



Endocrine profile from birth to puberty in buffalo calves and heifers

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ABSTRACT

The GH, FSH and LH were studied in female buffalo calves from birth to puberty and the endocrine profiles recorded at different stages of growth in female buffalo calves and heifers were compared. The minor fluctuation of GH upto 8–10 months of age may be due to gradual dilution of GH with increasing age and size of animals. The decreased GH concentrations at 21–24 months and 24–30 months is due to the endocrine changes that occur at puberty which is associated with a decrease in the number of GH binding sites and consequently the decline in serum circulation of GH. The FSH concentration did not differ significantly from birth to 15–18 months of age indicating that the cessation of early rise may be due to the establishment or enhancement of negative feedback regulation of gonadotrophin secretion by ovarian products. The higher levels of serum FSH from 18–21 months of age to 24–30 months of age could be indicative of the fact that the buffalo calves may have attained puberty. The higher levels of LH found after birth in buffalo calves might be presumably in response to the withdrawal of placental estrogen followed by a brisk surge of gonadotrophins. The gradual increase of gonadotrophin may be possibly due to gradual decrease in sensitivity to ovarian steroid inhibitory feedback, while decrease may be due to rise in sensitivity of GnRh secretion to the inhibitory effects of ovarian steroids. The highest concentration observed at 15–18 months suggests that a prepubertal increase in circulating LH concentration is the critical event leading to onset of puberty. 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.

Key words: Birth, Buffalo, Endocrine, Puberty

In the last two decades, considerable attention has been focused on understanding some of the causes for inherent limitations in reproduction among buffaloes by studying their reproductive endocrinology as well as developing biotechniques for augmenting their reproductive efficiency (Madan and Prakash 2007). Delayed age at calving, late maturity and onset of puberty in buffaloes depends on optimum hormonal levels and their proper interaction. The studies of endocrine and biochemical characters of animals under different physiological states are important since disturbance in physiology, significantly influences these parameters.

Information on the endocrine mechanisms that regulate the onset of puberty in buffalo heifers is scarce esp. on the pattern of plasma GH and LH concentrations in such animals. The difficulty in detecting onset of pubertal estrus by

expression of estrus behaviour in buffalo heifers is an important issue. Thus, a greater understanding of the endocrinology of puberty in buffalo heifers is urgently needed (Haldar and Prakash 2005).

The mechanism governing the control of the onset of puberty in farm animals is poorly understood. The information on hormones in relation to growth in buffalo calves and heifers is not adequate. The present study was therefore proposed with the following objectives: to study the endocrine profile viz. growth hormone, follicle stimulating hormone, and luteinizing hormone, in female buffalo calves from birth to puberty and to compare the endocrine profiles recorded at different stages of growth in female buffalo calves and heifers.

MATERIALS AND METHODS

The study was conducted on Murrah (168) female buffalo calves, new born to 30 months. The animals were maintained under standard conditions of feeding and management. The buffalo calves and heifers in accordance with their age, were divided into 14 groups, 12 in each as: group 1 – birth to 7 days, group 2–8 to 15 days, group 3–16 days to 1 month,

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group 4–1 to 2 months, group 5–2 to 4 months, group 6–4 to 6 months, group 7–6 to 8 months, group 8–8 to 10 months, group 9–10 to 12 months, group 10–12 to 15 months, group 11–15 to 18 months, group 12–18 to 21 months, group 13–21 to 24 months and group 14–24 to 30 months.

Blood samples (8–10 ml) were collected aseptically from the jugular vein in the separate sterile glass vials without anticoagulant. Samples were collected in morning hours between 8 a.m. and 10 a.m. Serum was separated by centrifugation. The serum samples were stored at -20°C until assayed. Before collection of blood samples, the body weights of the experimental animals were recorded (Sastry *et al.* 1998). During the experiment the average ambient maximum and minimum temperature was $30.25 \pm 0.49^{\circ}\text{C}$ and $20.30 \pm 0.12^{\circ}\text{C}$, respectively with relative humidity of $88 \pm 0.71\%$.

Analysis of variance of the data was done using complete randomised design. Differences in means were tested using critical difference (CD) test. To study the relationship of age, body weight and hormones, correlation coefficients were calculated.

The serum levels of follicle stimulating hormone and luteinizing hormone were estimated by radioimmunoassay using the classical double antibody technique of Greenwood *et al.* (1963) as modified by Midgley (1966). Growth hormone was determined using adsorption coating technique (Kumarasamy 2001) with modifications wherein, the anti-bGH raised in monkey was coated to polystyrene (PS) tubes as per the method developed in Radiation Medicine Centre, Babha Atomic Research Center (BARC), Mumbai. PS tubes were heat activated at 60°C for 2 h. About 400 μl bovine GH antiserum at 1: 25, 000 dilutions was added into each tube. These tubes were kept for incubation at room temperature for 6 hours. After incubation the excess antibody in the tube

was aspirated and then the tubes were washed twice with 0.1 M sodium bicarbonate buffer. PBS (1 ml) with 0.5% BSA, pH 7.5 as blocking buffer was added. The tubes were incubated for 3 h at room temperature. The blocking buffer was aspirated and the tubes were stored dry to set up the assay.

RESULTS AND DISCUSSIONS

Hormone patterns from birth to puberty

Growth hormone: The pattern of serum GH recorded in the present study is in agreement with Singh *et al.* (1993) who had observed similar trend up to 4 months of age in Murrah buffalo calves, however, the levels of GH recorded in the present study were relatively low. Badinga *et al.* (1991) found decreased GH up to 1 month which did not change thereafter for 1 year in dairy bull calves, while Purchas *et al.* (1970) reported relatively constant GH concentration after falling from high levels at birth. The accelerated growth that occurs after birth may be related to the rise in the number of hepatic GH receptors. Perhaps endocrine changes that occur at puberty are associated with decrease in the number of GH binding sites and consequently the decline in serum circulation of GH as seen in the present study from 21–24 months and 24–30 months of age.

Garcia *et al.* (2003) recorded decrease in the serum GH concentrations in beef heifers about 15 weeks before puberty and just before that it showed a higher concentration which is similar to the present study. The rise in serum GH concentration recorded in the present study from 10 month onwards is in accordance with Mondal and Prakash (2004) in Murrah buffalo heifers. The GH concentration appears to rise as the animals were reaching advanced stages of pubertal development, thereby suggesting the animals must be reaching peripubertal period. The minor fluctuation of GH

Table 1. Serum growth hormone, follicle stimulating hormone and luteinizing hormone profile (ng/ml) from birth to puberty in buffalo calves and heifers

Age groups	Age of buffalo calves/heifer	GH (ng/ml)	FSH (ng/ml)	LH (ng/ml)
1	0–7 days	$1.68^{\text{h}} \pm 0.08$	$0.17^{\text{c}} \pm 0.01$	$0.14^{\text{g}} \pm 0.01$
2	8–15 days	$2.54^{\text{defg}} \pm 0.18$	$0.15^{\text{c}} \pm 0.01$	$0.13^{\text{g}} \pm 0.01$
3	16 days–1 month	$2.07^{\text{gh}} \pm 0.19$	$0.17^{\text{c}} \pm 0.01$	$0.15^{\text{fg}} \pm 0.01$
4	1–2 months	$2.32^{\text{efg}} \pm 0.27$	$0.16^{\text{c}} \pm 0.04$	$0.17^{\text{defg}} \pm 0.01$
5	2–4 months	$2.17^{\text{fgh}} \pm 0.21$	$0.16^{\text{c}} \pm 0.01$	$0.20^{\text{cdef}} \pm 0.01$
6	4–6 months	$2.26^{\text{fg}} \pm 0.09$	$0.17^{\text{c}} \pm 0.01$	$0.22^{\text{cde}} \pm 0.01$
7	6–8 months	$2.89^{\text{cd}} \pm 0.18$	$0.15^{\text{c}} \pm 0.01$	$0.28^{\text{ab}} \pm 0.01$
8	8–10 months	$2.79^{\text{cde}} \pm 0.33$	$0.16^{\text{c}} \pm 0.01$	$0.22^{\text{cde}} \pm 0.01$
9	10–12 months	$3.20^{\text{c}} \pm 0.12$	$0.16^{\text{c}} \pm 0.01$	$0.22^{\text{cd}} \pm 0.02$
10	12–15 months	$4.09^{\text{b}} \pm 0.14$	$0.16^{\text{c}} \pm 0.01$	$0.17^{\text{efg}} \pm 0.01$
11	15–18 months	$4.72^{\text{a}} \pm 0.10$	$0.16^{\text{c}} \pm 0.01$	$0.30^{\text{a}} \pm 0.03$
12	18–21 months	$4.03^{\text{b}} \pm 0.11$	$0.22^{\text{b}} \pm 0.01$	$0.24^{\text{bc}} \pm 0.02$
13	21–24 months	$2.67^{\text{def}} \pm 0.25$	$0.24^{\text{ab}} \pm 0.01$	$0.21^{\text{cde}} \pm 0.01$
14	24–30 months	$2.28^{\text{efg}} \pm 0.16$	$0.26^{\text{a}} \pm 0.01$	$0.21^{\text{cde}} \pm 0.01$

Means with at least one common superscript do not differ significantly ($P < 0.05$)

up to 8–10 months of age might be due to gradual dilution of GH with increasing age and size of animals. The vigorous growth occurred only so long as the ratio of circulating GH to unit body tissue is sufficiently high to stimulate protein synthesis, abundant enough to cause bone and muscle growth and when available hormone becomes diluted, the animal can maintain its gain and do repair jobs, but it is incapable of actual true growth. Skeletal growth is again possible until the epiphysis fused with the shaft of the long bone. It therefore may be suggested that exogenous GH or GHRH may be administered to increase blood GH, thereby higher growth, during the growing stage. Halder and Prakash (2005) had also observed gradual increase in GH concentrations 6 months prior to puberty, as seen in the present study, but reported this increase until the month of puberty. Irrespective of sex, McCarthy *et al.* (1979) also observed significantly higher GH concentrations in prepubertal bulls, but Mondal *et al.* (2007) reported much greater concentrations from birth to 3 years of age in Mithun than in other species.

With regard to GH profile, recorded in the present study the levels showed highest concentration at 12–15 months through 18–21 months (4.03 ± 0.11 to 4.72 ± 0.10 ng/ml). Halder and Prakash (2005) reported a gradual increase of mean GH concentrations to peak level 1 month prior to puberty indicating gradual development as suggested in cattle (Jones *et al.* 1991). Active immunization of crossbred heifers against growth hormone releasing factor reduced the circulating concentrations of GH, and consequently, delayed puberty (Armstrong *et al.* 1993, Schoppee *et al.* 1996). GH may exert its effect on the reproductive organs either directly or by local production of IGF-I (Davoren and Hsueh 1986), which may promote FSH-supported granulosa cell proliferation (Jia *et al.* 1986), progesterone synthesis (Apa *et al.* 1994) and/or aromatase activity for estradiol production (Jimenez-Krassel *et al.* 1999). These actions are thought to reflect endocrine roles of pituitary GH and complementary autocrine and paracrine roles of GH produced within reproductive tissue. The high concentration of GH during the pubertal process may facilitate the diversion of nutrients to the reproductive organs for their development and maturation (Bauman and Currie 1980 and Burton *et al.* 1994).

If the GH status of an animal is considered as the quantity of growth hormone effectively utilized by the tissues, then it probably is a function of the concentration of GH and/or the turnover role of serum/plasma GH. If GH utilization is determined primarily by serum GH concentration, then the results suggests that there is relationship between GH status and growth as serum GH concentration and specific rate is correlated (Purchas *et al.* 1970).

Follicle stimulating hormone: The trend of serum FSH recorded in the present study is in accordance with Presicce *et al.* (2003) in buffalo calves. They attributed these fluctuations due to association of the hormone with regressing dominant follicle, which may be sufficient to trigger the

development of a new wave. The hypophyseal and ovarian hormonal fluctuation accompanies the development and regression of main follicular waves. The similar trend was also observed by McCarthy *et al.* (1979) who found no change in serum FSH with age in bulls up to 10 months of age.

Our findings that FSH concentration did not differ significantly from birth to 15–18 months of age is similar to those reported by Evans *et al.* (1994b) in Hereford heifers up to 36 weeks of age but there was rise in concentration from 2 to 14 weeks of age. The cessation of early rise may be due to the establishment or enhancement of negative feedback regulation of gonadotrophin secretion by ovarian products. These could have been produced in increasing amounts by enhanced gonadotrophin stimulation of large ovarian follicles. The ovarian inhibitory mechanism, controlling gonadotrophin secretion develops between 2 and 12 weeks of age. Between 2 and 8 weeks of age, there is an increase in the functional capacity of granulosa cell membranes in response to gonadotrophin stimulation. The concentration of FSH is high when number of ovarian follicles and maximum diameter of follicles is increased. Thus early rise in gonadotrophin secretion plays an important role in stimulating ovarian follicle growth in heifers and follicular products may be involved in terminating the early rise in gonadotrophin secretion. The time of early rise in gonadotrophin secretion may reflect a critical developmental stage in the reproductive maturation of heifer calves.

Our observation that higher FSH levels recorded during prepubertal and pubertal buffalo heifers is in accordance with Evans *et al.* (1994a). The change in plasma FSH is associated with waves of follicular development during ovulatory cycles of mature heifers. The endocrine mechanism leading to emergence, growth and selection of large follicles are similar between waves in prepubertal anovulatory period and waves during normal ovulatory cycles in mature animals. The secretion of FSH during sexual maturation is not the limiting factor preventing the first ovulation. The higher levels of serum FSH from 18–21 months of age to 24–30 months of age could be indicative of the fact that the buffalo calves may have attained puberty. However greater mean FSH concentration ($P < 0.05$) in heifer calves was observed by Rawlings *et al.* (2005) at 10, 20, 22–26, 30 and 56 weeks of age.

Lacau-Mengido *et al.* (2000) found mean FSH levels invariable throughout development from birth to 42 weeks of age in buffalo calves, which is in agreement with previous studies performed in beef heifers (Dodson *et al.* 1988 and Wolfe *et al.* 1989). No association between FSH levels and time of puberty was described in heifers (Kinder *et al.* 1995) and in sheep (Pappa-Michailidou *et al.* 1999). It was suggested that FSH secretion during sexual maturation is not the limiting factor related to first ovulation (Kinder *et al.* 1995).

The excitatory amino acids may be involved in the regulation of FSH release and its effects may be age related, developing in the early postnatal period and reaching their maximum in the mid to late prepubertal period sometime before first ovulation; besides even nitric oxide to some extent is involved in the regulation and release of FSH (Honaramooz 1999).

The low levels of FSH were reported by MacDonald *et al.* (1991) in Holstein bull calves and concluded that age related changes in plasma FSH occurred in bulls. In contrast, Jorio *et al.* (1999) reported the FSH concentration to increase progressively from birth until 17 weeks in ewe lambs.

Luteinizing hormone: LH concentration increased from 0–7 days to 6–8 months and this is in accordance with Jain (1988) in Murrah buffalo calves. He, however recorded highest LH concentration at 12–15 months of age. The higher levels of LH found after birth in buffalo calves might be presumably in response to the withdrawal of placental estrogen followed by a brisk surge of gonadotrophins. The other possibilities for a higher concentration of LH in neonates could be the presence of large follicles on the ovaries, secreting sufficient amount of estradiol to trigger the adenohypophysis through the activation of hypothalamus to secrete gonadotrophins.

The gradual and significant increase from 0–7 days to 6–8 months was also reported in buffalo heifers calves (Mondal and Prakash 2004) and Hereford heifers (Evans *et al.* 1994a, Evans *et al.* 1994b). Evans *et al.* (1994b) suggested that the early rise in gonadotrophin secretion plays an important role in stimulating ovarian follicle growth in heifers and follicular products may be involved in terminating the early rise in gonadotrophin secretion. The time of the early rise in gonadotrophin secretion may reflect a critical developmental stage in the reproductive maturation of heifer calves. Evans *et al.* (1994a) opined that throughout the prepubertal period, estradiol acts with strong negative feedback effects to suppress LH pulse frequency. The gonadostat hypothesis suggested that in the later stages of sexual maturation the responsiveness of the hypothalamo pituitary unit to estradiol decreases, allowing increased LH secretion. The increase in LH secretion is necessary to induce cyclicity.

In the present study, the LH concentration decreased significantly from 18–21 months of age. Irrespective of sex, Hemeida *et al.* (1985) reported gradual increase of LH concentration in bull calves between 8 and 15 months of age and then decreased with advancing age in 17–19 months of age. The change in LH concentration could be due to pulsatile pattern of release of LH. However, Dodson *et al.* (1988) found LH concentration decreasing between 3 and 15 weeks and then increased by 35 weeks of age in cattle heifers. The gradual increase of gonadotrophin may be possibly due to gradual decrease in sensitivity to ovarian steroid inhibitory feedback, while decrease may be due to rise in sensitivity of GnRh secretion to the inhibitory effects of ovarian steroids.

The fall in LH concentration may be the reflection of changes in basal LH concentrations. But subsequent rise in mean concentration may be due to increase in LH episodic frequency. Even seasonal factors can influence the pattern of gonadotrophin after birth. LH concentrations are reported to be higher in neonate born in autumn than those in spring. The changes in mean LH concentration could reflect the initial development of an inhibitory mechanism controlling gonadotrophin secretion, followed by a gradual escape from inhibition.

The mean LH concentration was highest in 15–18 months of age group and almost similar in age groups 18–21 months, 21–24 months and 24–30 months. This result is in agreement with Wolfe *et al.* (1989), Kumar and Rattan (1992) and Shah *et al.* (1993), in cow and buffalo heifers, respectively, who observed the LH values increased during the prepubertal period. Wolfe *et al.* (1989) suggested that the increase in LH concentrations as the puberty approached may be due to decrease in negative feedback of estradiol. Similar increase in mean circulating concentration of LH 4–5 months preceding onset of puberty was reported by Halder and Prakash (2005) in buffalo heifers. This increase preceding onset of puberty suggests that a prepubertal increase in circulating LH concentration is the critical event leading to onset of puberty and also the establishment of the mature pattern of LH secretion in buffalo heifers is not a sudden event but a gradual one. The gradual increase may be the result of a stepwise decreased response of the hypothalamus-pituitary axis to estradiol negative feedback action. Also the distinct elevation of progesterone concentration before onset of puberty may serve as primer for further maturation of the hypothalamus-pituitary-ovarian axis. As heifers mature and enter the peripubertal phase, the high frequency mode of LH release stimulated growth and maturation of ovarian follicles to the preovulatory stage, leading to onset of puberty and ovulation. 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. Lunstra *et al.* (1978) supports the hypothalamic desensitization theory of sexual maturation with gradual increase of LH as bulls approached puberty. Also the alpha adrenergic neuronal system has stimulatory effects on LH release, especially during the late prepubertal period (Honaramooz 1999).

However, McCarthy *et al.* (1979), Lacroix and Pelletier (1979) and Aravindakshan *et al.* (2000) found higher values at 4 months and 2–5 months of age respectively in bull calves and did not differ among other months upto 10 months of age, while Lunstra *et al.* (1978) observed increased LH concentration between 7 and 13 months of age in bull calves and Rawlings *et al.* (2005) reported high LH concentrations at 6, 10, 12, 40, 42 and 56 weeks of age in bull calves. The age related increase in concentration of LH is related to age related differences in the numbers and secretory activity of

gonadotrophs located within the pars distalis.

In contrast no differences between groups were found in LH concentrations in Holstein heifers (Lacau-Mengido *et al.* 2000). However the author favours the gonadostat hypothesis of puberty onset that the sensitivity of the hypothalamo-hypophyseal unit to negative feedback of estradiol decreases during prepuberty allowing increasing LH secretion that in turn stimulates an increase in estradiol production by the ovary.

Hormone patterns in relation to body weight: The body weights of experimental buffalo calves and heifers aged 0–7 days to 24–30 months, measured from 27.50 to 361.42 kg. Probably all hormones directly or indirectly influence growth by altering biochemical reactions, many of them influence size of specific tissues and organs. Based on body weights, the buffalo calves and heifers were divided into 4 groups, having body weight as 27.5–100, 101–200, 201–300 and 301–400 kg. The hormone pattern in these buffalo calves and heifers in relation to body weight is presented in Tables 2.

Growth hormone: The serum GH concentration did not significantly differ in different body weight groups. The serum GH concentration did not differ significantly, and it is in accordance with reported observation in Murrah buffalo heifers (Singh *et al.* 1993). These workers reported that the circulating levels of GH did not increase linearly with body weight and age and had negative correlation (-0.27) though nonsignificant. They further reported that mode of feeding did not have any significant effect on the concentration of GH. Haldar and Prakash (2005) reported highly positive correlation between serum GH concentration and body weight in buffaloes approaching puberty. The authors opined that the puberty onset is a dynamic state wherein 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. Since GH is the primary growth regulating hormone, the highly positive correlation between serum GH concentration and body weight in buffaloes 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.

Follicle stimulating hormone: Identical levels of serum

Table 2. Serum growth hormone profile (ng/ml) in relation to body weight in buffalo calves and heifers

Body weight (kg)	GH (ng/ml)	FSH (ng/ml)	LH (ng/ml)
27.5–100	2.16±0.13	0.16 ^b ±0.00	0.16 ^b ±0.00
101–200	2.57±0.31	0.16 ^b ±0.00	0.25 ^a ±0.02
201–300	3.36±0.38	0.16 ^b ±0.00	0.20 ^{ab} ±0.01
301–400	3.42±0.57	0.22 ^a ±0.02	0.24 ^a ±0.02

NS, Nonsignificant; Figures with different superscript within a column differ significantly.

FSH were recorded at 27.5–100 kg to 201–300 kg body weight. The serum FSH concentration significantly ($P < 0.05$) increased and reached to a value of 0.22 ± 0.02 ng/ml at 301–400 kg body weight. These results of the serum FSH concentration in relation to body weight could not be compared as no published report could be traced.

Luteinizing hormone: The average serum LH concentration recorded at 27.5–100 kg body weight was 0.16 ng/ml. The LH concentration significantly ($P < 0.05$) increased at 101–200 kg body weight (0.25 ± 0.02 ng/ml) and remained almost similar up to 301–400 kg body weight (0.24 ± 0.02 ng/ml). Lunstra *et al.* (1978) in their study in bulls of different breeds reported that serum LH concentration increased linearly ($P < 0.01$) between 7 and 13 months of age for all bulls combined. Breed differences in LH concentrations were not observed between 7 and 11 months of age (i.e. through puberty). Mean LH concentration averaged over the entire evaluation period was higher for Red Poll than for Hereford bulls. On the basis of the observation that LH increased gradually, as bulls approached puberty supported the hypothalamic desensitization theory of sexual maturation (Odell and Swerdloff 1974). Haldar and Prakash (2005) reported that the LH concentration 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 indicating 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.

Relationships of age, body weight and hormones: The intercorrelations matrix of age, body weight and serum hormones in buffalo calves and heifers is presented in Table 3. The body weight was highly positively correlated with age of buffalo calves and heifers ($r = 0.97$, $P \geq 0.01$). The serum hormones GH ($r = 0.47$), FSH ($r = 0.47$) and LH ($r = 0.49$), were positively correlated with age. The relationship of GH, FSH and LH, with age however, was statistically nonsignificant. However, Singh *et al.* (1993) reported that the circulating levels of GH did not increase linearly with age and, had negative correlation though nonsignificant. Pappa-

Table 3. Intercorrelations matrix of age, body weight and serum hormones in buffalo calves and heifers

Attributes	Age	Body wt	GH	FSH	LH
Age	-	0.97**	0.47 ^{NS}	0.47 ^{NS}	0.49 ^{NS}
Body Wt	-	-	0.62*	0.68**	0.60*
GH	-	-	-	-0.02 ^{NS}	0.59*
FSH	-	-	-	-	0.12 ^{NS}
LH	-	-	-	-	-

Number of pairs, 14; Figures with different superscript within a column differ significantly.

Michailidoe *et al.* (1999) however, reported no relationship between prepubertal FSH and age at puberty. The positive correlation of serum LH with age was in accordance with Evans *et al.* (1994a) and they reported steady increase in mean LH concentration with advancement of age in heifers.

With regards to the relationship of hormones with body weight, serum GH ($r = 0.62$, $P \geq 0.05$), FSH ($r = 0.68$, $P \geq 0.01$) and LH ($r = 0.60$, $P \geq 0.05$) were positively correlated with body weight of buffalo calves and heifers. Our observation that serum GH was positively correlated with body weight is in accordance with reported observation in Murrah buffalo heifers (Halder and Prakash 2005). They opined that GH is the primary growth regulating hormone. The positive correlation between GH and body weight in buffalo heifers approaching puberty suggested that the high concentration of GH is necessary not only for the final development of skeleton and body mass, but also for maturation of gonads. Singh *et al.* (1993) however, in buffalo calves and heifers, reported negative relationship of GH with body weight, although nonsignificant. In the study, serum LH concentration was positively correlated with body weights, is in accordance with reported observation in Murrah buffalo heifers during the prepubertal period (Halder and Prakash 2005). These authors indicated 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.

As regards the relationships amongst the hormones studied, the serum GH concentration was positively correlated with serum LH ($r = 0.59$, $P \geq 0.05$) and negatively correlated with serum FSH ($r = -0.02$) and were statistically nonsignificant. The serum FSH was positively correlated and statistically nonsignificant with LH ($r = 0.12$). The observation that the serum GH was positively correlated with serum LH is in agreement with Halder and Prakash (2005). They demonstrated a positive correlation between GH and LH during the prepubertal period in buffalo heifers. They indicated that the GH may enhance follicular survival and cell proliferation by potentiating LH action. They further added that the role of GH may appear to "fine tune" LH secretion, which is essential for initiating pubertal onset (Ogilvy-Stuart and Shalet 1992). 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.

In conclusion, the gradual rise and highest GH concentration at prepubertal period indicates gradual development and maturation of reproductive organs. Thus, GH is an important regulator in the process of sexual differentiation and pubertal maturation in buffalo heifers. The increase in serum FSH in prepubertal and pubertal heifers

indicated that the heifers had attained puberty and this change in FSH could be associated with waves of follicular development during ovulatory cycles. Further, the high concentration of serum LH in prepubertal heifers is a critical event which leads to onset of puberty and helps to trigger initiation and organization of cyclic ovarian activity in buffalo heifers.

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