

PERIPHERAL PATTERNS OF GROWTH HORMONE, LUTEINIZING HORMONE, AND PROGESTERONE BEFORE, AT, AND AFTER PUBERTY IN BUFFALO HEIFER

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□ *Buffalo, the premier dairy animal in India, suffers from slow growth rate, delayed puberty, and silent heat. It is not known whether the delay in puberty in such animals is due to the delay in expression of hypothalamus-pituitary-gonadal functions. To determine the changes in growth hormone (GH), luteinizing hormone (LH), and progesterone before, at, and after puberty of Murrah buffalo heifers, six Murrah buffalo heifers (21.92 ± 1.09 months of age, 269.67 ± 7.97 kg body weight) were assigned to well-ventilated individual pens and fed a roughage-concentrate diet to provide weight gain of 0.4 kg/day. Blood samples were collected at 3-day intervals during a period of 12 months, and plasma harvested from blood samples was assayed for progesterone, LH, and GH. The day that plasma progesterone was greater than 1 ng/mL for three consecutive sampling days was defined as the day of puberty. Heifers attained puberty at an average age of 31.53 ± 0.88 months with a body weight of 380.67 ± 6.42 kg. Progesterone levels were very low (0.20 to 0.30 ng/mL) during the pre-pubertal period. There were two distinct elevations before the day of puberty onset. Plasma LH and GH concentrations increased ($P < 0.05$) during the months preceding puberty and were highest during the month before puberty. GH and LH were positively correlated ($P < 0.05$) prior to ($r = +0.59$) as well as after puberty ($r = +0.42$). A positive correlation ($P < 0.05$) between LH and body weight during the pre-pubertal period ($r = +0.61$) and thereafter, negative correlation ($P < 0.05$) during post-pubertal period ($r = -0.64$) was noted. GH and body weight showed positive correlation both before puberty ($r = +0.92$, $P < 0.01$) and after puberty ($r = +0.32$, $P < 0.05$). Results suggest that both GH and LH are equally important and vital cues in inducing onset of ovarian functions in buffalo heifers.*

Keywords Buffalo, Puberty, Progesterone, Luteinizing hormone, Growth hormone

INTRODUCTION

The onset of puberty is the result of a series of complex developmental events that include the completion of growth and the maturation of the

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reproductive system. It would appear that while growth hormone (GH) has a fundamental role in the growth process, the action of GH on the reproductive axis is more akin to "fine tuning" than that of a major player (1). There is convincing evidence that GH plays many roles in terms of gonadotrophin release and responsiveness, folliculogenesis, and steroidogenesis during the pubertal period in cattle (2,3), sheep (4), humans (5,6), and monkeys (7). However, no information is available regarding the GH role during pubertal development in buffalo heifers, which needs to be addressed.

Buffalo are the mainstay of the milk production system in India amounting to about 54% of the total milk production (8). However, buffalo suffer from slow growth rate (9) and delayed puberty (10). In particular, Indian Murrah, an important dairy breed of buffalo, has been recorded to attain puberty at an age as high as 33 months (11).

Puberty, the final maturation of the sexual phenotype, is the consequence of increased secretion of gonadal steroids, driven by increased production and secretion of gonadotrophins (12). Changes in the hypothalamic-hypophysial-ovarian axis before puberty have been investigated in cattle (13,14) and sheep (15,16). There have been a few reports on the peripheral levels of LH and progesterone in buffalo heifers (17–20); however, those studies did not address the regular trend of hormonal dynamics associated with growth and puberty in this species.

Information on the endocrine mechanisms that regulate the onset of puberty in buffalo heifers is scarce. In particular, information is limited on the pattern of plasma GH and LH concentrations in such animals. However, the endocrine profiles of GH, LH, and progesterone in growing buffalo calves have been reported recently (21). The difficulty in detecting onset of pubertal estrus by expression of estrus behavior in buffalo heifers is an important issue. Thus, a greater understanding of the endocrinology of puberty in buffalo heifers is urgently needed. Hence, the present study has been taken up with the aim to investigate the changes in pituitary and gonadal hormone (GH, LH, and progesterone) profiles in the blood plasma of buffalo heifers occurring before, at, and after the onset of puberty.

EXPERIMENTAL DESIGN AND METHODS

Animals and Management

Six growing female prepubertal Murrah buffaloes were selected from the National Dairy Research Institute Farm, Karnal, which is situated in the northern belt in Haryana State of India, located 249 meters above the mean sea level in the Indo-Gangetic Plain on 29°42'N latitude and 72°02'E longitude. The agro-climatic situation is dry and tropical. The animals selected for the study were free from any anatomical, physiological, or infectious

disorders. The growing buffaloes averaged 21.92 ± 1.09 months of age and 269.67 ± 7.97 kg body weight. Animals were housed in individual pens with brick flooring and asbestos roofing, having a courtyard for free movement. They were fed daily a concentrate mixture (C.P. 18.54% and T.D.N. 72.7%) consisting of 30% maize, 10% wheat, 5% oats, 17% GNC, 10% mustard cake, 15% wheat bran, 10% rice bran, 1% common salt, 2% vitamin-mineral mixture and green fodder (oats, maize, or jowar according to the availability in the farm) to achieve approximately 0.4 kg body weight gain daily according to the Kearn standard (22). Fresh and clean water was supplied to each animal throughout the day. Live body weight was recorded fortnightly to estimate the growth rate and body weight at puberty. Estrus was checked with teaser bull between 0600 and 0700 AM and between 0600 and 0700 PM daily. The experimental protocol and animal care met the approval of the Institutional Animal Care and Use Committee (IACUC) regulations.

Blood Sampling

Animals were haltered and restrained for the collection of blood samples by jugular vein puncture at 3-day intervals during a study period of 12 months. Before the daily feeding, blood samples were collected at 0800 AM in heparinized polypropylene tubes (20 IU heparin/mL of blood) and were immediately placed in an ice box and carried back to the laboratory. Then the blood samples were centrifuged at $500 \times g$ for 30 minutes, and plasma was separated and collected in the storage vials of 2 mL capacity and stored at -20°C until assayed for GH, LH, and progesterone.

Assay Methods

Plasma progesterone concentrations were analyzed by a direct RIA method developed in the laboratory using 20 μL of plasma in duplicate (23). Mean RIA sensitivity for progesterone was 4 pg/tube and the 50% binding limit being 70 pg/tube. The intra-assay and inter-assay coefficient of variance of plasma progesterone were 7.2% and 11.6%, respectively.

100 μL and 20 μL plasma samples in duplicate were assayed for GH and LH by enzyme immunoassay (EIA) techniques described by Prakash et al. (24) and Prakash et al (25), respectively. Mean EIA sensitivities for GH and LH were 0.40 and 0.31 ng/mL, respectively. The intra-assay and inter-assay coefficients of variance were 3.68% and 6.89%, 5.26% and 8.36%, for GH and LH, respectively.

Determination of Onset of Puberty

Plasma progesterone concentration was monitored regularly to determine the onset of puberty. Pubertal age was calculated relative to when the

first ovulation was presumed on the basis of plasma progesterone profiles. The criterion for cyclicity commencement was the plasma progesterone level reaching 1.0 ng/mL or more and staying high for at least three consecutive samples as has been considered in cattle (26,27). Ovulation date was assumed when the progesterone concentration remained basal (< 0.4 ng/mL) prior to the initial rise. The day on which ovulation first occurred was considered as the day of puberty and designated as day 0. Monitoring of plasma progesterone level was continued after attainment of puberty to study the cyclicity for at least three cycles in all animals.

Statistical Analysis

Periods were assigned to either days or months in relation to puberty for statistical comparisons. The endocrine data was initially normalized to a period of 210 days prior to puberty to 75 days after puberty. The effect of period (month) on hormone profiles was analyzed by least squares analysis of variance, and pairwise mean difference probabilities were compared by Fisher's least-significant difference test, using SYSTAT 0.7 Software package (1997, SPSS, Inc., USA). Pearson's correlation between the different hormones and body weight were run using Microsoft® Excel 2000 Software package (Microsoft Corporation, USA). Data are reported as the mean \pm the standard error of the mean (SEM). The hormone concentration in blood samples collected during a particular month were utilized to find out the mean (\pm SEM) concentration of hormone for that month.

RESULTS

Peripubertal Body Weight Gain

The average body weight at 15-day intervals from 210 days (7 months) prior to puberty up to 75 days (2.5 months) post-puberty is presented in Fig. 1. Buffalo heifers attained puberty on an average at 31.53 ± 0.88 months of age. The mean body weight at the time of puberty was 380.67 ± 6.42 kg. The average daily gain (ADG) in buffaloes was 0.46 ± 0.02 kg/day during the 3 months before puberty and 0.43 ± 0.03 kg/day for the 3 months after puberty (Fig. 2).

Plasma Progesterone (P4) profiles

The overall progesterone profiles (mean \pm SEM) from -210 day to +75 day is presented in Fig. 1. Throughout the 7 months prior to puberty, progesterone concentration showed a continuous basal level ranging from 0.20 to 0.56 ng/mL in all the heifers. Five of six heifers showed a first short-term

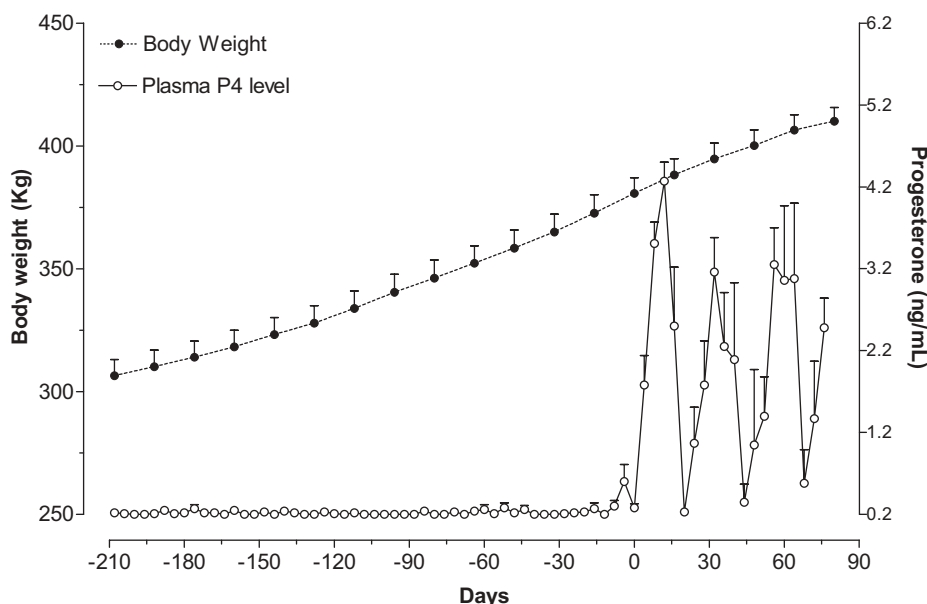


FIGURE 1 Mean (\pm SEM) body weight (kg) at 15-day interval, and plasma progesterone profiles (ng/mL) at 3-day interval, of buffalo heifers from 210 days prior to puberty up to 75 days post-puberty. "0" indicates the day of onset of puberty. Blood samples were collected at 3-day intervals.

elevation of progesterone ranging from 0.55 to 1.58 ng/mL, which occurred 16 to 4 days before the onset of puberty. The mean (\pm SEM) of progesterone concentration in the first elevation was 0.82 ± 0.20 ng/mL. The second rise of progesterone concentration (≥ 1 ng/mL) continued for at least three consecutive samples, reaching a mean peak value of 4.64 ± 0.31 ng/mL (range was 3.90 to 5.83 ng/mL). The date of commencement of the first progesterone cycle was taken as the day of puberty onset. The first estrus was not detected either by visual observation or by the teaser bull. The first progesterone elevation (0.60 ± 0.21 ng/mL, Fig. 1) was for a shorter duration in magnitude and lower ($P < 0.05$) in concentration than the second (4.27 ± 0.23 ng/mL, Fig. 1). Cyclic changes in progesterone profiles subsequently were characteristic of the known progesterone profiles recorded during the estrus cycles. The mean (\pm SEM) post-pubertal estrus cycle lengths were 20.33 ± 1.33 (range, 15 to 23 days), 19.80 ± 0.88 (range, 19 to 23 days) and 20.60 ± 1.60 (range, 19 to 27 days) days for the first, second, and third cycles, respectively.

Plasma LH profiles

The overall LH profiles (mean \pm SEM) from -210 day pre-puberty to +75 day post-puberty are presented in Fig. 3. Plasma LH concentrations changed ($P < 0.01$) over days or months approaching puberty. The LH

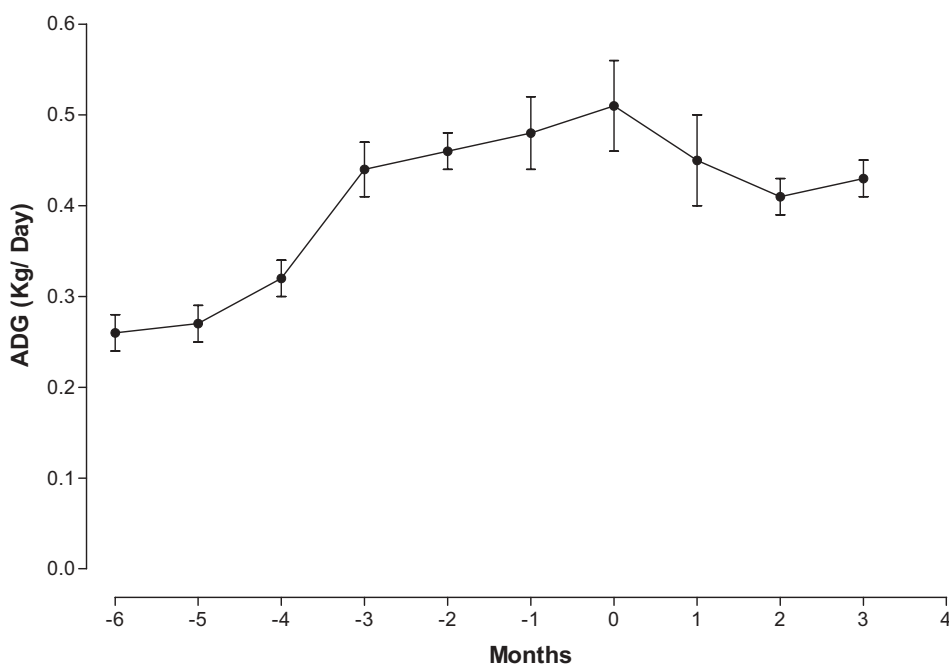


FIGURE 2 Monthly changes of mean (\pm SEM) ADG (kg/day) in buffalo heifers from 6 months prior to puberty up to 3 months post-puberty. "0" indicates the month of puberty.

concentration was 0.45 ± 0.03 ng/mL 6 months prior to puberty. Then it increased ($P < 0.05$) to 0.59 ± 0.05 ng/mL 4 months prior to puberty. Two months later, the LH concentration further increased ($P < 0.05$) to 0.83 ± 0.09 ng/mL and fluctuated within narrow limits thereafter prior to the month of puberty. The hormone level was 0.73 ± 0.06 ng/mL during the month of puberty and then declined ($P < 0.05$) to 0.57 ± 0.05 ng/mL 2 months post-puberty. The LH concentrations 3 to 4 months before and 2 months after puberty were found to be similar ($P > 0.05$). The LH concentrations were positively correlated to body weight before puberty ($r = +0.61$, $P < 0.05$) and negatively correlated ($r = -0.64$, $P < 0.05$) after puberty.

Plasma GH profiles

The overall GH profiles (mean \pm SEM) from -210 day pre-puberty to $+75$ day post-puberty are presented in the Fig. 3. Plasma GH concentrations changed ($P < 0.01$) over time (days or months). The GH concentration was 11.25 ± 0.64 ng/mL 6 months prior to puberty. It increased ($P < 0.05$) gradually to 15.21 ± 0.81 ng/mL 4 months prior to puberty. There was a further increase ($P < 0.01$) of GH concentrations (17.91 ± 0.96 ng/mL) 3 months prior to puberty. The mean GH level fluctuated

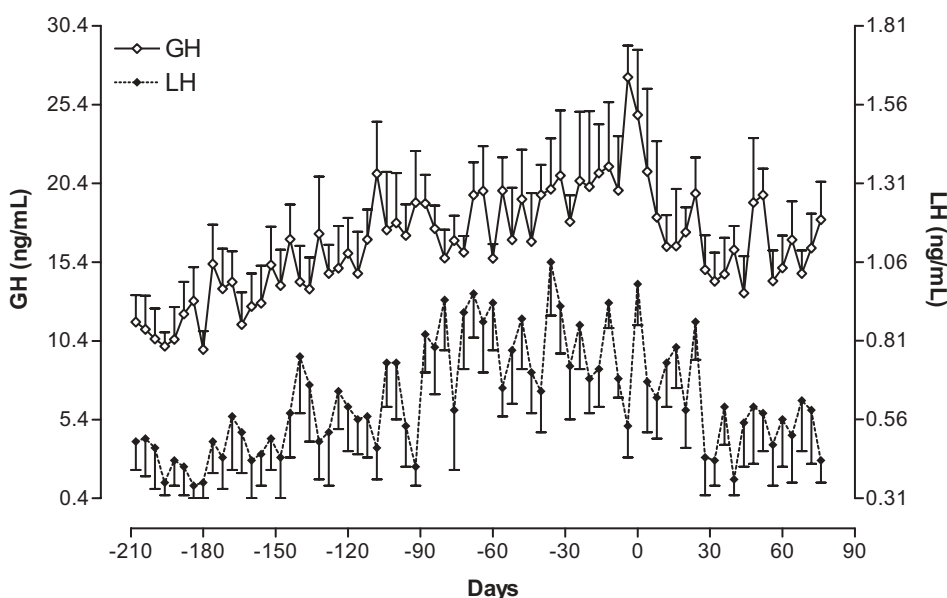


FIGURE 3 Mean (\pm SEM) plasma GH and LH concentrations (ng/mL) in buffalo heifers from 210 days prior to puberty up to 75 days post-puberty. "0" indicates the day of onset of puberty. Blood samples were collected at 3-day intervals.

narrowly thereafter until the month of puberty when the highest ($P < 0.05$) mean GH level was 21.73 ± 1.49 ng/mL. After puberty, the GH level declined ($P < 0.01$) to 16.23 ± 0.77 ng/mL one month post puberty and stayed low for the next month also (Fig. 3). The mean concentration of GH was positively correlated to LH levels both before puberty ($r = +0.59$, $P < 0.05$) and after puberty ($r = +0.42$, $P < 0.05$). A positive correlation between GH and body weight was noted during the pre-pubertal as well as the post-pubertal period. But, the correlation coefficient was higher during pre-puberty ($r = +0.92$, $P < 0.01$) as compared to the value during post-puberty ($r = +0.32$, $P < 0.05$).

DISCUSSION

Low plasma progesterone concentrations ranging between 0.20 and 0.30 ng/mL were observed from 7 months before puberty up to 16 to 4 days prior to first estrus in the present investigation. Low pre-pubertal plasma progesterone concentrations have also been recorded in Egyptian buffaloes (28). In bovine heifers, two distinct elevations of progesterone concentration before onset of puberty have been reported (26,29). We report a transient increase of mean progesterone level of 0.60 ± 0.21 ng/mL with a range between 0.55 and 1.58 ng/mL in buffalo heifers 16 to 4 days before puberty (Fig. 1). The

second rise of progesterone concentrations (4.27 ± 0.23 ng/mL, Fig. 1) signaled the commencement of cyclicity since progesterone concentrations remained ≥ 1 ng/mL for at least three consecutive samples collected at every 3-day interval. The source of progesterone secretion for the first elevation is not known. It may either be of adrenal origin (14) or luteal tissue origin embedded within the ovary (29) as postulated for the rise seen in cattle heifers. The short luteal cycle may exist in the heifers used in this study, and the source of progesterone may be corpus luteum. The first progesterone elevation was usually briefer and lower in magnitude than the second. The first elevation of progesterone may have a vital role in sensitizing the ovaries to LH for initiation of puberty (30). Thereafter, the high progesterone concentration (≥ 1 ng/mL) indicates the cyclic luteal function in heifers (31).

The gradual increase in plasma LH level with approaching puberty observed in buffalo heifers confirms the earlier observations recorded in peripubertal heifers (32) and sheep (16). In the present study, it appears that the establishment of the mature pattern of LH secretion in buffalo heifers is not a sudden event but a gradual one. The gradual increased mean circulating concentrations of LH during the 125–140 days preceding onset of puberty in buffalo heifers suggests that a prepubertal increase in circulating LH concentration is the critical event leading to onset of puberty as also seen in dairy heifers (13). It has also been documented that precocious puberty can be induced by repeatedly injecting purified LH to increase episodic LH frequency artificially in sheep (33). The gradual increase in mean concentration of LH may be the result of a stepwise decreased response of the hypothalamus-pituitary axis to estradiol negative feedback action (34). Secondly, the distinct elevation of progesterone concentration before onset of puberty in buffalo heifers (in the present study) may serve as primer for further maturation of the hypothalamus-pituitary-ovarian axis (35). As the heifers mature and enter the peripubertal phase, the high frequency mode of pulsatile LH release stimulates growth and maturation of ovarian follicles to the preovulatory stage, leading to onset of puberty and ovulation (36). Thus, the high concentration of LH prior to puberty onset may be an important event in the trigger for initiation and organization of cyclic ovarian activity in buffalo heifers.

A positive correlation between LH and body weight during the prepubertal period also indicates that the mean LH concentration in the peripheral circulation increases as the buffalo heifers gradually grow to attain a critical body weight associated with the development of gonads and reproductive organs and preparing the animal to come into puberty and exhibit estrus. Interestingly, it appears that plasma LH concentrations show negative correlation with body weight after puberty. Correlations between LH and progesterone have been reported to be negative for the period 0 to 12 days after estrus ($r = -0.48$; $P < 0.01$) in sows (37) and for the period 1 to

19 days post estrus ($r = -0.26$; $P < 0.01$) and during the luteolytic phase ($r = -0.39$; $P < 0.05$) in cows (38). Thus, the negative correlation between LH and body weight in the present investigation may explain a negative feedback mechanism of progesterone on LH release from the pituitary during the post-pubertal estrus cycles in buffalo heifers.

To the best of our knowledge, our data on GH profiles during peripubertal development may be the first information on buffalo heifers. A gradual increase of mean GH concentrations to peak level 1 month prior to puberty is indicative of gradual development as suggested by reports on cattle (39). GH deficiency in female rats led to delay puberty and puberty could be advanced by GH treatment (40). The importance of GH in sexual maturation is demonstrated by the ability of exogenous GH to accelerate sexual maturation in GH-depleted monkeys (7). In the United Kingdom, 97 boys and 37 girls with isolated idiopathic GH deficiency (IGHD) exhibited a marked delay in the onset of puberty, which was corrected by GH administration (5). Active immunization of crossbred heifers against growth hormone-releasing factor (GRF) has been found to reduce the circulating concentrations of GH, and consequently, delay puberty (41,42). Puberty is similarly delayed in a large proportion of GH receptor (GHR) knockout mice. It may be corrected after GH administration (43). GH may exert its effect on the reproductive organs either directly or by local production of IGF-I (44), which may promote FSH-supported granulosa cell proliferation (45), progesterone synthesis (46), and/or aromatase activity for estradiol production (47). These actions are thought to reflect endocrine roles of pituitary GH and complementary autocrine or paracrine roles of GH produced within reproductive tissues. The high concentrations of GH during the pubertal process may facilitate the diversion of nutrients to the reproductive organs for their development and maturation (48,49). The mechanism for this effect is not known.

Researchers examining the relationship between GH and reproduction found that GH is positively correlated with LH secretion (50). Our data also demonstrate a positive correlation between GH and LH during the peripubertal period in buffalo heifers. GH may enhance follicular survival and cell proliferation by potentiating LH action, since GH deficiency is associated with decreased LH receptor gene expression and LH responsiveness in rats and GH administration corrects both defects (40). In previous study (21), a definite pattern of change in plasma GH levels with a small amount of fluctuation in plasma LH concentrations in buffalo calves during growth has been depicted, while a clear change in plasma GH and LH concentrations during the peripubertal stage in buffalo heifers was observed in the present study. The role for GH may appear to "fine tune" LH secretion, which is essential for initiating pubertal onset (1). Since the onset of puberty is the result of a series of complex developmental events for the completion of growth and the

maturing of the reproductive system, GH is thus an important regulator in the process of sexual differentiation and pubertal maturation in buffalo heifers.

Puberty onset is such a dynamic state because a tremendous change in all aspects takes place in the body to attain maturity in the sense of reproduction. Change in the growth curve represents an inflection point that coincides with onset of puberty, and thereafter growth rate is diminished (51). Since GH is the primary growth regulating hormone, the highly positive correlation ($P < 0.01$) between plasma GH concentration and body weight in buffalo heifers approaching puberty suggests that the high concentration of GH is necessary not only for the final development of skeleton and body mass, but also for the maturation of gonads. Thereafter, the lower positive correlation between GH and body weight may explain the diminishing phase of growth during post-puberty, since the average daily gain in buffaloes is 0.43 ± 0.03 kg/day during 3 months after puberty versus 0.46 ± 0.02 kg/day during 3 months before puberty (Fig. 2).

The results of the present investigation suggest that the onset of puberty in buffalo heifers is, at least in part, a result of coordinated action of GH and LH on the ovary. The probability exists that additional mechanisms, such as estradiol negative feedback mechanism, also may contribute to control pubertal development and thus it is an area that warrants further investigation. Information on the coordinated action of GH and LH on the ovary during pubertal development in buffalo heifers could be useful for some endocrinological manipulations to augment puberty in buffalo species.

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