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# STUDY ON ROLE OF KISSPEPTIN IN REPRODUCTIVE DEVELOPMENT AND INTER-RELATIONSHIP WITH ENDOCRINE MARKERS FOR NILI-RAVI BUFFALO BULLS (*BUBALUS BUBALIS*)

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## ABSTRACT

Kisspeptin, a neuropeptide, is known to be a regulator of the hypothalamic-pituitary-gonadal axis and is considered to have a role in the onset of puberty. The objective of the present study was to investigate the potential role of kisspeptin and its correlation with anthropometric parameters, testicular biometry, serum profiles of reproductive as well as selected metabolic hormones in prepubertal and postpubertal Nili-Ravi buffalo bulls (*Bubalus bubalis*). Anthropometric parameters and testicular biometry were measured, and blood samples were collected from prepubertal (n = 20) and postpubertal (n = 20) buffalo bulls during slaughtering. Serum samples were analysed for the assessment of circulating levels of kisspeptin, gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, triiodothyronine (T3), thyroxin (T4), and insulin-like growth factor (IGF-1). Results demonstrated that there was a non-significant change in serum concentration of kisspeptin between prepubertal and postpubertal animals. However, concentrations of GnRH ($P \leq 0.001$), FSH ($P \leq 0.05$), T3 ($P \leq 0.01$) and most of the anthropometric parameters were higher in the postpubertal calves than the prepubertal calves. Correlation analysis revealed that LH had significant correlation ($P \leq 0.05$) with testicular developmental parameters and IGF ($P \leq 0.01$) in postpubertal calves. Kisspeptin showed positive correlations ($P \leq 0.05$) with the width of the chest and width of the pelvis in prepubertal and postpubertal calves respectively. The IGF, testosterone, and T3 also have shown associations with some of the anthropometric parameters showing their roles in the pubertal development of calves. In conclusion, the circulating level of kisspeptin did not influence either reproductive or selected metabolic hormones suggesting that there are some other unknown mechanism(s) that influence the reproductive developments of buffalo bulls which need to be addressed in further studies.

**Keywords:** Anthropometric parameters, *Bubalus bubalis*, hormones, kisspeptin, puberty

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## INTRODUCTION

Buffalo is highly adapted to the sub-tropic region compared to other livestock animals that are unable to survive in the same environment. They are economically more productive due to having many homeo-kinetic mechanisms to maintain their critical body functions in a diversified environment. However, buffalo also exhibit some of the known reproductive constraints including delayed onset of puberty, poor estrus expression, and longer postpartum ovarian quiescence (Warriach *et al.*, 2015). Buffalo has a variable age of puberty ranging from 18 to 46 months due to differences in gaining their 60% body weight at different ages (Warriach *et al.*, 2015). Delayed maturity is a common issue in both males and females buffalo. There may be several reasons for this constrain such as hormonal deficiencies, poor development of reproductive organs, and genetic anomalies. Metabolic, as well as reproductive hormones, have significant interventions in pubertal development. During puberty, secondary sex characteristics appear, the adolescent physical growth spurt, and reproductive competency is achieved (Pinyerd *et al.*, 2005). Bulls having early puberty and greater scrotal circumference produce the progeny which attains their puberty at an early age and ultimately a higher percentage of heifers can be seen in the cycling stage in the breeding season (Moser *et al.*, 1996). Hypothalamic-pituitary-gonadal (HPG) axis in conjunction with other metabolic hormones, plays a vital role to transform animals from sexual latency to the active sexual phase.

Kisspeptins, regulated by the KISS1 gene, are neuropeptides of varying length of amino acids (10-54 in number) and are very effective stimulators of gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus. Kisspeptins have a stimulatory effect on testosterone release in cattle (Ahmed *et al.*, 2009). Kisspeptin neurons are located in close apposition with GnRH neurons, which express KISS1r (Clarkson *et al.*, 2006). Hypogonadotropic hypogonadism and delayed puberty have been correlated with the functional absence of GPR54 protein (Seminara *et al.*, 2003). Neuronal activation of GnRH neurons has a significant role in pubertal initiation. Previously, our group has reported higher expression of the KISS1 gene in postpubertal buffalo bulls (Rehman *et al.*, 2019) which shows its importance in pubertal development. It is believed that stimulation of GnRH neurons is essential for kisspeptin-GPR54 signaling in the GnRH neuronal network for the initiation of puberty (Seminara *et al.*, 2003). Central administration of kisspeptin has shown to stimulate the release of GnRH and ultimately secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in cattle and buffalo (Pottapenjera *et al.*, 2018).

There is a vast body of evidence that suggests kisspeptin variations (Chaikhun-Marcou *et al.*, 2019; Pottapenjera *et al.*, 2018) and its hormonal interaction with reproductive development and semen quality in buffalo bulls (Shahzad *et al.*, 2021). However, most of these studies have investigated the effects of exogenous kisspeptin on the secretion of the hypothalamus and pituitary. Our previous study showed that the serum concentration of kisspeptin remained similar in various seasons in breeding Nili-Ravi buffalo bulls (Shahzad *et al.,* 2021). To the best of our knowledge, no data are available on the endogenous level of kisspeptin and its association with other reproductive and metabolic hormones regarding pubertal development in Nili-Ravi buffalo bulls Therefore, the objective of this study was to assess circulating levels of kisspeptin at a prepubertal and postpubertal stage in Nili-Ravi buffalo bulls, and to assess its relationship with anthropometric and endocrine attributes. The present study will not only enhance our existing knowledge about the endocrine pattern but may also provide a basis for the understanding of kisspeptin physiology in the puberty achievement in buffalo bulls.

## MATERIALS AND METHODS

**Geo-location of study:** This study was conducted in the Department of Meat Science and Technology, University of Veterinary and Animal Sciences-Lahore (Latitude 31.1° 3′ 45.5′′ N) and Longitude 73.9° 51′ 59.3′′ E) and Tazij Meat and Food Industry (Latitude 31.3° 15′ 15.8′′ N and Longitude 74.2° 10′ 51.3′′ E), Kasur, Pakistan.

**Study animals:** The study animals (n = 40) were distributed into two groups *i.e*., prepubertal (n = 20) with an age below 2 years and postpubertal (n = 20) with an age range of 3 to 4 years, based on tooth eruption pattern (Ahmad *et al.*, 1989). Antemortem and postmortem inspections of animals were carried out by qualified veterinarians and only those animals were included in this study, those were free of all abnormalities.

**Anthropometry:** Anthropometric parameters include live weight (kg), brain weight (grams), height at withers (cm), height at the pelvis (cm), width of the chest (cm), depth of chest (cm), chest girth (cm), width of the pelvis (cm), body length (cm), cross body length (cm), scrotal circumference (cm), the weight of full testis (grams), the weight of epididymis (grams), and length of testicles (cm), were determined using a measuring tape and weighing scale at the slaughterhouse.

**Blood collection:** Blood was collected from the jugular vein during slaughtering. The serum was separated by centrifugation at 3,000 rpm for 10 minutes and stored in Eppendorf tubes at-40°C until analysed for hormonal estimation.

**Ethical consideration:** All procedures were conducted after due approval from the Ethical Review Committee, University of Veterinary and Animal Sciences, Lahore with serial No. 1978.

**Serum hormonal profiles:** Serum concentrations of kisspeptin were measured using bovine kisspeptin ELISA kit (Cat No: PRS-B5005; Nanjing Paars Biochem Co, Ltd. China). Concentrations of GnRH were determined using the ELISA kit (Cat No: E-EL-0071, Elabscience® Biotechnology Inc. USA). Testosterone (Cat No: EB0049), FSH (Cat No: EB0141), LH (Cat No: EB0142), and IGF-1 (Cat No: EB0068) were estimated with the help of commercial ELISA kits (Fine Biotech Co., Ltd, Wuhan, China). Triiodothyronine (T3; product code 125-300) and thyroxine (T4; product code 225-300) were analysed by the ELISA kits from Accu-Bind ELISA microwells, Monobind Inc., Lake Forest, CA. Final readings of ELISA plates were taken by using Epoch™ microplate spectrophotometer (Biotek Instruments Inc., Winooski, USA).

**Statistical analysis:** Data (mean ± SE) were analysed to assess the normal distribution using Kolmogorov–Smirnov test using SPSS (Version 22.0 IBM Corp, Armonk, USA). Normally distributed data of prepubertal and postpubertal groups were compared using an independent sample t-test. On the other hand, the Mann-Whitney-U test was employed to compare non-normal data between prepubertal and postpubertal groups. Pearson's correlation for normal data and Spearman's correlation test for non-normal data, was used for correlation analyses among kisspeptin, hormonal

concentration, and anthropometric parameters. The significance level was pre-determined at $P \leq 0.05$.

## RESULTS

The differences in endocrine levels and anthropometric parameters of prepubertal and postpubertal animals are presented in Table 1. Kisspeptin levels were non-significant ($P > 0.05$) in both groups. The concentrations of GnRH ($P \leq 0.001$), FSH ($P \leq 0.05$), and T3 ($P \leq 0.01$) were higher in the postpubertal group compared to the prepubertal group (Table 1). Anthropometric parameters showed that the postpubertal animals had significantly higher ($P \leq 0.05$) live weight, brain weight, width of chest, depth of chest, chest girth, body length, the weight of full testicles, the weight of epididymis, and length of testicle than the prepubertal group.

The anthropometric correlation with endocrine status is shown in Table 2. Kisspeptin was positively correlated with the width of the chest ($r = 0.673$ ; $P \leq 0.05$) in the prepubertal group and the width of the pelvis ($r = 0.767$ ; $P \leq 0.05$) in the postpubertal group. GnRH was negatively correlated with body length ($r = -0.853$ ; $P \leq 0.05$) and cross body length ($r = -0.758$ ; $P \leq 0.05$) in postpubertal animals. FSH was negatively correlated with body weight ($r = -0.837$ ; $P \leq 0.05$) depth of chest ($r = -0.764$ ; $P \leq 0.05$), chest girth ($r = -0.764$ ; $P \leq 0.05$), body length ($r = -0.847$ ; $P \leq 0.05$), cross body length ($r = -0.791$ ; $P \leq 0.05$), and weight of epididymis ($r = -0.762$ ; $P \leq 0.05$), in the postpubertal group.

There was a positive correlation of LH with height at pelvis ($r = 0.582$ ; $P \leq 0.05$), the width of chest ($r = 0.683$ ; $P \leq 0.05$), weight of full testicles ($r = 0.615$ ; $P \leq 0.05$), the weight of epididymis ($r = 0.639$ ; $P \leq 0.05$), testicular width ($r = 0.597$ ; $P \leq 0.05$), in the prepubertal group. The LH also had a positive correlation in the postpubertal group with the width of chest ($r = 0.786$ ; $P \leq 0.05$), weight of full testicles ($r = 0.821$ ; $P \leq 0.05$), the weight of epididymis ($r = 0.793$ ; $P \leq 0.05$), testicular length ($r = 0.786$ ; $P \leq 0.05$), and testicular width ($r = 0.786$ ; $P \leq 0.05$). Testosterone was positively correlated with the weight of testicles ($r = 0.756$ ; $P \leq 0.05$) in postpubertal group. IGF-1 was positively correlated with the weight of epididymis ($r = 0.607$ ; $P \leq 0.05$), body length ($r = 0.578$ ; $P \leq 0.05$), in the prepubertal group. T3 was positively correlated with height at withers ($r = 0.625$ ; $P \leq 0.05$), in prepubertal groups.

**Table 1. Mean (±SE) values for age-wise comparison of endocrine and anthropometric attributes in Nili-Ravi buffalo bulls.**

| | Endocrine Attributes | | |
|---|---|---|---|
| | **Prepubertal** | **Postpubertal** | **P Value** |
| **Kisspeptin (ng/L)** | 31.93 ± 1.20 | 33.50 ± 1.22 | 0.367 |
| **GnRH (pg/mL)** | 67.39 ± 1.92 | 77.81 ± 1.30 | 0.001 |
| **FSH (mIU/mL)** | 7.28 ± 0.37 | 8.51 ± 0.30 | 0.014 |
| **LH (mIU/mL)** | 6.74 ± 0.56 | 6.52 ± 0.58 | 0.781 |
| **Testosterone (ng/mL)** | 1.51 ± 0.07 | 1.65 ± 0.09 | 0.199 |
| **IGF-1 (ng/mL)** | 250.74 ± 9.04 | 246.84 ± 9.51 | 0.187 |
| **T3 (ng/mL)** | 1.93 ± 0.16 | 2.84 ± 0.20 | 0.001 |
| **T4 (µg/dL)** | 4.51 ± 0.21 | 4.57 ± 0.31 | 0. 889 |
| | **Anthropometric Attributes** | | |
| **Live weight (kg)** | 281.42 ± 8.60 | 363.64 ± 26.34 | 0.006 |
| **Brain weight (grams)** | 500.92 ± 12.22 | 540.58 ± 9.13 | 0.013 |
| **Height at withers (cm)** | 130.50 ± 1.67 | 138.30 ± 3.16 | 0.036 |
| **Height at pelvis (cm)** | 134.00 ± 1.76 | 140.36 ± 2.86 | 0.055 |
| **Width of chest (cm)** | 53.76 ± 1.01 | 46.38 ± 2.32 | 0.010 |
| **Depth of chest (cm)** | 77.83 ± 0.76 | 82.59 ± 2.00 | 0.035 |
| **Chest girth (cm)** | 152.10 ± 2.81 | 165.18 ± 4.01 | 0.011 |
| **Width of pelvis (cm)** | 52.06 ± 1.07 | 43.83 ± 5.28 | 0.283 |
| **Body length (cm)** | 128.10 ± 1.90 | 140.78 ± 3.01 | 0.004 |
| **Cross body length (cm)** | 133.33 ± 1.06 | 139.50 ± 5.19 | 0.806 |
| **Scrotal circumference (cm)** | 20.82 ± 0.39 | 23.00 ± 1.38 | 0.193 |
| **Weight of full testis (grams)** | 59.68 ±3.92 | 96.58 ± 9.81 | 0.002 |
| **Weight of epididymis (grams)** | 14.37 ± 0.73 | 22.84 ± 2.44 | 0.003 |
| **Length of testicles (cm)** | 62.03 ± 2.34 | 73.70 ± 3.08 | 0.006 |

Groups are significantly different at $P \leq 0.05$. GnRH = Gonadotropin releasing hormone, FSH = Follicle-stimulating hormone, LH= Luteinizing hormone, T3 = Triiodothyronine, T4 = Thyroxin, IGF-1 = Insulin like growth factor

**Table 2. Correlation coefficient (r) between endocrine and anthropometric attributes in the prepubertal (n = 20) and postpubertal (n = 20) Nili-Ravi buffalo bulls.**

| | Kisspeptin | GnRH | FSH | LH | Testosterone | IGF-1 | T3 | T4 |
|---|---|---|---|---|---|---|---|---|
| | | | | **Prepubertal** | | | | |
| **Live weight** | 0.218 | 0.327 | 0.015 | 0.370 | 0.437 | 0.507 | 0.350 | 0.301 |
| **Brain weight** | 0.384 | 0.102 | 0.106 | -0.030 | 0.280 | 0.558 | -0.072 | 0.053 |
| **Height at withers** | 0.099 | 0.126 | -0.089 | 0.481 | 0.410 | -0.025 | 0.625* | 0.465 |
| **Height at pelvis** | 0.357 | 0.372 | 0.015 | 0.582* | -0.074 | -0.094 | 0.125 | 0.224 |
| **Width of chest** | 0.673* | 0.070 | 0.521 | 0.683* | 0.301 | 0.028 | 0.396 | 0.418 |
| **Depth of chest** | -0.196 | 0.350 | -0.494 | -0.183 | 0.064 | -0.398 | -0.265 | 0.472 |
| **Chest girth** | -0.196 | 0.350 | -0.494 | -0.183 | 0.064 | -0.398 | -0.265 | 0.472 |
| **Width of pelvis** | 0.380 | 0.242 | -0.192 | 0.154 | 0.366 | -0.166 | 0.387 | 0.508 |
| **Body length** | 0.216 | 0.295 | -0.205 | 0.314 | 0.399 | 0.578* | 0.482 | 0.232 |
| **Cross body length** | -0.028 | 0.336 | -0.322 | 0.219 | 0.184 | 0.318 | 0.421 | 0.226 |
| **Scrotal circumference** | 0.465 | 0.298 | 0.460 | 0.536 | -0.247 | 0.290 | -0.011 | 0.071 |
| **Weight of testicles** | 0.422 | 0.261 | 0.405 | 0.615* | -0.214 | 0.440 | 0.292 | -0.100 |
| **Weight of epididymis** | 0.170 | 0.191 | -0.014 | 0.639* | -0.074 | 0.607* | 0.526 | -0.097 |
| **Length of testicle** | 0.282 | 0.282 | 0.419 | 0.173 | -0.380 | 0.190 | -0.227 | -0.276 |
| **Width of testicle** | 0.510 | 0.174 | 0.355 | 0.597* | -0.098 | 0.405 | 0.280 | 0.048 |
| | | | | **Postpubertal** | | | | |
| **Live weight** | -0.357 | -0.321 | -0.837 | 0.571 | 0.179 | 0.536 | 0.357 | 0.286 |
| **Brain weight** | 0.370 | 0.337 | 0.157 | -0.500 | -0.061 | -0.459 | 0.469 | 0.373 |
| **Height at withers** | -0.306 | -0.450 | -0.400 | 0.649 | 0.216 | 0.559 | 0.108 | 0.378 |
| **Height at pelvis** | 0.169 | -0.369 | 0.167 | 0.259 | -0.005 | 0.471 | -0.258 | 0.157 |
| **Width of chest** | 0.050 | -0.305 | -0.373 | 0.786* | 0.479 | 0.701 | 0.056 | 0.372 |
| **Depth of chest** | -0.087 | -0.496 | -0.764* | 0.685 | 0.516 | 0.327 | 0.359 | 0.263 |
| **Chest girth** | -0.087 | -0.496 | -0.764* | 0.685 | 0.516 | 0.327 | 0.359 | 0.263 |
| **Width of pelvis** | 0.767* | 0.393 | 0.576 | 0.299 | 0.337 | 0.449 | 0.131 | 0.711 |
| **Body length** | -0.157 | -0.853* | -0.847* | -0.054 | 0.526 | -0.207 | -0.207 | -0.169 |
| **Cross body length** | -0.291 | -0.758* | -0.791* | 0.162 | 0.432 | -0.119 | -0.236 | -0.348 |
| **Scrotal circumference** | -0.093 | -0.668 | -0.483 | 0.543 | -0.040 | 0.097 | 0.534 | -0.019 |
| **Weight of testicles** | 0.045 | -0.467 | -0.630 | 0.821* | 0.756* | 0.516 | -0.157 | 0.249 |
| **Weight of epididymis** | -0.023 | -0.603 | -0.762* | 0.793* | 0.722 | 0.361 | -0.082 | 0.138 |
| **Length of testicle** | 0.128 | -0.319 | -0.534 | 0.786* | 0.625 | 0.623 | 0.214 | 0.295 |
| **Width of testicle** | 0.006 | -0.356 | -0.499 | 0.786* | 0.659 | 0.604 | -0.184 | 0.312 |

* represents significant level at $P \leq 0.05$. GnRH = Gonadotropin releasing hormone, FSH = Follicle-stimulating hormone, LH= Luteinizing hormone, T3 = Triiodothyronine, T4 = Thyroxin, IGF-1 = Insulin like growth factor

## DISCUSSION

Kisspeptin has a potential role in the onset of puberty by acting as a secretagogue of GnRH which is essential for the initiation of normal pubertal development. Kisspeptin regulates the tonic as well as the pulsatile release of GnRH which may have a role in the spermatogenesis and fertility of buffalo bulls (Hussain *et al.,* 2020; Okamura *et al.,* 2013). Though a few studies have already described the endocrine changes in relation to pubertal development in bovines (Habeeb *et al.*, 2016), however, to the best of our knowledge, we are describing for the first time, the role of kisspeptin in pubertal development and its correlation with reproductive as well as metabolic hormones in Nili-Ravi buffalo bullsIn the current study, serum concentrations of kisspeptin showed a non-significant difference between prepubertal and postpubertal age groups. This apparent lack of changes in the concentrations of kisspeptin in both groups shows that there might be some other unknown factors influencing the release of kisspeptin from hypothalamic neurons. One factor might be the nutritional status of the animals that mediate the metabolic signals of leptin receptors present on kisspeptin neurons. Generally, the animals are kept off-feed about 12-16 hours prior to slaughter. This feed withdrawal might decrease the level of leptin that is responsible for the release of kisspeptin (Shashank *et al.,* 2018). Our hypothesis has also been supported by the fact that short-term fastening decreased serum leptin level in cattle (Chelikani *et al.,* 2004). It seems that kisspeptin neurons are not only a single factor that regulates puberty in buffaloes. Rather, this may help in GnRH pulse generation which is necessary for the activation of the HPG axis.

The GnRH is released both in pulsating and tonic patterns and it is believed that LH is also released in the same pulsatile fashion (Okamura *et al.,* 2013). In the current study, the serum level of GnRH was elevated in the postpubertal calves compared to prepubertal calves. Higher concentration of GnRH irrespective of non-significant change of kisspeptin may be due to the involvement of GnRH pulse generation from KNDy neurons i.e., Kisspeptin, neurokinin B (NKB) and dynorphin A (Dyn) located in the ARC of the hypothalamus (Navarro, 2012). Unlike other animals, the ARC has a low level of KISS1 gene expression than POA of the hypothalamus of buffaloes (Rehman *et al.*, 2019).

In the present study, we could not find any difference in the serum level of LH or any pronounced correlation between GnRH and LH in both prepubertal and postpubertal calves. Although there is an increase in the concentration of GnRH with age, the concentration of LH does not change in adult bulls. Our results are supported by a study in which the exogenous administration of GnRH had no effect on LH concentration in prepubertal bulls (Ronayne *et al.,* 1993). Exogenous administration of GnRH also shows a differential effect on LH and FSH of cows by having no effect on LH in cows when infused with an interval of every 4 hours (Vizcarra *et al.,* 1997). The current study revealed positive correlations of LH with body development and testicular parameters in both prepubertal and postpubertal suggesting a regulatory role of LH in reproductive development in bulls. In a previous study, the administration of GnRH at an early age increased the reproductive development in the bull calves (Madgwick *et al.,* 2008).

Presently, we also found a strong positive correlation ($r = 0.964$) between the LH and IGF-1 in postpubertal calves. It has been suggested that IGF-1 might be involved in the regulation of gonadotropins (Brito *et al.,* 2007) and IGF-1 has its receptors both in the hypothalamus in GnRH neurons (Daftary *et al.,* 2004) as well as in testes (Villalpando *et al.,* 2008). Moreover, a positive correlation of IGF-1 with anthropometric parameters and LH in the current study also suggests that IGF-1 has potential regulatory roles on testicular tissues. Our results are also consistent with the study in which an exogenous administration of IGF-1 causes an increase in the secretion of LH in sheep (Adam *et al.,* 1998).

We observed higher levels of FSH in adult bulls compared with the prepubertal bulls which are consistent with previous studies (Amann *et al.*, 1983). The elevated level of serum FSH is due to a lower circulating level of inhibin, a hormone that negatively controls the secretion of FSH (Dixit *et al.,* 1998). Prepubertal bulls have a higher level of inhibin and it decreased with the advancement in the age which leads to an increase in FSH in adult bulls (Miyamoto *et al.*, 1989). Besides, we have also found a negative correlation of FSH with body development parameters in the postpubertal calves which might be due to the reason that inhibin stops controlling the release of FSH and testosterone take over the control of FSH secretion after puberty (Miyamoto *et al.*, 1989).

The thyroid hormones play a significant role in the growth of buffalo bulls because these hormones directly control the basal metabolic rate of the body. We have observed a higher level of T3 in the postpubertal group than prepubertal while the T4 level remained unchanged in both groups. Our results are similar to the study in which circulating T3 level was higher at puberty while the T4 level did not change with the advancement of age in Murrah buffalo calves (Ingole *et al.,* 2012). The effects of thyroid hormones on body growth in bovines showed inconsistent results. A few studies demonstrated no difference in serum concentrations of thyroid hormones in young and adult male calves (Habeeb *et al.*, 2016). While others described that serum T3 concentration decreased with age and T4 increased as the age advances in female Mithun calves (Lalsangpuii *et al.*, 2015). It seems quite difficult to compare sporadic data from various authors because of different environmental conditions and analytical methods.

**Conclusion:** The higher serum concentrations of GnRH, FSH, and T3 levels in the postpubertal group and positive correlation of kisspeptin, LH, testosterone, IGF-1, and thyroid hormone with anthropometric attributes and testicular biometry show their significant role in puberty. Although, the serum concentration of kisspeptin was not significantly different in age groups, however, a pronounced association of kisspeptin with some anthropometric parameters is suggestive of some indirect mechanism that may influence the developmental process by interacting with reproductive as well as metabolic hormones. Nevertheless, this study would help to better understand the physiology of the reproductive system and to understand the mechanism of late maturity in the buffalo bulls.

**Table 3. Correlation coefficient (r) within selected endocrine attributes in the prepubertal (n = 20) and postpubertal (n = 20) Nili-Ravi buffalo bulls**

| **Prepubertal** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Kisspeptin** | **GnRH** | **FSH** | **LH** | **Testosterone** | **IGF-1** | **T3** | **T4** |
| Kisspeptin | 1.000 | -0.496 | 0.440 | 0.268 | 0.532 | 0.002 | 0.007 | 0.325 |
| GnRH | | 1.000 | -0.288 | 0.189 | -0.402 | 0.194 | 0.133 | -0.170 |
| FSH | | | 1.000 | 0.412 | 0.007 | 0.190 | -0.064 | 0.123 |
| LH | | | | 1.000 | -0.096 | 0.120 | 0.395 | 0.305 |
| Testosterone | | | | | 1.000 | 0.115 | 0.335 | 0.391 |
| IGF-1 | | | | | | 1.000 | 0.382 | -0.303 |
| T3 | | | | | | | 1.000 | 0.220 |
| T4 | | | | | | | | 1.000 |
| **Postpubertal** | | | | | | | | |
| Kisspeptin | 1.000 | -0.065 | 0.306 | 0.071 | 0.507 | 0.377 | -0.094 | 0.407 |
| GnRH | | 1.000 | 0.671 | -0.036 | -0.368 | 0.187 | 0.072 | 0.007 |
| FSH | | | 1.000 | 0.234 | -0.480 | 0.300 | -0.197 | -0.176 |
| LH | | | | 1.000 | 0.250 | 0.964** | -0.250 | -0.071 |
| Testosterone | | | | | 1.000 | 0.315 | -0.299 | 0.426 |
| IGF-1 | | | | | | 1.000 | 0.752 | 0.836 |
| T3 | | | | | | | 1.000 | 0.314 |
| T4 | | | | | | | | 1.000 |

** represents significant level at $P \leq 0.01$. GnRH = Gonadotropin releasing hormone, FSH = Follicle-stimulating hormone, LH= Luteinizing hormone, T3 = Triiodothyronine, T4 = Thyroxin, IGF-1 = Insulin like growth factor.

Correlation among hormonal levels is shown in Table 3. There was no correlation between the kisspeptin and other hormones in the prepubertal and postpubertal groups. However, LH showed a positive correlation ($r = 0.964$ ; $P \leq 0.001$), with IGF-1 in the postpubertal animals.

**Conclusion:** The higher serum concentrations of GnRH, FSH, and T3 levels in the postpubertal group and positive correlation of kisspeptin, LH, testosterone, IGF-1, and thyroid hormone with anthropometric attributes and testicular biometry show their significant role in puberty. Although, the serum concentration of kisspeptin was not significantly different in age groups, however, a pronounced association of kisspeptin with some anthropometric parameters is suggestive of some indirect mechanism that may influence the developmental process by interacting with reproductive as well as metabolic hormones. Nevertheless, this study would help to better understand the physiology of the reproductive system and to understand the mechanism of late maturity in the buffalo bulls.

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