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Effects of Episodic Administration of Gonadotropin-Releasing Hormone on Luteinizing Hormone Concentrations and Libido in Pubertal Male Kundhi Buffalo Calves

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ABSTRACT

This study investigated the effects of gonadotropin-releasing hormone (GnRH) administration on luteinizing hormone (LH) concentrations and libido in pubertal male Kundhi buffalo calves. **Objective:** To determine the effects of GnRH administration on LH concentrations and libido in pubertal male Kundhi Buffalo calves. **Methods:** Eight calves, aged 16 months, were divided into two groups: Group A (treatment n=4), administration of GnRH analog (25ug lecirelin) and Group B (control n=4), there is not any administration. Each group consisting of four calves under a semi-intensive management system, the calves were fed a diet including 2 kg/day/calf of cotton seed cake, along with wheat bran, wheat straw, and green fodder, at the Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences. Results revealed a significant temporal change ($P < 0.000$) in the mean circulating concentration of LH over time after the intramuscular administration of GnRH analog (25ug lecirelin, Fatro®, International) three times. **Results:** The LH pulse was highest after the second GnRH dose (29.46±1.828), with significant differences in libido parameters, including mounting activity, erection, and sniffing, between the treatment (Group A) and control (Group B) groups. Statistical analysis revealed significant effects of episodic GnRH administration on both LH concentrations and libido. **Conclusion:** The episodic administration of GnRH significantly increased LH concentrations and improved libido in pubertal male Kundhi buffalo calves.

INTRODUCTION

The Kundhi breed emerges as the second most significant buffalo breed in Pakistan, primarily concentrated in the southern Sindh region. Its distribution spans from Kashmore to Shah Bandar along the Indus River, reaching urban centers such as Hyderabad, Karachi, Larkana, Nawabshah, Mirpurkhas, and extending into Quetta and the surrounding regions of Balochistan [1]. Attaining sexual maturity and optimal reproductive behavior in male Kundhi buffalo calves pose challenges, and limited research has delved into the effects of GnRH administration on Luteinizing Hormone (LH) concentrations and libido in this particular population. Examining the impact of episodic GnRH administration on LH concentrations and libido in pubertal male Kundhi buffalo calves is imperative for enhancing reproductive efficiency and optimizing livestock production, especially in regions where buffalo farming predominates, such as Pakistan. Sexual puberty in male buffalo calves represents the initiation of reproductive activities, which are oriented by a responsive fetal hypothalamus-pituitary-gonadal axis controlled hormonal cascade. LH is a key gonadotropin, and its secretion depends on the neuropeptide GnRH. LH, in its turn stimulates testosterone production impacting the development of secondary sexual characteristics and reproductive competence [2]. The general principles of GnRH action are understood and also LH elevation during

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puberty but regarding effect at episodic administration of GnRH on LH in pubertal male Kundhi buffalo calves is a missing link. Kundhi buffalo is endowed with distinctive features and economical importance, therefore it serves as a good model to understand the reproductive physiology intricacies. These results will provide new insights into the role of episodic GnRH administration on LH concentration during pubertal male Kundhi buffalo calves and likely to contribute reproductive biology in this important livestock. This can help to both better breeding allotment and thus improve agricultural productivity (Herbison, 2018). GnRH plays a critical role in the Hypothalamic-Pituitary-Gonadal (HPG) axis, controlling LH and FSH biosynthesis and secretion. Pulsatile release of GnRH is essential to maintain normal reproductive function that drives the pulsatile secretion of LH and FSH from the pituitary gland [3]. In males' bulls, failure of the libido may be due to low testosterone levels which leads to not only slower response but also less mounts a successful ejaculation, smaller ejaculate volume and reduced sperm per ejaculate. Knowledge of hormonal and physiological complexities, which underpin puberty and reproductive performance will aid in exploiting best field practices to harvest the full potential productivity efficiency from buffalo heifers into breeding system [4]. Conclusion Role of GnRH is well established in reproductive physiology among the species but here it may be proved and need to work more on certain effects like its stimulatory action upon libido in male pubertal Kundhi Buffalo calves. This research could be significant regarding the hormonal influence on sexual behaviors in this breed, which might help to improve breeding strategies and productivity of Kundhi Buffalo.

The objective of this study was to evaluate the effects of episodic administration of gonadotropin-releasing hormone (GnRH) on luteinizing hormone (LH) concentrations and to assess its impact on the libido of male pubertal Kundhi buffalo calves. By analyzing the hormonal response to GnRH administration, the study aims to determine variations in LH secretion patterns, which play a crucial role in reproductive physiology. Additionally, the research seeks to observe changes in sexual behavior and libido in response to GnRH treatment, providing insights into its potential role in enhancing reproductive performance in Kundhi buffalo calves.

METHODS

Management of Experimental Animals

Eight Kundhi buffalo calves of age 16 months were used and divided into two groups i.e group A treatment and group B control group and each group had four number of calves. These animals were kept under semi-intensive management system and were fed, and water was provided ad libitum to all experimental animals throughout the experimental phase at the Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences. Vaccination and drenching were given as per farm schedule. The calves in the treatment group (group A) received an intramuscular administration of 25ug lecirelin (GnRH analog) Fatro®, International. Administered the first dose on day 1, the second dose 48 hours following the first dose and the third dose after one month of second dose. Control Group (Group B): (n=4)– No administration.

Blood Sampling

Blood samples were drawn from the jugular vein then immediately transferred into the Sodium Citrate vacutainer tube with ± 3 mL blood. The blood samples were collected on day-0 from Group-B respectively, in order to obtain baseline LH measurements. On day 1, blood samples were collected from Group-A, this time, GnRH 25 µg lecirelin (GnRH analog) (Fatro®, International) was injected through IM immediately. Following giving injection, four times blood samples were collected for 2 hours at the interval of 30 minutes from animals individually. To increase the number of observations, the experiment was repeated after 48 hours and then after one-month interval.

Measurement of LH concentration and Libido Observation

The serum of all collected samples was analyzed for the measurement of LH levels (concentration) commercially. The all collected Samples were sent to Asian Institute of Medical Sciences Diagnostic and Research Lab, Tandojam. The concentration of luteinizing hormone was determined by using method cobas-411 Roche immunoassay analyzer. Libido was observed twice a day, morning, and evening immediately after administration of GnRH injection. The sexual parameters were monitored i.e mounting, erection (protrusion), sniffing, close contact (neck to neck), mounting with erection and mounting without erection.

The study was conducted over three months at the Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences. Date collected was tabulated in Excel sheet and analyzed for statistical difference between group and with group using Excel state version statistics 8.1. Data analysis was performed using ANOVA LSD test and Descriptive statistics.

RESULTS

In present study eight Kundhi buffalo calves were used and aimed to assess the effect of episodic administration of GnRH on luteinizing hormone level (concentrations) and to observe the influence of GnRH on the libido of male pubertal Kundhi Buffalo Calves.

Luteinizing Hormone Level (Concentration)

The figure 0.1 shows that after episodic administration of GnRH, the level of luteinizing hormone in control group was

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0.922 ng/ml, on day-0 the level was 1.095 ng/ml. Whereas, after 48 hours the level was 1.385 ng/ml, after one month, the level was 1.057 ng/ml. The highest level was found after 48 hrs. The significant differences lie in the incremental increases in LH levels at each time point, with the highest level (1.385 ng/ml) observed after 48 hours, indicating a peak response to the GnRH administration. The decrease in LH level after one month suggests a return to near-baseline levels.

The Comparisons Between The Control and The 1st Dose Of GnRH

The figure 0.2 shows that after episodic administration of GnRH, the level of luteinizing hormone was 0.922 ng/ml in the control group, whereas it was 1.ng/ml on day 0 after 1st dose, increased by 0.17 ng/ml or 18.5% from control. GnRH showed numerically the highest values after the first dose.

The Comparisons Between The Control and The 2nd Dose Of GnRH

The figure 0.3 show that after episodic administration of GnRH, the level of luteinizing hormone was 0.922 ng/ml in the control group, whereas it was 1.385 ng/ml in 2nd dose of GnRH after 48hrs increased by 0.465 ng/ml or 42.4% from control and 0.295 ng/ml or 27.0% from Day 0), the 2nd dose of GnRH highest values of concentration was observed.

The Comparisons Between The Control and The 3rd Dose Of GnRH

The Figure 0.4 shows that after episodic administration of GnRH, the level of luteinizing hormone was 0.922 ng/ml in the control group, whereas it was 1.057 ng/ml 3rd dose after one month, increased by 0.13 ng/ml or 14.1% from control, but decreased by 0.335 ng/ml or 24.2% from 48 hours the significate difference values were observed after 3rd dose of GnRH.

The Comparisons Between The 1st and The 2nd Dose Of GnRH

The Figure 0.5 show that after episodic administration of GnRH, the level of luteinizing hormone was 0.922 ng/ml in the 1st dose, whereas it was 1.095 ng/ml in 2nd dose, after 48 hours +0.295 ng/ml 27% increase after the 2nd dose of GnRH showed numerically the highest values.

The Comparisons Between The 1st, 2nd and 3rd Dose Of GnRH

The Figure 0.6 show that after episodic administration of GnRH, the level of luteinizing hormone 1.095 ng/ml in 1st dose on day-0 whereas, after 48 hours it was 1.385 ng/ml, +0.295 ng/ml (27% increase) and after one month the level was 1.057 ng/ml, -0.335 ng/ml (24% decrease from 2nd dose) and Day 0 vs. 1 month: -0.04 ng/ml (4% decrease). The highest level of 1.385 ng/ml was found after 48 hours.

Libido Observation

The mean of libido was observed from group A and B as shown in Table 1.1. The comparison of libido parameters between treatment group A and control group B reveals significant differences, with group A showing numerically higher values across all measured parameters. The specific findings are: The behavioral parameters observed in the study indicate that Group A exhibited significantly higher frequencies of mounting (2.82±0.054 vs. 1.42±0.046), mounting with erection (2.37±0.043 vs. 1.16±0.030), erection (2.07±0.057 vs. 1.05±0.018), close contact neck to neck (2.63±0.051 vs. 1.40±0.046), and sniffing (2.92±0.055 vs. 1.38±0.045) compared to Group B. The result shows that treatment group A consistently exhibited higher mean values across all libido parameters measured compared to control group B. These differences were significant and suggest that the GnRH treatment administered to group A effectively enhanced various aspects of sexual behavior. The consistency in the higher mean values for mounting, mounting with erection, erection, close contact (neck to neck), and sniffing highlights the potential efficacy of the treatment in increase male libido and sexual behaviors. Table 1 presents the standard values of libido (Mean ± % SEM) in Group A (GnRH treatment) and Group B (control), highlighting significant differences in reproductive behaviors.

Table 1: Standard Values of Libido

Observation	Treatment Group A (Mean ± SD)	Control Group B (Mean ± SD)
Mounting	2.82 ± 0.054[a]	1.42 ± 0.046[b]
Mounting with Erection	2.37 ± 0.043[a]	1.16 ± 0.030[b]
Erection (Protrusion)	2.07 ± 0.057[a]	1.05 ± 0.018[b]
Close Contact Neck To Neck	2.63 ± 0.051a	1.40 ± 0.046[b]
Sniffing	2.92 ± 0.055[a]	1.38 ± 0.045[b]

[a.b]Different Superscripts within the column show significant difference(P<0.5)

Fgure 1 shows a significant difference between the control and all three doses of GnRH.

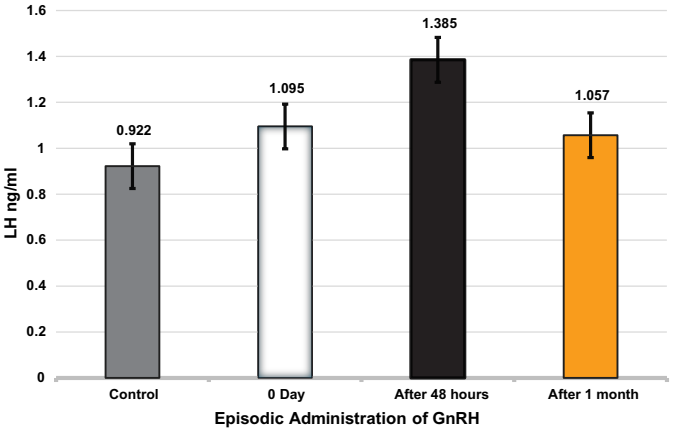

Figure 1: Significant difference between control and 1st, 2nd, and 3rd doses of GnRH

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Figure 2 demonstrates a significant difference between the control and the first dose of GnRH,

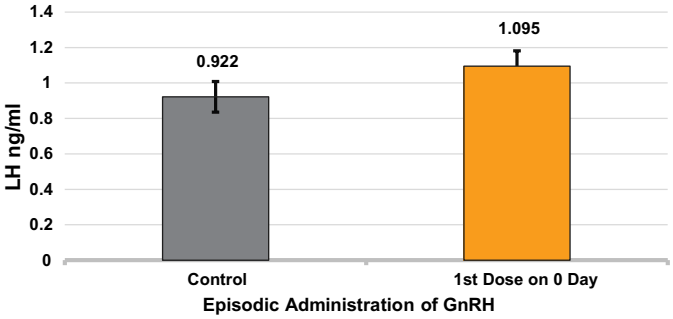

Figure 2: Significant difference between control and first dose of GnRH

Figure 3 highlights a significant difference between the control and the second dose

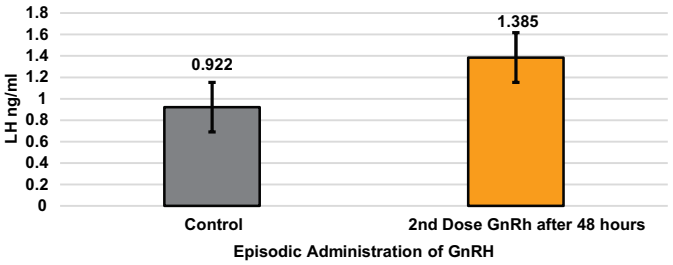

Figure 3: Significant difference between control and second dose of GnRH

Figure 4 indicates a significant difference between the control and the third dose of GnRH

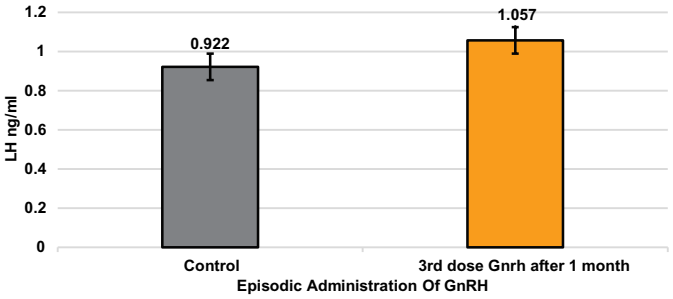

Figure 4: Significant difference between control and third dose of GnRh

Figure 5 illustrates a significant difference between the first and second doses of GnRH.

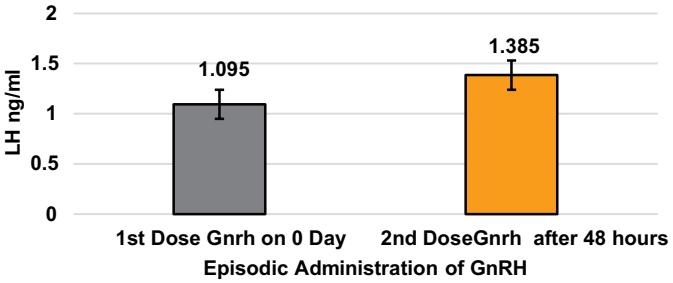

Figure 5: Significant difference between first and second dose of GnRH

Figure 6 shows a significant difference among the first, second, and third doses of GnRH

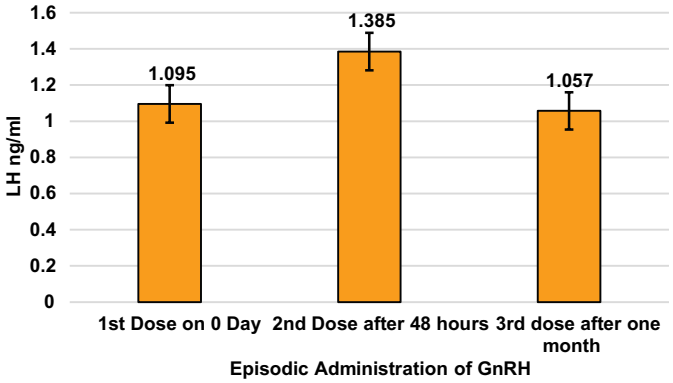

Figure 6: Effect of Episodic GnRH Administration on Luteinizing Hormone (LH) Levels in Buffalo Calves

DISCUSSION

In the present study the effects of episodic administration of GnRH on Luteinizing Hormone (LH) concentrations and libido in pubertal male Kundhi buffalo calves were investigated. In the treatment group, a notable increase in LH levels was observed compared to baseline levels and the control group. Specifically, the initial administration of GnRH resulted in a surge in LH concentration, peaking at 1.385 ng/ml after 48 hours, suggesting a strong hormonal response to exogenous GnRH stimulation. Subsequent doses maintained elevated LH levels, although with fluctuations observed over time. The findings of this study show that administration of GnRH significantly increased the level of LH and libido in the animals of the treatment group compared to the control group. The results of present study are consistent with Kumar BB *et al.*, in 2016, it has been shown that in bull calves GnRH stimulated the secretion of LH [5]. Also, in other male animals like male rats studied by Funabashi T *et al.*, in 2002 who given a subcutaneous GnRH injection (450 ng/100 g body weight) which resulted in enhanced libido, FSH, LH, and testosterone concentrations, testicular volume and testicular weight and scrotal circumferences were also increased [6]. Similar reports were documented in bubaline species that GnRH analogue administration has significantly improved the endocrinological profiles, libido and semen quality profiles, by El-Khawaga AR *et al.*, in 2011 [7]. These results are consistent with previous studies indicating the stimulatory effect of GnRH on LH secretion in male Ganjam Goat Nayak J *et al.*, in 2022 [8]. The highest LH level observed 48 hours after the first dose of GnRH suggests a temporal response to GnRH administration, which has implications for optimizing reproductive management in buffalo breeding programs. Our findings revealed the significant variations in LH concentrations following episodic administration of GnRH. The hormonal and behavioral responses have important implications for

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understanding the process of puberty and reproductive performance in male buffalo calves. GnRH, originating from the hypothalamus, is at the top of a cascade that triggers LH secretion and in many ways serves as a master signal for reproductive function, so our results may also collectively suggest exogenous GnRH can be an effective means to stimulate LH release and thereby boost libido among pubertal males. This preprint explored how cholinergic and GABAergic co-transmission regulates GnRH neuron activity and luteinizing hormone secretion [9]. In another study, weekly injection of GnRH significantly raised the gonadal androgen concentration and scrotal circumference in bovine species Ali S *et al.*, in 2012 [10]. The injection of exogenous GnRH resulted in a significantly increased testosterone concentration in the bovine species, as reported by Kumar BB *et al.*, in 2016 [5]. These findings suggest that GnRH administration may be a useful strategy for improving reproductive efficiency in buffalo breeding programs. GnRH treatment has been suggested to influence male sexual behavior through the elevation of testosterone levels in the bloodstream McDonnell SM, in 1992 [11]. Research indicates that in male rats, a rapid rise in testosterone levels, induced by GnRH treatment, promptly reduces the time taken to initiate mounting behavior (James and Nyby, 2002). In addition to hormonal changes, libido-related behaviors in response to GnRH administration were observed. Mounting behavior, a key indicator of sexual arousal and activity, exhibited significant differences between the treatment and control groups. Calves receiving GnRH demonstrated higher levels of mounting compared to the control group, indicating an enhancement of sexual behavior following hormonal intervention. This article provided a historical overview of methods and advancements in measuring GnRH levels in pituitary portal blood, highlighted their significance in understanding reproductive neuroendocrinology [12]. These effects may contribute to accelerated sexual maturation and improved reproductive efficiency in male buffalo calves, potentially facilitating breeding programs and livestock management practices, Kumar BB *et al.*, in 2016 [5]. It is important to acknowledge the limitations of current study, including the small sample size and the short-term nature of the intervention. Further studies with longer observation periods to fully elucidate the effects of episodic GnRH administration on reproductive physiology and behavior in male buffalo calves may be done. Additionally, investigating the long-term implications of GnRH intervention on fertility and reproductive performance would provide valuable insights into its potential applications in livestock breeding programs as stated by Kumar *et al.*, 2016; Singh AK, in 2016 [5, 13]. This paper provides a detailed physiological and pharmacological overview of gonadotropin-releasing hormone (GnRH), exploring its mechanisms of action and potential therapeutic applications [14]. In another study investigated the effects of episodic GnRH administration in buffalo bulls, showing that it stimulates testosterone secretion and testicular growth without negatively impacting semen quality [15]. These findings align with Herbison (2018), who underscored the critical role of the GnRH pulse generator in modulating reproductive function [16]. The broader implications of GnRH in animal reproduction have been extensively reviewed. Singh, (2022) discussed subclinical mastitis in dairy animals, drawing attention to hormonal influences on immune function and fertility [13]. Goodman RL *et al.*, (2022) provided insights into how testosterone rapidly affects copulatory behavior, reinforcing the significance of hormonal regulation in male reproductive strategies [17]. Hassanein *et al.*, (2024) detailed the structural and biosynthetic aspects of GnRH and its role in estrous synchronization, a crucial aspect of reproductive management [18]. Additionally, Gupta and Barański *et al.*, explored the effects of GnRH analogues on LH and testosterone concentrations in buffalo bulls, particularly during the non-breeding season [19, 20]. At the molecular level, Stamatiades and Kaiser (2018) elucidated the signaling pathways and gene expression changes induced by pulsatile GnRH administration, providing a mechanistic understanding of its regulatory role in reproductive endocrinology [21]. These insights collectively support the potential of GnRH therapies in optimizing reproductive efficiency, particularly in managed breeding programs for buffalo and other livestock species.

CONCLUSION

In conclusion, episodic GnRH administration positively influences LH secretion and libido in pubertal male Kundhi buffalo calves, offering potential benefits for reproductive management. This approach may enhance breeding efficiency and fertility in buffalo farming. However, further research is needed to refine protocols and explore its therapeutic applications, particularly for infertile or sub-fertile animals.

Authors Contribution

Conceptualization: FHJ
Methodology: PK
Formal analysis: FHJ
Writing, review and editing: HS, FR, RA, SA

All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

All the authors declare no conflict of interest.

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