COMPARISON OF THE EFFECTS OF KISSPEPTIN-10 OR GNRH ON LUTEINIZING HORMONE SECRETION DURING THE LUTEAL PHASE OF THE OESTROUS CYCLE IN SWAMP BUFFALO COWS

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ABSTRACT

This study compared the effect of kisspeptin-10 or GnRH administration on LH release in swamp buffalo during the luteal phase of the estrous cycle. Six animals were treated with a single intravenous injection of 1,000 pmol/kg b.w. of kisspeptin-10 or a single intramuscular injection of 10 µg/cow of GnRH agonist buserelin. For plasma LH analysis blood samples were collected every 15 minutes, 1 h before and 6 h after kisspeptin-10 and GnRH administration. An increase in LH plasma concentrations was observed after GnRH administration but not after kisspeptin-10 administration. The results of this study indicate that during the luteal phase of the estrous cycle, administration of GnRH, but not kisspeptin-10, stimulate LH secretion.

Keywords: *Bubalus bubalis*, buffalo, kisspetin-10, GnRH, LH, progesterone, swamp buffalo

INTRODUCTION

In water buffalo cows, as in other domestic animals, attempts to control follicular development during the estrous cycle have been the subject of several studies. However, in buffalo, the neuroendocrine mechanisms that control the reproductive axis is not yet well known (El-Wishy, 2007; Qureshi and Ahmad, 2008). Better understanding of the mechanisms regulating the activity of the hypothalamic-pituitary-ovarian (HPO) axis would contribute to the improvement of fertility or to the development of novel agents that would control reproductive activity.

In buffalo, gonadotropin-releasing hormone (GnRH) plays a pivotal role in controlling reproductive functions and GnRH administration induces LH secretion with a stimulating effect on ovarian activity (Chaikhun *et al*., 2010; Nasir *et al*., 1986; Singh *et al*., 1984). Recently, it has been observed that kisspeptin-10 produced by the hypothalamic neurons and Kisspetin-10 receptors are highly expressed in GnRH neurons (Irwig *et al*., 2004; Smith *et al*., 2011). Therefore, these neurons are considered to be a master regulator of reproduction in many mammalian species (Oakley *et al*., 2009; Okamura *et al*., 2013; Messanger *et al*., 2005; Hashizume *et al*., 2010; Tanaka *et al*., 2012). Indeed, it has been shown that kisspeptin-10 administration can stimulate GnRH and LH secretion in rats (Irwig *et al*., 2004), mice (Gottsch *et al*., 2004; Messanger *et al*., 2005), humans (Dhillo *et al*., 2007; George *et al*., 2012; Jayasena *et al*., 2015), sheep (Caraty *et al*., 2007), goats (Hashizume *et al*., 2010; Matsui *et al*., 2004),

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cattle (Kadokawa *et al*., 2008; Whitlock *et al*., 2008; Ezzat *et al*., 2009, 2010), river-type buffalo (Macedo *et al*., 2014) and is able to induce ovarian activity and ovulation (Caraty *et al*., 2007, Matsui *et al.,* 2004; Sebert *et al*., 2010).

Recent data *in vitro* data show in buffalo the presence of kisspeptin-10 receptor expression in GnRH neurons (Chaikhun *et al*., 2016; Chaikhun-Marcou *et al*., 2016; Chaikhun-Marcou *et al*., 2018) and therefore has been suggested that exogenous administration of kisspeptin-10 could induce LH release and can stimulate ovarian activity.

The aim of this study has been to investigate if kisspetin-10 administration can induce LH release during the luteal phase of oestrous cycle, when the plasma progesterone concentrations are high. and investigate if this effect is different with GnRH administration. If this hypothesis is correct, then the use of kisspetin-10 can be considered for controlling the estrous cycle in swamp buffalo.

MATERIAL AND METHODS

Ethical approval detail

This project has been reviewed and approved by the Certification of Institutional Animal Care and Use Committee (IACUC) in accordance with Chulalongkorn University Animal Care and Use Committee regulations and policies governing the care and use of laboratory animals. The Animal use protocol and approval number is 13310007. The review followed the guidelines documented in Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes, edited by the National Research Council of Thailand.

Animals and treatment

Six healthy swamp buffalo cows (*Bubalus bubalis*) between the ages of 4 and 6 years (determined by using the dental age estimation technique; Moran, 1992) with a mean body weight 360 kg were selected for this study. The experiment was done between December 2016 and February 2017.

During the experimental period the animals were housed in individual pens and fed fresh grass and hay and allowed to graze freely in the fields and bath in the area's natural pounds.

To allow frequent blood sampling for LH determination, all cows were fitted with an indwelling jugular vein catheter 4 to 6 h before the start of blood sampling.

The buffalo were treated during the midluteal phase of the estrous cycle (i.e. day 10 to 11 from estrus). Evaluation of the luteal phase of the estrous cycle was determined by detection of estrus. Estrus was detected by visual observation twice a day. The luteal phase was also confirmed by ultrasound evaluation of the corpus luteum morphology and confirmed, retrospectively, by the determination of progesterone plasma concentrations.

During the luteal phase of oestrous cycle, each animal received a single intravenous administration in the jugular vein of kisspeptin-10 (human metastin 45 to 54 (YNWNSFGLRF-NH2), 4389-V2, Peptide Institute Inc., Osaka, Japan) consisting of 1,000 pmol/kg b.w. or 1.3 µg/kg b.w. per dose dissolved in 2 ml distilled water (Chaikhun-Marcou *et al*., 2014). This method of administration was chosen based on suggestion that peripheral administration of kisspeptin-10 would not allow all the kisspeptin-10 to pass the blood-brain barrier (d'Anglemont de Tassigny *et al*., 2010; Ezzat *et al*., 2010; Suzuki *et al*., 2008) and was calculated on the basis of previous studies in ovariectomized cows (Whitlock *et al*., 2008). To test the effect of GnRH administration, to each animal was given a single intramuscular injection of 10 µg GnRH (Buserelin, Receptal, Intervet, Netherlands) during the luteal phase ofthe following estruos cycle in which was administered kisspeptin.

Blood sample collection

Blood was collected at 15 minutes intervals 1 h before and for 6 h after Kispeptin-10 or GnRH administration. After centrifugation at 3000 x g for 5 minutes, plasma was harvested and stored at -20^{o}C until analysis for plasma LH and progesterone concentrations were effectuated.

LH and progesterone determination

Plasma samples were measured for LH concentrations by a commercial enzymatic immunoassay test kit (EIA) (LH DETECT for Buffalo, Repropharm, France), following the kit's instruction. The LH intra- and inter-assay coefficients of variation were 32.16% and 39.53%, respectively and the sensitivity was 0.25 ng/ml.

Plasma progesterone concentration was analyzed by radioimmunoassay (RIA). The progesterone intra- and inter-assay coefficients of variation were 7.7 and 13.9, respectively and the sensitivity was 0.01 ng/ml.

Statistical analysis

The effect of treatment on LH plasma concentrations were tested for period (pre- or post-treatment), and treatment by period interaction using ANOVA procedures for repeated measures. The LH data in each treatment group were presented as mean±SEM. Level of significant was $P<0.05$.

RESULTS

The LH plasma profile for each cow after Kisspetin-10 or GnRH analogue administration is represented in Figure 1 and Figure 2, respectively. After Kisspetin-10 administration, there was no increase in LH mean concentrations during the whole sampling time whereas after GnRH administration mean plasma LH concentrations significantly increased ($P<0.05$) beginning about 30 minutes after treatment and lasting for about 130 minutes (Figure 2). The mean plasma LH concentrations were greater ($P<0.05$) for the first 3 h after GnRH compared to kisspeptin-10 administration (Figure 3).

DISCUSSION

Kisspeptin-10 administration stimulates LH secretion in humans (Jayasena *et al*., 2015), female rats (Adachi *et al*., 2007), prepubertal heifers (Kadokawa *et al*., 2008), ovariectomized ewes (Caraty *et al*., 2007), ovariectomized cows (Whitlock *et al*., 2008) and ovariectomized river buffalo (Macedo *et al*., 2014). Secretion of LH after Kisspetin-10 administration is reduced when compared to GnRH administration. However, the interaction between progesterone and kisspetpin-10 effect on GnRH and LH secretion has been not clarified. In one study in goats (Hashizume *et al*., 2010) during the luteal phase of estrous cycle, there was an increase in LH secretion after kisspeptin-10 administration, but lower compared to GnRH. Our study is the first to examine the effect of kisspeptin-10 on LH secretion in the presence of elevated progesterone palsma concentrations, as it occurs during the luteal phase of the estrous cycle. The results show that kisspetin-10 does not induce

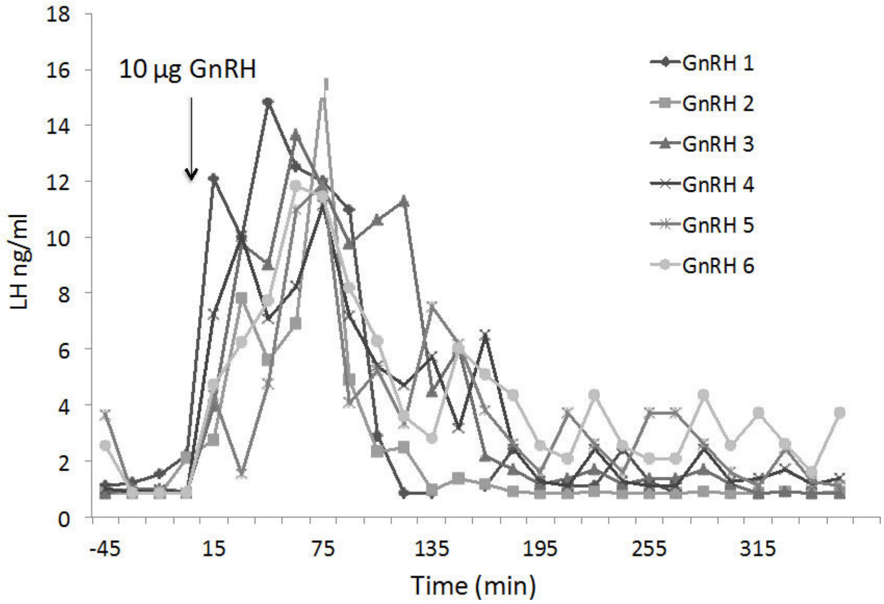

Figure 1. Response of circulating LH in swamp buffalo during the luteal phase of oestrus cycle after administration (arrow) of GnRH analogue (10 µg of Buserelin, Receptal). Time 0 = administration of GnRH.

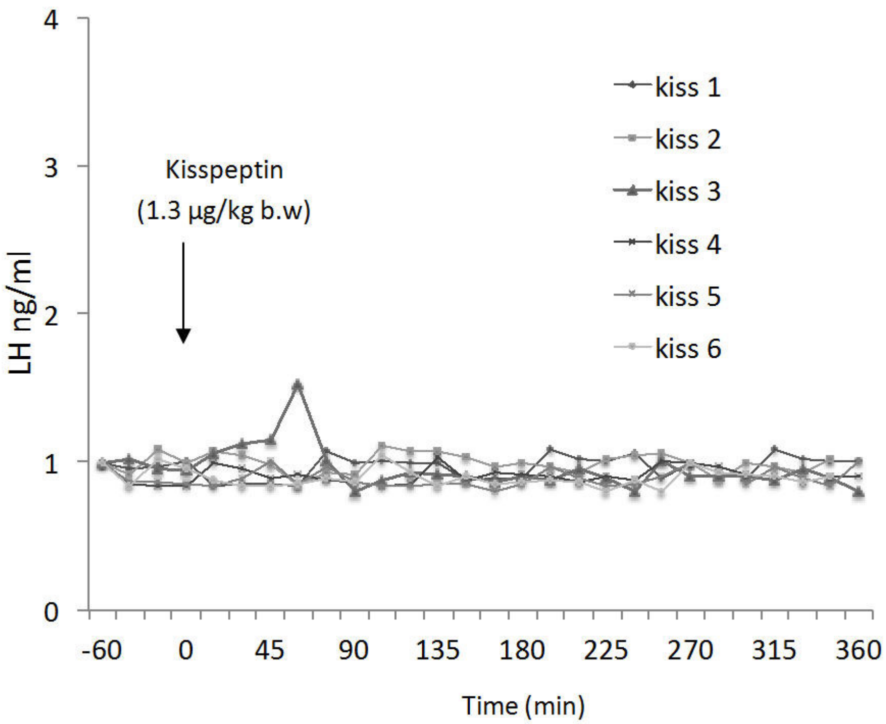

Figure 2. Response of circulating LH in swamp buffalo during the luteal phase of the oestrous cycle after administration (arrow) of Kisspeptin (1.3 µg/kg b.w). Time 0 = administration of kisspeptin.

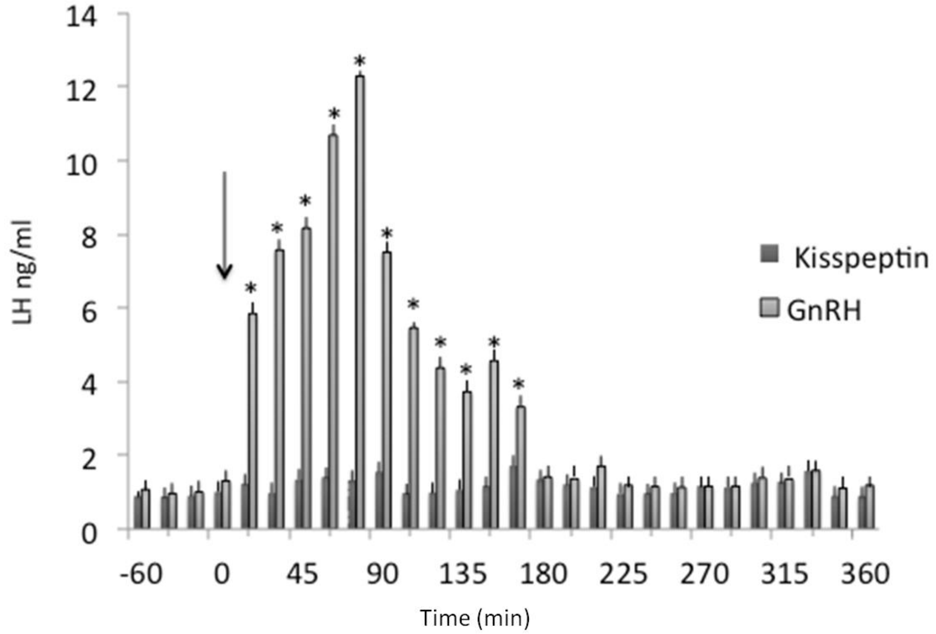

Figure 3. Response of circulating LH (mean ± SEM) in swamp buffalo during the luteal phase of the oestrous cycle after administration of Kisspeptin or GnRH. *indicate differences (P<0.05) in mean LH between treatments.

any significant LH release in presence of elevated plasma concentrations of progesterone. The difference between studies is difficult to explained but could be related to the species utilized (swamp buffalo *vs* goats) and the dose utilized because we injected 1.3 µg/kg b.w compare 5,0 µg/kg b.w. dose of kisspeptin in Hashizume *et al*. (2010) study.

The fact that in overiectomized cows the maximum LH-releasing effect to the i.v. injection of Kp10 was observed at 0.13 µg /kg b.w. (Whitlock *et al*., 2008). However, Whitlock *et al*. (2008) study were utilized ovariectomized cows while in our study we utilized cows during the luteal phase of estrous cycle.

In conclusion, our study indicate that GnRH, but not kisspeptin-10 administration during the luteal phase of the estrous cycle can stimulate LH secretion.

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