



Plasma micronutrients status and gonadotrophin hormone profiles during peripubertal period in female Black Bengal goat

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ABSTRACT

The aim of the study was to address the micronutrients' status and hormonal profiles and their relationships during the sexual maturity in Indian prolific Black Bengal goats. Female Black Bengal kids (12) were selected at day 1 of age and subjected to the weekly blood samplings from the day 1 age up to 8 weeks post puberty. Live body weight was recorded weekly. The occurrence of estrus was checked by a teaser buck, followed by transrectal ultrasonographic (USG) examination on 10th day post puberty. Plasma samples harvested from the blood samples were assayed for progesterone to detect luteal function and confirm the timing of puberty onset. The female Black Bengal goats attained puberty at an average age of 25.47±3.84 weeks with a mean body weight of 7.93±0.87 kg. The plasma samples were further utilized for estimating zinc (Zn), iron (Fe), copper (Cu), manganese (Mn), follicle stimulating hormone (FSH) and luteinizing hormone (LH). Plasma Zn, Fe and FSH levels increased during the months preceding puberty onset. Plasma Cu and Mn levels remained higher during the month of puberty. The significant correlations of body weight with plasma FSH, LH, Cu, Zn and Mn levels and between micronutrients and gonadotrophin hormones before puberty onset might have important functions of micronutrients and gonadotrophin hormones for bringing about puberty onset in prolific Black Bengal goats.

Key words: Black Bengal goat, Follicle stimulating hormone, Luteinizing hormone, Micronutrients, Puberty

Puberty is a process encompassing morphological, physiological and behavioral development for the attainment of the functional capability for producing offspring (Ebling 2005). Since the timing of onset of puberty determines the reproductive capacity of an animal for the whole life, it has huge economic importance in livestock farming. Though the goat plays a significant role in Indian agriculture for its remarkable contribution in rural economy, least care is practically paid to the goats under the prevailing management and nutritional practices. Probably, goat is the most neglected animal among the livestock species. Evidences from the literature (Hostetler *et al.* 2003, Yokus *et al.* 2004) suggest nutritional factors; especially micronutrients are perhaps the most crucial, in terms of their effects on reproductive phenomenon, acting either directly on the gonads and other reproductive organs, or indirectly via the hypothyseal-pituitary-gonadal axis (Smith and Akinbamijo 2000). Hence, it is necessary to understand

the micronutrients' status and hormonal profiles during sexual maturity in goats. Some works on micronutrients' status (Kadzere *et al.* 1996, Ahmed *et al.* 2001) and hormone profiles (Gaffar *et al.* 2005, Khanum *et al.* 2008, Kandiel *et al.* 2010) were reported in few goat breeds during growing period, estrous cycle, pregnancy and lactational period under different agro-climatic and managerial situations. However, micronutrients profiles and hormonal mechanisms that regulate the onset of puberty have not been explored in any prolific goat breed like Black Bengal during the peripubertal period. Hence, the present investigation was undertaken to address the issues of micronutrients' and gonadotrophin hormones' profiles during pubertal process in prolific Black Bengal breed of goats. The objectives of the present study were to (i) elucidate micronutrients' changes around the peripubertal period, (ii) investigate the gonadotrophin hormone profiles during the peripubertal period, and (iii) explore the relationships among the physiological variations of micronutrients' concentrations and puberty-related gonadotrophin hormone profiles and body weight in female Black Bengal goats

MATERIALS AND METHODS

Newborn female Black Bengal kids (12) were selected for the study from goat farm of the ICAR complex, Tripura. The animals selected for the study were free from any anatomical, physiological, or infectious disorders. Newborn

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kids were raised with their mothers until weaning at 8 weeks of age. As the kids grew, they started to eat green grass/ cut green leaves (hybrid napier, congo signal, mulberry or jackfruit leaves) and commercially available pelleted concentrate feeds along with their mothers. After weaning, the kids were fed according to the recommendations of NRC (2007) with an access of grazing on natural pasture and supplementary concentrate feeding. Fresh drinking water was available *ad lib* to all the kids. A vasectomised (teaser) buck was allowed to run with the growing kids after the attainment of 3 months of age for recording the estrus behavior. Live body weight of all the experimental animals was recorded weekly.

Blood samples were collected weekly in heparinised polypropylene tube (20 IU heparin/ml of blood) from all the experimental animals by venipuncture between 0800 and 0900 h before feeding starting from the day 1 age up to 8 weeks after onset of puberty. The blood samples were centrifuged (2,500×g for 10 min at 4°C) and the plasma samples collected and stored at -20°C until the analysis of micronutrients and hormones.

Some estrus behavioral signs were recorded for initial identification of the goats that were in puberty onset. The goats that exhibited puberty onset were subjected to transrectal ultrasonographic (USG) examination at 10 days after observed puberty, using 7.5 MHz linear transducer with B-mode to observe the presence of corpus lutea (CL) on ovaries (Orita *et al.* 2000). It was further confirmed by progesterone concentrations in weekly plasma samples (Sakurai *et al.* 2004).

Plasma levels of Zn, Fe, Cu and Mn were determined by atomic absorption spectrophotometry using 1 ml of plasma sample collected at 2 weeks interval and following the method described by Sandel (1950) as modified by Arenze *et al.* (1977). Twenty microliter of plasma sample was assayed for estimating plasma progesterone concentrations by a radio immunoassay (RIA) using the technique of Kamboj and Prakash (1993). Mean RIA sensitivity for progesterone was 0.2 ng/ml. The intra- and inter-assay coefficients of variation (CVs) were 5.3% and 9.7%, respectively. Eighty microliter of plasma sample was run in 96-well microtiter plate using a double antibody enzyme immunoassay (EIA) as previously validated in this laboratory (Haldar *et al.* 2009) for determining plasma LH concentrations. The intra- and inter-assay coefficients of variation (CVs) were 7.8% and 10.8%, respectively. The sensitivity of the EIA assay for LH was 0.3 ng/ml. Fifty microliter of plasma sample was used to quantify plasma FSH concentrations by an EIA using ELISA kit as reported elsewhere (Haldar *et al.* 2013). The sensitivity of the assay for FSH was 0.5 ng/ml. The intra- and inter-assay coefficients of variation (CVs) were 8.8% and 11.8%, respectively.

Data are presented as mean± standard error of the mean (SEM). The time period was assigned to day in relation to the day of puberty for statistical comparisons. The data generated on body weight, micronutrients and hormones

was initially normalized to a period of 26 weeks before and 8 weeks after the onset of puberty. The effect of time period (week) on body weight, micronutrients' and hormones' data was determined by an ANOVA for repeated measures technique with post hoc analysis using SPSS statistical software. Pearson's correlation among micronutrients, hormones and body weight was also determined.

RESULTS AND DISCUSSION

The observed estrus behaviors are presented in Fig. 1. The major estrus symptoms expressed were tail wagging and frequent urination, followed by buck mounting, bleating and swollen vulva. Other estrus symptoms were mucus discharge from the vagina and restlessness. In the present study, the estrus behaviors of female Black Bengal goat noted are in agreement with the earlier observations (Thibier *et al.* 1981). Further, transrectal USG examination on day 10 after the commencement of estrous cycle indicated the presence of CL on the ovary. Puberty onset was finally confirmed by plasma progesterone profiles to detect luteal function. A representative profile of change in plasma progesterone concentrations in peripubertal female Black Bengal goat during the period from -22 week prepuberty to 8 weeks postpuberty is presented in Fig. 2. Throughout 22 weeks prior to puberty, plasma progesterone concentrations showed a continuous low level ranging from 0.20 to 0.59 ng/ ml and sometimes it was non- detectable. The first time elevated progesterone levels (> 1.0 ng/ml) for at least 1 week confirmed the onset of puberty in female Black Bengal goats and provided evidence for the first estrous cycle to commence the puberty as reported previously (Sakurai *et al.* 2004).

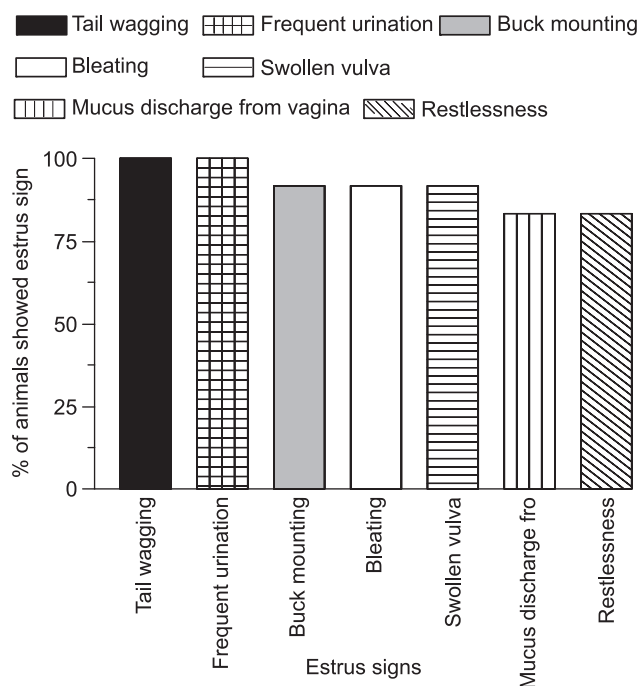


Fig. 1. Incidence of various behavioral estrus signs in pubertal goats (n= 12).

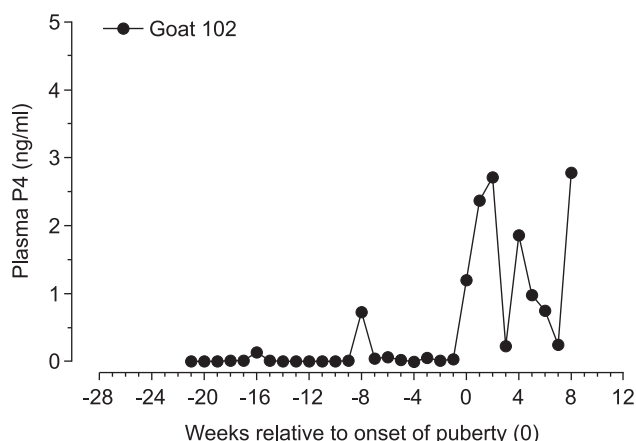


Fig. 2. A representative profile of plasma progesterone concentrations (ng/ml) from 22 weeks prior to puberty up to 8 weeks post puberty. '0' indicates the week of onset of puberty. Blood samples were collected weekly.

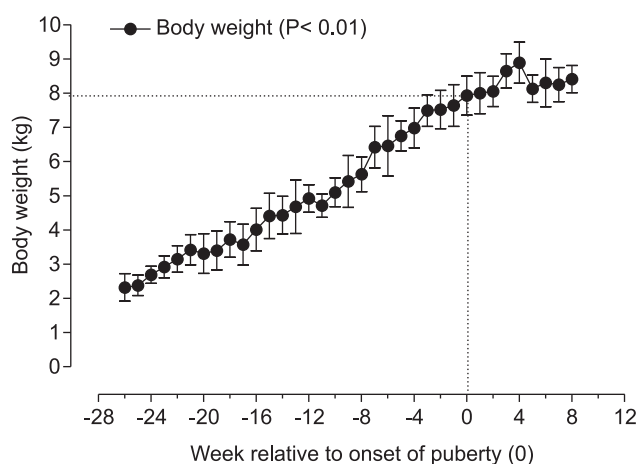


Fig. 3. Weekly mean ($\pm$ SEM) body weight (kg) of female Black Bengal goats ($n=12$) from 26 weeks prior to puberty up to 8 weeks post puberty. '0' indicates the week of onset of puberty. Mean ($\pm$ SEM) body weight at the time of puberty was 7.93 ± 0.87 kg at the age of 25.47 ± 3.84 weeks.

The change in average body weight at weekly intervals from 26 weeks prior to puberty up to 8 weeks post puberty is presented in Fig. 3. In the present study, the age at onset of puberty in female Black Bengal goats was 25.47 ± 3.84 weeks which was earlier than other goat breeds (Freitasa *et al.* 2004, Chentouf *et al.* 2011). The mean body weight at the time of puberty was 7.93 ± 0.87 kg. Body weight changed significantly ($P < 0.01$) over time for the attainment of puberty in female Black Bengal goats (Fig. 3). The female Black Bengal goat attained puberty at a lighter body weight (7.93 ± 0.87 kg) as compared to other goat breeds (Sakurai *et al.* 2004, Chentouf *et al.* 2011). The large variability between individuals in the onset of puberty indicated that the timing of puberty is not simply a function of chronological age (Ebling 2005). Rather, sufficient body growth is a consequence of metabolic changes occurring before and around the onset of puberty and blood-borne

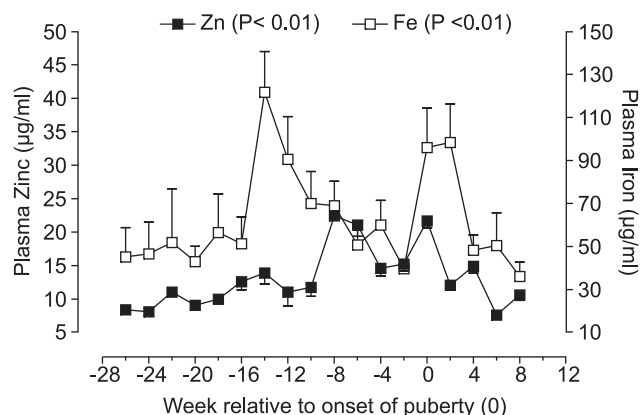


Fig. 4. Mean ($\pm$ SEM) plasma Zn and Fe concentrations ($\mu\text{g/ml}$) of female Black Bengal goats ($n=12$) from 26 weeks prior to puberty up to 8 weeks post puberty. '0' indicates the week of onset of puberty. Blood samples were collected weekly.

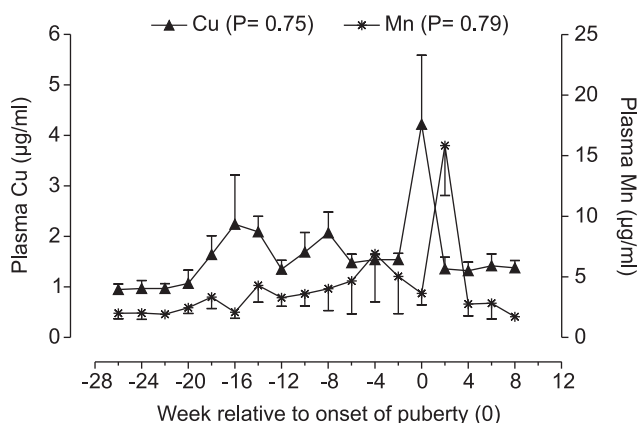


Fig. 5. Mean ($\pm$ SEM) plasma Cu and Mn concentrations ($\mu\text{g/ml}$) of female Black Bengal goats ($n=12$) from 26 weeks prior to puberty up to 8 weeks post puberty. '0' indicates the week of onset of puberty. Blood samples were collected weekly.

substances, which may be metabolites, hormones or a combination, influence the reproductive system and initiate puberty (Suttle *et al.* 1991). The positive correlations of body weight with plasma FSH ($r = 0.61$, $P < 0.05$), LH ($r = 0.52$, $P < 0.05$), Zn ($r = 0.76$, $P < 0.01$), Cu ($r = 0.60$, $P < 0.05$) and Mn ($r = 0.89$, $P < 0.01$) during pre-pubertal period indicated that FSH, LH, Zn, Cu and Mn concentrations reached to certain levels in peripheral circulation, when female goats attained to a certain critical body weight necessary for puberty onset. Thus, the body weight might be a simple index to predict the timing of puberty in female Black Bengal goats.

The mean ($\pm$ SEM) plasma Zn, Fe, Cu and Mn profiles in female goats during the period from 26 weeks prior to puberty up to 8 weeks post puberty are presented in Figs 4 and 5. Plasma Zn concentration increased ($P < 0.05$) gradually, 8 weeks prior to puberty and thereafter, it decreased ($P < 0.05$) gradually after puberty onset. Available reports indicated that Zn is necessary for carbohydrate

metabolism, protein synthesis and nucleic acid metabolism, bone and blood formation (Georgievskii *et al.* 1982, Vergnes *et al.* 1990), growth and development of germinal and somatic cells (Underwood and Somers 1969). Zn could induce a significant increase in FSH-supported progesterone synthesis (Paksy *et al.* 1997). Inadequate Zn levels are associated with impaired secretion of FSH and LH, abnormal ovarian development and decreased fertility in cattle (Bedwal and Bahuguna 1994). In the present study, a gradual increasing demand of Zn from kid stage to pubertal stage indicates certain role of Zn for bringing about puberty onset in prolific Black Bengal goats.

Unlike plasma Zn profile, plasma Fe concentration increased ($P < 0.05$) suddenly, 14 weeks prior to puberty and thereafter, the level decreased ($P < 0.05$) progressively just 2 weeks prior to puberty (Fig. 4). Again, plasma Fe concentration increased ($P < 0.05$) on puberty onset (week 0) and remained same up to 2 weeks postpuberty and thereafter, declined ($P < 0.05$) gradually. Higher concentration of plasma Fe was recorded in calf when compared to that of adult cattle (Van Aken *et al.* 1991). The trend in concentrations of plasma Fe during the peri-pubertal period in goats suggested that demand of Fe may be higher for more synthesis of haemoglobin and thus more oxygen transport to the growing cells for growth and development during the younger age as well as during the puberty onset. In the present study, plasma Fe registered significant positive correlations with plasma LH level during prepubertal period ($r = 0.60$, $P < 0.05$) as well as, during postpubertal period ($r = 0.99$, $P < 0.01$). These findings suggested that plasma Fe might have role on secretion of LH for bringing about puberty onset in goats.

Plasma Cu and Mn concentrations remained within a very low range without any change ($P > 0.05$) during the peripubertal period (Fig. 5). Remarkably, the higher ($P < 0.05$) concentrations of plasma Cu and Mn were noted during the time of onset of puberty. Plasma Cu registered significant positive correlations with plasma LH level ($r = 0.81$, $P < 0.01$) only during prepubertal period. The increased Cu level was recorded to be associated with higher concentrations of circulating estrogens and progesterone in sheep (Yokus *et al.* 2004). Plasma Mn showed significant positive correlations with plasma FSH level ($r = 0.60$, $P < 0.05$) during prepubertal period and with plasma LH level ($r = 0.98$, $P < 0.01$) during postpubertal period. Pine *et al.* (2005) reported that Mn could stimulate specific puberty-related hormones like FSH, LH and estradiol to facilitate the normal onset of puberty.

The mean ($\pm$ SEM) plasma FSH and LH profiles (mean $\pm$ SEM) from -26 weeks prior to puberty up to 8 weeks postpuberty are presented in Fig. 6. Plasma FSH concentrations changed ($P < 0.01$) over weeks approaching puberty. Plasma FSH concentration increased ($P < 0.05$) slowly with some fluctuations, 12 weeks prior to puberty and then it increased ($P < 0.05$) and remained high 1 week prior to puberty and thereafter, it declined ($P < 0.05$) progressively through puberty onset. The present findings

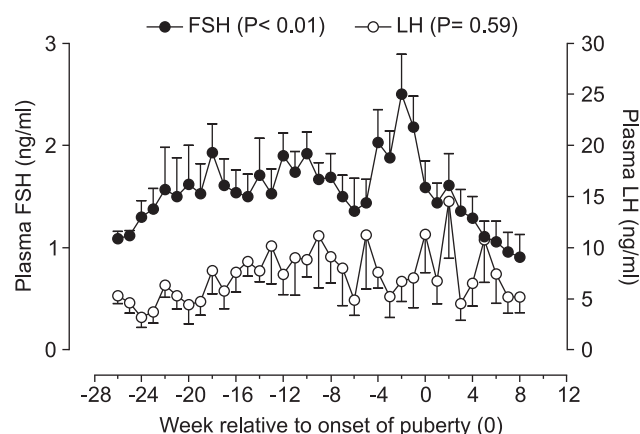


Fig. 6. Mean ($\pm$ SEM) plasma FSH and LH concentrations (μ g/ml) of female Black Bengal goats ($n = 12$) from 26 weeks prior to puberty up to 8 weeks post puberty. '0' indicates the week of onset of puberty. Blood samples were collected weekly.

confirm the earlier observations recorded in peripubertal sheep (Mahdi and Khallili 2008) and cattle (Evans *et al.* 1994). It appears that the establishment of the mature pattern of FSH secretion in female Black Bengal goats is not a sudden event, but a gradual increase during the prepubertal period.

Though the change of plasma LH concentration was not significant ($P = 0.59$) over weeks approaching puberty, there was a regular increasing trend of plasma LH concentrations with some fluctuations during the peripubertal period in female Black Bengal goats (Fig. 6). The present result agreed well with the observations in peripubertal cattle (Schillo *et al.* 1982) and buffalo (Halder and Prakash 2005). The increasing pattern of plasma FSH and LH concentrations during peripubertal period may be the result of a step-wise decreased response of the hypothalamus-pituitary axis to estradiol negative feedback action (Rawlings *et al.* 2003). As the female goats mature and enter the peripubertal phase, the reactivation of GnRH secretory networks may lead to the stimulation of the pituitary gland resulting increased concentrations of blood FSH and LH which further promote the growth and maturation of the ovarian follicles to reach to the preovulatory stage, leading to initiation of cyclic ovarian activity.

It may be concluded from the present study that female Black Bengal goats reach puberty at the age of 25.47 ± 3.84 weeks with body weight of 7.93 ± 0.87 kg, which has considerable importance in the selection of early pubertal goat breed. The significant change in plasma Zn, Fe and FSH concentrations over time, positive correlations of plasma Cu with plasma LH and plasma Mn with plasma FSH during the prepubertal period suggested that plasma Zn, Fe, Cu and Mn play a vital role in initiation of puberty in prolific Black Bengal goats.

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