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Luteinizing Hormone Release in Kisspeptin treated Pre-pubertal Murrah Buffaloes

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ABSTRACT

Kisspeptin is a neuropeptide that governs the reproductive axis upstream to GnRH. Present study was undertaken to compare the effect of different doses of kisspeptin on release of luteinizing hormone (LH) in Murrah buffaloes during pre-pubertal period. Twenty four pre-pubertal buffalo heifers were selected and divided into 4 groups (Control, T1, T2 and T3), having 6 animals in each group. Kisspeptin-10 was administered at the dose of 2 μg/kg body weight, 1.5 μg/kg body weight, 1μg/kg body weight respectively in T1, T2 and T3 group along with administration of equal volume of NSS in control group. Blood sampling was done at every 20 minutes interval beginning with zero minutes up to 2 hours and analyzed for LH concentration. Results indicated that LH concentration was significantly (p<0.05) higher in T1 group. The peak level of LH was recorded at 20 minutes post administration and falls to basal level at 60 minutes post administration. Although increased LH concentration was observed in other treatment groups but it was lower than T1 group. No LH peak or rise in LH level was observed in control group. The result of this study indicates that during the pre-pubertal period, administration of Kisspeptin causes dose dependent increase in LH concentration and peak level was seen at 2 μg/kg body weight of dose.

Key words: Buffalo, Kisspeptin, LH, Pre-pubertal

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INTRODUCTION

Major factors, which limit reproductive efficiency of buffaloes are delayed puberty, higher age at first calving, poor expression of the estrus signs, silent heat, poor conception rate and prolonged inter-calving interval (Madan, 1988; Terzanoet *al.,* 2012). Delayed puberty is a major problem which limits the lifetime production of buffaloes, as heifers calved at early age will produce more amount of milk in her life time. The Indian buffalo (*Bubalusbubalis)* usually

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attain puberty when they reach 16 to 40 months of age but the average age of puberty is more than 2.5 years (CIRB Annual Report, 1999-2000). Optimal secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus is essentially required for the onset of puberty, by its action on the release of gonadotropin hormones i.e. luteinizing hormone (LH) and follicular stimulating hormone (FSH) from the pituitary gland and sex steroids from the gonads. An increase in the pulsatile secretion of GnRH/LH appears to be an important event leading to puberty in all prepubertal rats, human and heifers (Kinder *et al*., 1995). Recently a new neuropeptide "Kisspeptin" (KP) emerge as a master regulator in animal reproduction by stimulating GnRH release and mediate sex steroid feedback mechanism on reproductive axis (Caraty and Franceschini, 2008). Administration of exogenous KP induce LH secretion in many species including bovines (Whitlock *et al*., 2011; Naniwa *et al*., 2013), pigs (Lents *et al*., 2008), ewes (Wang *et al*., 2012), mares (Wilborn, 2008) and canines (Albers-Wolthers *et al*., 2014), by stimulation of GnRH secretion and are ultimately responsible for maturation of gonads for onset of puberty and ovulation. Literature regarding effective dosage of kisspeptin for induction of cyclicity in buffaloes is scarce. Therefore, objective of this study was to investigate whether kisspeptin-10 administration can induce LH release during pre-pubertal period in Murrah buffalo heifers and investigate if this effect is different with different dose of kisspeptin-10.

MATERIALS AND METHODS

Selection of animals

Twenty four pre-pubertal Murrah buffalo heifers were selected from Livestock Research Center, ICAR-National Dairy Research Institute, Karnal, Haryana. The mean body weight of experimental animals was in range of 230-250 kg and their age were between 18-24 months. Non cyclic stage of all 24 Murrah Buffalo heifers was confirmed by AI records of LRC. It was further confirmed by per rectal palpation and ultrasound examination in which no corpus luteum (CL) was found in any pre-pubertal buffalo heifers. The animals were randomly divided into 4 groups having 6 animals in each group.

Preparation and administration of Kisspeptin injection

Bovine Kisspeptin (Tyr-Asn-Trp-Asn-Ser-Phe-Gly-Leu-Arg-Tyr-NH2), a decapeptide, was commercially procured (Biotech Desk). Stock solutions were prepared at the 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 2.5ml volume. After confirming pre-pubertal stage of heifers and grouping of buffaloes, three different dosage of Kisspeptin 10 was used (Figure 1). First group of buffalo heifers was administered 2.0 µg kisspeptin/kg body weight (T1), second group of heifers was administered 1.5 µg kisspeptin/kg body weight (T2), third

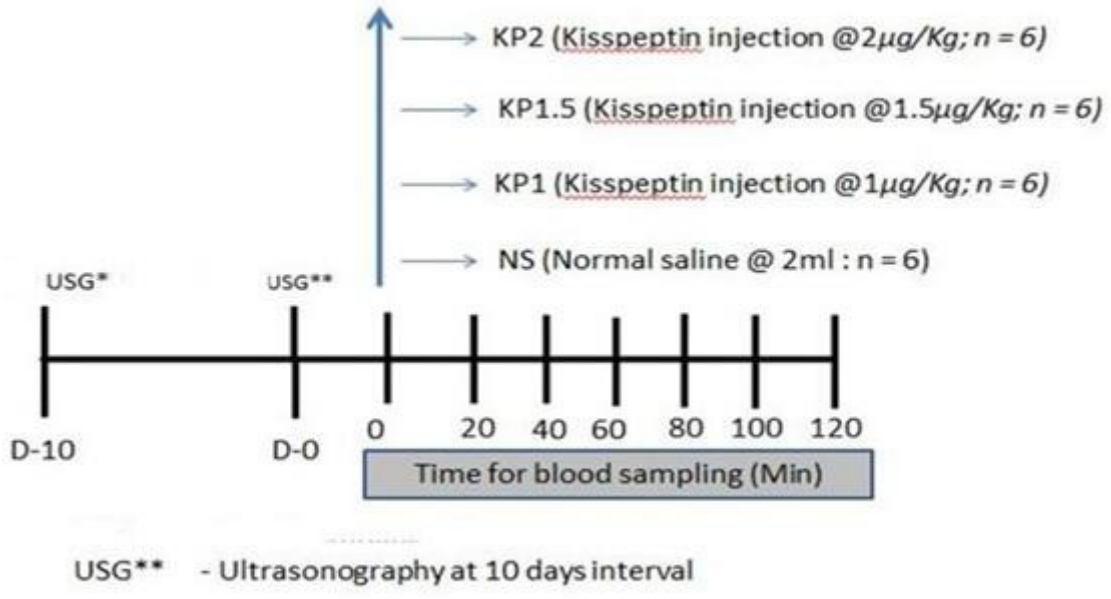

Fig. 1: Groups of Murrah buffalo heifers and blood sampling interval

group with 1.0 µg kisspeptin /kg body weight (T3) and fourth group was given equal volume of normal saline solution. Fourth group served as control group in this experiment. Kisspeptin 10 was administered by intravenous route in all animals. On the day of administration of drugs, the buffaloes were fitted with intravenous catheter in jugular vein enabling sequential sampling.

Blood sampling

Blood samples were collected from the start of the experiment and then at every 20 minutes interval i.e. 0, 20, 40, 60, 80, 100 and 120 minutes. Collected samples were kept in ice box, brought to laboratory and centrifuged at 3000 rpm for 20 minutes. Plasma was separated, kept in 2 ml collecting vial and stored at –20°C until analysis. Estimation of luteinizing hormone (LH) was by using sandwich ELISA Kit (Bovine LH ELISA Kit Bioassay Technology Laboratory, China).

Statistical analysis

The effect of treatment on LH plasma concentrations were tested for different time, and treatment time interaction using two-way RM ANOVA, followed by multiple comparison tests. The LH data in each treatment group were presented as mean±SEM. Level of significant was P<0.05.

RESULTS AND DISCUSSION

Mean (±SE) profile of LH at every 20 minutes interval starting from zero minutes till 120 minutes after treatment with different doses of Kisspeptin has been presented in Fig 2. During pre-treatment period (0 minutes), almost similar LH concentrations were seen in all of the groups. LH concentration was at basal level in control group and all other treatment group with minor variations between the groups. LH level was at basal level in control group in entire post treatment period starting from 0 minute up to 2 hr. There was no significant difference in LH concentration between different time intervals. However, in treatment groups, there was significant (p<0.05) difference in LH level in 20 and 40 minutes post treatment. Maximum peak level of LH was observed at 20 minutes post treatment in all treatment groups followed by a second LH peak at 40 minutes, but of lower amplitude (p<0.05). LH level then further decreases and reached to basal level at 60 minutes post treatment in all treatment groups and remain at basal level till 2 hours (Fig.1). Although significantly higher LH level was observed at 20 minutes in all treatment groups, treatment group 1 in which 2µg KP/kg BW of Kisspeptin-10 was injected shows maximum peak level of LH and it was 1.83±0.03ng/ml.Results of present study are in agreement with Pottapenjera *et al.* (2018) who also found peak level of LH within 15 minutes of injection in pre-pubertal buffaloes. However, the dose used by them was 5µg, 10 µg and 20µg/kg. Highest concentration of LH in their experiment was 17.4 ng/ml at 15 min, while in our study maximum concentration of LH was 1.83± 0.03 as 2 µg/kg dose was highest dose in our experiment. Similar to our study, they also observed basal level of LH at 60 minutes. Sudden rise and decline of LH level observed in different studies may be because of shorter half-life of kisspeptin.

This dose dependent release and short peak of LH after kisspeptin administration was also reported in different studies. Kadokawa *et al.* (2008) found peak level

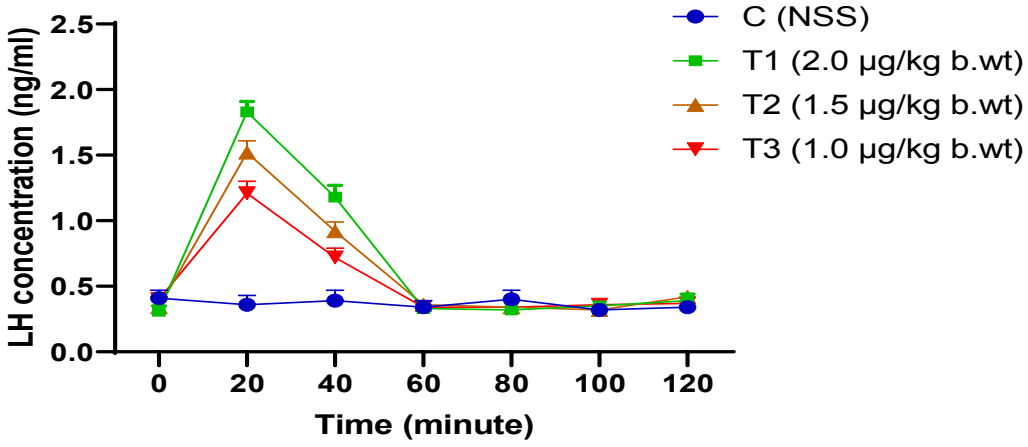

Fig 2: Mean (±SE) plasma LH concentration in four groups of Murrah buffalo heifers at every 20 minutes starting from 0 minutes pre-treatment to 120 minutes post treatment

of LH at 27 minutes by administration of 1 mg KP-10 in pre-pubertal Holstein heifers and concentration of LH was 5.0±0.9ng/ml. Leonardi *et al*. (2018) reported peak concentration of LH (1.24±0.40ng/ml) by 1mg KP-10 by IV administration, at 15 min after injection in Herford heifers during luteal phase. It is quite evident that KP increases LH level by binding with its receptors present in GnRH neurons; induce secretion of GnRH from these neurons, which in turns stimulate LH secretion from pituitary. The dose dependent release of LH was also reported in some *in vitro* studies where exposure of different doses of kisspeptin to gonadotropic tissue explants released LH in dose dependent manner (Kotani *et al.,* 2001; Navarro *et al.,* 2005 and Richard *et al.,* 2008).

In contrast to above studies, KP-53 at the dose of 0.2 or 2nmol/kg in Japanese Black beef cows induced LH peak after 2 hours of injection (Naniwa *et al*., 2013) whereas Chaikhun-Marcou *et al*. (2019) did not observed any effect of kisspeptin on LH surge at 1.3µg/kg body weight dose in swamp buffalo. This discrepancy in results might be due to the fact that the dose used in their experiment was low or conduction of experiment was in luteal phase where progesterone might have interfered with Kisspeptin action.

CONCLUSIONS

It can be concluded from present study that Kisspeptin-10 is very effective at the dose of 2µg/kg body weight to induce LH release after IV administration in Murrah buffalo heifers. This dose could be clinically used for hastening of onset of puberty in Murrah buffaloes.

CONFLICT OF INTEREST

None

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