese black beef cows.

The dose-dependent release of LH was also reported in some *in vitro* studies, in which exposure of gonadotropic tissue explants to different doses of kisspeptin resulted in LH release in a dose-dependent manner (Suzuki *et al.,* 2008). Chaikhun-Marcou *et al.* (2019) did not observe any significant effect of kisspeptin on LH surge at a 1.3 µg/kg dose as compared to Buserelin in swamp buffalo. Caraty *et al.* (2007) also reported a surge in LH concentration in ovariectomized anoestrus ewes during the non-breeding period within 2 h of continuous Kp administration. This discrepancy in results might be due to the very low dose used in their experiment, or to the response being studied in the luteal phase, where progesterone interferes with kisspeptin action. Our present study suggests that Kp10 is very effective at the dose of 15 µg/kg body weight to induce LH release after IV administration, and this delayed LH surge may be due to a prolonged anoestrus period (absence of progesterone priming influences gonadotropin release) or may be due to the pharmacological (composition) effect of the drug.

From the above studies, it is quite obvious that kisspeptin raises LH levels through binding with its receptors present in GnRH neurons (hypothalamus): the prime established pathway (Redmond *et al.,* 2011); a second potential pathway could be a GnRH-independent direct action on pituitary gonadotrope cells (Smith *et al.,* 2008) which was evidenced from *in vitro* cultured bovine anterior pituitary cells (Suzuki *et al.,* 2008).

Table 1: Mean (±SE, n=6) LH concentration (ng/mL) in four groups of postpartum anoestrus buffaloes at different periods following treatment

Time (min)	Control	T_1 (10 µg/kg)	T_2 (15 µg/kg)	T_3 (20 µg/kg)
0	0.77^{Aa}±0.02	0.78^{Aa}±0.01	0.79^{Aa}±0.02	0.76^{Aa}±0.02
20	0.79^{Aa}±0.01	1.42^{Bb}±0.03	1.65^{Cb}±0.01	1.49^{Db}±0.02
40	0.82^{Aa}±0.01	1.57^{Bc}±0.02	2.15^{Cc}±0.02	1.61^{Dc}±0.04
60	0.81^{Aa}±0.01	1.32^{Bd}±0.01	1.52^{Cd}±0.02	1.28^{Dd}±0.03
80	0.79^{Aa}±0.01	0.82^{Aa}±0.01	0.84^{Aa}±0.01	0.81^{Aa}±0.01
100	0.78^{Aa}±0.02	0.81^{Aa}±0.02	0.83^{Aa}±0.01	0.83^{Aa}±0.02
120	0.80^{Aa}±0.02	0.82^{Aa}±0.01	0.81^{Aa}±0.02	0.79^{Aa}±0.01

Means bearing different superscripts in upper-case letters in rows and lower-case letters in columns differ significantly ($p<0.05$).

Conclusion

This research study finds that IV administration of kisspeptin at 15 µg/kg body weight may be more beneficial for LH release and could serve as a therapeutic strategy for treating fertility disorders (postpartum anoestrum) in buffaloes.

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