Journal of Animal and Veterinary Advances 13 (12): 732-739, 2014
ISSN: 1680-5593

Patterns of Plasma Hormone Concentrations in Mithun Bulls Under a Semi-Management

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Abstract: The present study was aimed to investigate the patterns of plasma hormones including oestradiol-17β (E_2), Progesterone (P_4), Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Testosterone (T) profiles in Mithun bulls (*Bos frontalis*) kept together with females under a semi-management. Blood plasmas of 16 Mithun bulls at 1, 2, 3, 4 and 8 years were collected by jugular venipuncture, respectively. Plasma E_2, P_4, FSH, LH and T were measured by Radioimmunoassay (RIA). As plasma T levels were increasing with age and reached a peak at 8 years (11.39±0.26 ng mL^{-1}) ($p<0.01$) with significant positive correlation between plasma T concentration and bull ages ($r = 0.933$, $p<0.01$), body weight ($r = 0.726$, $p<0.01$). Plasma P_4 concentration was gradually decreasing with age and the peak of P_4 concentration was observed at 1 year (0.43±0.14 ng mL^{-1}) while it stayed the lowest level at 8 years (0.13±0.02 ng mL^{-1}) ($p<0.05$). The plasma P_4 concentrations had a significant negative relationship with age ($r = -0.519$, $p<0.05$), body weight ($r = -0.468$, $p<0.05$) in Mithun bulls. Also the plasma E_2, FSH and LH concentrations trended to decrease with age ($p>0.05$). The plasma FSH concentration had a significant positive correlation with P_4 concentration ($r = 0.621$, $p<0.01$), similar to a positive correlation between plasma LH and P_4 concentrations ($r = 0.259$, $p>0.05$). The negative correlation were found between the concentrations of plasma P_4 and E_2 ($r = -0.126$, $p>0.05$) and between T and P_4 levels ($r = -0.431$, $p = 0.057$), respectively.

Key words: Mithun (*Bos frontalis*), bull, age, hormone, venipuncture

INTRODUCTION

Mithun or gayal (*Bos frontalis*) is a rare semi-management *Bos* species (Mondal *et al*., 2005; He *et al*., 2009) believed to be the descendent of wild gaur (*Bos gaurus*) (Simoons, 1984) as a potential source of meat and milk (Mondal *et al*., 2006). Their population spread in the Northeast hill region of India and some parts of Bhutan, Myanmar, Bangladesh and China (Simoons, 1984; He *et al*., 2009; Mondal *et al*., 2010). There were a total population of 0.27 million Mithuns in India (Prakash *et al*., 2008) and some 34,000 Mithuns in Myanmar (Scherf, 2000).

In China, Mithun was named as Dulong cattle, inhabited Dulong River Valley and exclusively domesticated by Dulong tribe. Today, Dulong cattle is mainly found in the remote and dense forest along Dulong River and Nujiang River Valley in Yunnan Province, Southwestern China. It was listed in the National Preservation Record of Genetic Resource of Domestic Animal and Poultry in China and the World Watch List for Domestic Animal Diversity (Scherf, 2000). Dulong cattle was classified in an endangered status by Yunnan Livestock and Poultry Breeds due to the depletion of their population which were <100 animals in 1987 (Editorial Committee of the Breeds of Domestic Animal and Poultry in Yunnan, 1987). After the *in* or *ex-situ* conservation systems being established, its population became larger and about 3,000 animals (He *et al*., 2011; Qu *et al*., 2012).

The haemoglobin genotypes of mithun differentiated from those of zebu (*Bos indicus*) as well as the karyotype which was not same as European cattle (*Bos taurus*). However, they were considered to be similar to the gaurs (Mondal and Pal, 1999). The chromosome number of mithun was 2n = 58 which was greater than those of wild ox in Yunnan (*Bos gaurus readel*, 2n = 56) but fewer than those of zebu (*Bos indicus*) and taurine cattle (*Bos taurus*) (2n = 60) (Shan *et al*., 1980; Qu *et al*., 2012). The karyotype of generation F_1 derived from crossing

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between Mithun bull and Brahman cow was 2n = 59 with a rob (2; 28) Robertsonian translocation and F_1 showed better performances at rapid growth, wider adaptation and significant heterosis. And the hybrid female F_1 were entirely reproductive capacity and the F_1 bulls were infertile (He *et al*., 2011; Qu *et al*., 2012).

Hormones play a major role in the control of reproduction event in animal (Nanda and Sharma, 1985). The profiles of reproductive hormone in bulls had been clarified in many reports such as *Bos taurus* and its hybrid (Peirce *et al*., 1987; Miyamoto *et al*., 1989; Thompson *et al*., 1994; Pagano *et al*., 2001; Rota *et al*., 2002; Li *et al*., 2006; Kawate *et al*., 2011), *Bos indicus* and its crossbreeds (Bindon *et al*., 1976; Jimenez-Severiano *et al*., 1996) and buffalo (*Bubalus bubalis*) (Chantaraprateep *et al*., 1981; Ahmad *et al*., 1989; Shahab *et al*., 1993; Dixit *et al*., 1998; Malfatti *et al*., 2006). Reproductive hormone of Mithun cows in a normally reproductive cycle had been clarified in the previous reports (Dhali *et al*., 2005, 2006, 2007; Mondal *et al*., 2005, 2006, 2007). However, little was known on blood reproductive hormone levels in Mithun bulls according to the literatures and there is no more information concerning on E_2, P_4, FSH, LH and T concentrations in Mithun bulls kept together with females. The objective of present study was to establish the secretion pattern of E_2, P_4, FSH, LH and T at the different ages in Mithun bulls to obtain useful understandings on the fundamental regulation of reproductive hormone and the further reproductive management and breeding in Mithun.

MATERIALS AND METHODS

Experimental animal and management: The study was performed on the Dulong Cattle reserves located on the Phoenxi Mountain in Lushui County, Yunnan Province. Dulong cattle were maintained in semi-intensive condition allowing for free-grazing, free access to water and nature service under whole year. All animals mainly fed on bamboo, edible shrub and locally available grasses and leaves. Salt additives were given every 7-15 days during an experiment. The 16 mithun bulls were selected from the herd maintained on the Dulong Cattle Reserves of Phoenxi Mountain. The 16 Mithun bulls in the different ages (1, 2, 3, 4 and 8 years) were divided into 5 age groups with body weights (mean±SE) of bulls were 151.74±11.88, 218.73±8.88, 282.78±24.49, 327.25±18.28 and 446.70±47.23 kg, respectively. Mithun bulls were free-grazing with females during the investigation.

Blood sampling: Blood samples of the testing bulls were collected in April and June by jugular venipuncture and kept in the heparinized tubes (20 IU of heparin/mL of blood). All blood samples collected within 30 min were centrifuged at 2,500 rpm min^{-1} for 10 min. The plasma samples separated were rapidly labeled, frozen and stored at -20°C for hormone analysis.

Hormonal assay: The levels of plasma oestradiol-17β (E_2), progesterone (P_4), Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Testosterone (T) concentrations were measured in this experiment by Yu and Chen (1997), Dixit *et al*. (1998) and Xie (2005) the kit manuals. And the levels of plasma E_2, P_4, LH, FSH and Testosterone (T) were assessed by GC-1200 Gamma Radioimmunoassay Counter (USTC Chuanxin Co., Ltd.) and commercially available Radioimmunoassay (RIA) kit (Shenzhen Larewen Biomedical Engineering Technology Co., Ltd.) which were Iodine [^{125}I] Luteinizing Hormone RIA kit, Iodine [^{125}I] Estradiol RIA kit, Iodine [^{125}I] Follicle Stimulating Hormone RIA kit, Iodine [^{125}I] Testosterone RIA kit and Iodine [^{125}I], Progesterone RIA kit, respectively. The intra and inter-assay coefficients of variation, the sensitivity and range of hormone kit were showed in Table 1.

Statistical analysis: The data are described as means±SE. The basal E_2, P_4, FSH, LH and T concentrations were analyzed by means of repeated measure ANOVA in SPSS 13.0 procedure. The Duncan multiple-range test was used to detect the significant differences among means. To assess the correlation between the parameters presented in Table 2, the partial correlation analysis was performed according to the procedure of SPSS. All the results were considered at the significant levels of 5 or 1%.

Table 1: The intra- and inter-assay CV, the sensitivity and range of hormone kit

Hormone kit	^{125}I E_2 RIA kit	^{125}I LH RIA kit	^{125}I FSH RIA kit	^{125}I P_4 RIA kit	^{125}I T RIA kit
Intra-assay CV (%)	6.06	6.70	4.32	4.85	5.92
Inter-assay CV (%)	7.86	8.27	6.98	7.84	8.68
Assay sensitivity	0.5 (pg mL^{-1})	0.4 (mIU mL^{-1})	0.4 (mIU mL^{-1})	0.02 (ng mL^{-1})	0.2 (ng dL^{-1})
Range	0~1.000 (pg mL^{-1})	0~200 (mIU mL^{-1})	0~200 (mIU mL^{-1})	0~80 (ng mL^{-1})	0~1.600 (ng dL^{-1})

Table 2: Pearson correlation matrix among hormone, age and body weight in Mithun bulls

Mithun bulls	Bull age	E_2	P_4	FSH	LH	T
E_2	-0.138					
P_4	-0.519*	-0.126				
FSH	-0.113	0.202	0.621**			
LH	-0.282	0.095	0.259	0.076		
T	0.933**	-0.267	-0.431	-0.150	-0.170	
Body weight	0.844**	0.131	-0.468*	0.013	-0.295	0.726**

*, **Represented the significant differences at the levels of 5 and 1%, respectively

RESULTS AND DISCUSSION

The alteration of E_2 concentration at the different ages in Mithun bulls: The mean of plasma E_2 concentrations at the different ages in Mithun bulls were shown in Fig. 1a. E_2 concentration was increasing when the age increased. The peak of E_2 concentration was observed at 3 years (6.30±2.58 pg mL^{-1}) and thereafter gradually decreased to the lowest level at 8 years (2.38±0.87 pg mL^{-1}) that was lower than that at 1 year (2.71±1.08 pg mL^{-1}). There were no significant differences of the plasma E_2 concentration among the ages of bulls ($p>0.05$). There was not significantly negative correlation to Mithun bulls between E_2 concentrations and bull ages ($r = -0.138$, $p>0.05$), plasma E_2 and P_4 ($r = -0.126$, $p>0.05$), E_2 and T ($r = -0.267$, $p>0.05$), respectively (Table 2).

The alteration of plasma P_4 concentration at the different ages in Mithun bulls: The plasma P_4 concentrations at the different Mithun bull ages were described in Fig. 1b. The plasma P_4 concentration was gradually decreasing with age. The peak of P_4 concentration was observed at 1 year (0.43±0.14 ng mL^{-1}), the lowest level at 8 years (0.13±0.02 ng mL^{-1}) ($p<0.05$). The basal plasma P_4 concentrations had significant negative correlations with bull ages ($r = -0.519$, $p<0.05$), body weight ($r = -0.468$, $p<0.05$) and plasma T concentration ($r = -0.431$, $p = 0.057$), respectively (Table 2). There was significantly positive correlation between concentrations of P_4 and FSH ($r = 0.621$, $p<0.01$) (Table 2).

The alteration of FSH concentration at the different ages in Mithun bulls: The plasma FSH concentrations at the different Mithun bull ages were shown in Fig. 1c. The FSH concentration maintained constantly with minor fluctuation whichever age. The peak of FSH concentration reached at 4 years (0.78±0.33 mIU mL^{-1}) then gradually decreased to the lowest level at 8 years (0.53±0.14 mIU mL^{-1}). No significant difference of FSH concentrations was observed whichever age in Mithun bulls ($p>0.05$). There was a slightly negative correlation between plasma FSH level and bull ages ($r = -0.113$, $p>0.05$). Moreover, the plasma FSH concentration had a significantly positive correlation with P_4 concentration ($r = 0.621$, $p<0.01$) (Table 2).

The alteration of LH concentration at the different ages in Mithun bulls: The plasma LH concentrations at the different age groups in Mithun bulls were demonstrated in Fig. 1d. The plasma LH concentration was peaked at 2 years (3.12±0.80 mIU mL^{-1}) and slowly decreased when the age increased ranged from at 3-8 years (1.57-1.98 mIU mL^{-1}). No significant difference between LH concentration and bull ages were observed ($p>0.05$). There was a negative correlation between the plasma LH concentration and bull ages ($r = -0.282$, $p>0.05$) while a positive correlation was found between the concentrations of LH and P_4 ($r = 0.259$, $p>0.05$), respectively (Table 2).

The alteration of plasma T concentration at different ages in Mithun bulls: The plasma T concentrations at different Mithun bull ages were shown in Fig. 1e. The T concentration was increasing when the ages increased and reached one peak at 8 years (11.39±0.26 ng mL^{-1}) which was significantly higher than those at the other ages ($p<0.01$). Especially, the T concentration at 8 years in Mithun bulls was 13.1 times higher than that at 1 year in Mithun bulls. Furthermore, the plasma T concentration showed a significantly positive relation with both bull ages ($r = 0.933$, $p<0.01$) and body weight ($r = 0.726$, $p<0.01$) but had a negative correlation with P_4 levels ($r = -0.431$, $p = 0.057$) (Table 2).

The alteration of T concentration at the different ages in Mithun bulls: Testosterone controls a large suite of male-typical behaviors and morphological characteristics (Longpre and Katz, 2011) which generated an important function of male reproductive physiological animals on developing and maturity of procreative organ, keeping the second male characters, promoting sperm generation, growth and function of accessory sex gland, sexual behavior and adjusting substance metabolizing (Dixson, 1998). The relationship between T concentration and reproductive competition of male animals was observed on mating motivation, kingdom behavior, attacking behavior and showing off behavior (Wingfield *et al*., 1990; Dixson, 1998). However, high T concentrations may costly be energetically or even detrimental to survival (Pierce and Parsons, 1981). It was found that Plasma T concentration had the significant differences from cattle breeds, ages, sexes and seasons (Chantaraprateep *et al*., 1981; Peirce *et al*., 1987; Miyamoto *et al*., 1989; Shahab *et al*., 1993;

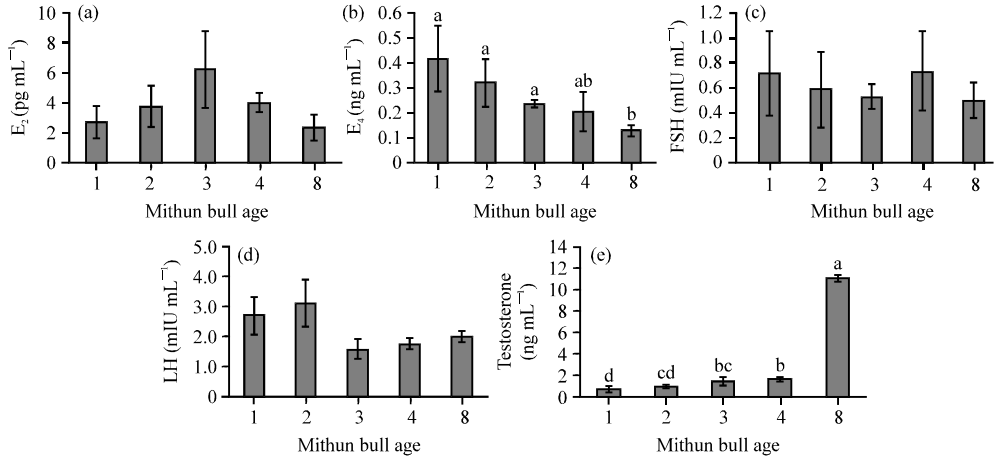

Fig. 1: The patterns of a) E_2; b) P_4; c) FSH; d) LH and e) and Testosterone concentration at the different ages in Mithun bulls. The numbers of abscissa in Fig. 1 denoted the ages at 1 = (n = 4), 2 = (n = 4), 3 = (n = 4), 4 = (n = 4) and 8 years = (n = 4) in Mithun bulls, respectively

Thompson *et al*., 1994; Jimenez-Severiano *et al*., 1996; Dixit *et al*., 1998; Rota *et al*., 2002; Li *et al*., 2006; Malfatti *et al*., 2006; Kawate *et al*., 2011). Especially, T concentration in *Bos taurus* and its crossbreeds were higher than those in *Bos indicus* and its crossbreeds and in River buffalo (*Bubalus bubalis*) higher than in Swamp buffalo. T concentration in Mithun bulls at 1-2 years were lower than that in Holstein bulls of 10-18 months of age (3.0-4.5 ng mL^{-1}) (Peirce *et al*., 1987; Rota *et al*., 2002), Japanese Black bulls (Kawate *et al*., 2011), 1 year three way crosses of *Bos taurus* breeds bulls (0.9-19.13 ng mL^{-1}) (Thompson *et al*., 1994) and 12-18 months Nili-Ravi buffalo bulls (1.3-3.3 ng mL^{-1}) (Shahab *et al*., 1993), higher than that in 1-2 years Swamp buffalo bulls (0.10-0.55 ng mL^{-1}) (Chantaraprateep *et al*., 1981) and 1 year zebu crossbred bulls (0.10-0.15 ng mL^{-1}) (Jimenez-Severiano *et al*., 1996). T concentration at 3-4 years in Mithun bulls was also lower than those at 4 years in Limousin (2.31-18.74 ng mL^{-1}) and Charolais (4.42-12.44 ng mL^{-1}) (Li *et al*., 2006) and the approach of 3.6-4.7 years Mediterranean buffalo bulls (0.79-3.20 ng mL^{-1}) (Malfatti *et al*., 2006). T concentration at 8 years in Mithun bulls was higher than that at 7-8 years in Murrah bulls (0.19-2.99 ng mL^{-1}) (Dixit *et al*., 1998).

Changing patterns of T concentration in Mithun bulls with age changes were observed in accordance with the models of Holstein and buffalo bulls (Peirce *et al*., 1987; Ahmad *et al*., 1989; Wingfield *et al*., 1990; Rota *et al*., 2002). However, the trend of T levels in Mithun bulls rapidly increased with bull ages increasing showing that T levels rapidly grew up than that in buffalo bulls which was possibly related to the cattle breeds or wild-living style.

The plasma T concentration in adult buffalo, Limousin, Charolais and crossbred bulls were observed to be obvious fluctuation (Thompson *et al*., 1994; Dixit *et al*., 1998; Li *et al*., 2006; Malfatti *et al*., 2006). The ranges of T concentration at 1, 2, 3, 4 and 8 years in Mithun bulls were 0.89-1.32, 0.52-1.31, 0.95-2.3, 1.55-2.12 and 10.89-11.90 ng mL^{-1}, respectively. The plasma T concentration was observed to be large fluctuation at 1-3 years, the minor fluctuation at >4-8 years of individual bulls which was just at growth period and significantly difference at 1-3 years individual bulls. Some reports showed that T concentration in bulls was influenced along with season changes. T concentrations at 4 years in Limousin and Charolais bulls in Spring and Autumn were 15.57 and 8.22 ng L^{-1}, significantly higher than those in Summer (4.15 ng L^{-1}) and Winter (3.36 ng L^{-1}), respectively (Li *et al*., 2006). Serum T concentrations at 3.6-4.7 years in Italian Mediterranean buffalo bulls in Spring (2.05 ng mL^{-1}) and Summer (2.11 ng mL^{-1}) were significantly higher than those in Autumn (1.23 ng mL^{-1}) and Winter (1.19 ng mL^{-1}). As a result of decline on synthesis of plasma testosterone was adjusted due to high temperature in Summer (Malfatti *et al*., 2006). In the study, plasma T concentrations at 3-4 years in Mithun bulls in Spring and Summer was lower than those at 4 years in Limousin and Charolais bulls (Li *et al*., 2006) in same season and the approach with 3-5 years buffalo bulls (Malfatti *et al*., 2006). The season changes with low temperature (The average daily temperature ranged from 11.47-16.85°C in Summer) maybe did not researh on T concentration changes in Mithun bulls collected from April to June when Mithun population freely moved and grazed to highland in the forest (Elevation ranged from

2,400-3,500 m). However, the pattern of T concentration of bulls needs to disclose in the different seasons later.

Malfatti *et al.* (2006) reported that T concentration with mixed or detached bulls and cows distinctly changed in different seasons especially T concentration with disjoining bull and cow in Autumn and Summer were significantly lower than those in bulls following the pubescent cows, however, T levels did not differ among the individual bulls following cows. The management of the contact with females affected testosterone values. In this study, T concentration of individual bulls were found minor influence with mixed grazing under the whole year which may be related to cow sexual attraction or bull properly displaying sexual behavior. It indicated the difference of T levels in bulls might be related to the different breeds, environment, feeding and management.

The alteration of plasma E_2 and P_4 concentrations at the different ages in Mithun bulls: To the knowledge, few papers described blood E_2 (Shahab *et al.*, 1993) and P_4 (Thompson *et al.*, 1994) levels in bulls. Shahab *et al.* (1993) reported overall E_2 concentrations significantly varied between the different age groups (12, 18 and 52 months were 8.5, 4.1 and 9.4 pg mL^{-1}, respectively) and suggested its role in the activation of hypothalamic-pituitary axis during sexual development in the male buffalo. Herein, the plasma E_2 concentration did not significantly differ among the different age groups in Mithun bulls, indicating plasma E_2 concentration at the different ages in Mithun bulls generally displayed less fluctuation. The differences were still unclear from cattle breeds, management, growth development and body maturation. However, the information concerning the function of estrogens in the bull is available. Interestingly, a phenomenon was observed that a decline of average E_2 level in Mithun bulls ranged from 4-8 years which E_2 was accompanied by an increase in plasma T concentrations.

The determination of ontology of estrogen receptors in the hypothalamus and anterior pituitary might be helpful for an understanding of the physiological role of peripheral estrogen concentrations during sexual maturation (Shahab *et al.*, 1993). The present results provided the information about the pattern secretion of estradiol at the different ages in Mithun bulls and its physiological role in the process of sexual maturation. It was suggested that the concentrations of estradiol constituted the components of the feedback mechanism of T secretion in the developmental cycle in Mithun bulls.

P_4 concentrations varied widely (ranged from 21-1070 pg mL^{-1}) in three-way crossbreeds of *Bos taurus* breeds bull at 1 year (Thompson *et al.*, 1994). P_4 concentrations were the highest to the yearly Mithun bulls, ranged from 0.20-0.78 ng mL^{-1} which were not similar to the ranges of P_4 levels fluctuation at 1 year in three-way crossbred bulls, related to level off on breeding environment and feeding (Thompson *et al.*, 1994). But no more documents about P_4 levels at other ages in bulls were available under breeding management, except for P_4 concentration to the yearly bull by Thompson *et al.* (1994).

Researchers found that plasma P_4 concentration in Mithun bulls significantly declined with age indicating that P_4 concentration expressed distinctly diversity among the different ages in bulls, especially the age near to adult showing lower P_4 levels. P_4 was considered as a synergic effect with FSH and antagonistic effect with T when bulls were growing in the investigation, displaying that a high T level inhibited the secretion of P_4, FSH and LH.

The alteration of FSH and LH concentrations at the different ages in Mithun bulls: The function of FSH and LH cooperate for the process of sperm's development and maturity in male animals (Gharib *et al.*, 1990). Serum FSH levels were significant higher at 2 months than those at 1 and 3-8 months and LH levels were higher at 3 months than those at other ages in beef cattle bulls (Miyamoto *et al.*, 1989). FSH is the principal regulator of inhibin production in the testis during the 1st 5 months of age in bulls, testosterone might play a dominant role in suppression of FSH from the pituitary after the onset of puberty (Miyamoto *et al.*, 1989). FSH level in adult (7-8 years) Murrah buffalo bulls was 1.66 ng mL^{-1} (ranged from 0.95-3.61 ng mL^{-1}) whereas the LH levels was 3.33 ng mL^{-1} in peripheral circulation (ranged from 0.92-9.91 ng mL^{-1}) which displayed the parallelism in FSH and LH secretion pattern. It might be due to the presence of appreciable amounts of peripheral inhibin (Dixit *et al.*, 1998). FSH levels of Holstein bulls at 18 months was the highest in Autumn (27.3 ng mL^{-1}), lowest in Spring (8.0 ng mL^{-1}) and the concentrations of LH and FSH were positively correlated in Holstein bulls (Peirce *et al.*, 1987).

In this study, secretion pattern of plasma FSH at the different age groups in Mithun bulls were described to be stayed lower in 1-3 years then gradually increased from at 4 years, once decreased at 8 years. The plasma FSH levels showed a slight fluctuation at the different ages. And whole FSH levels trended to decline with bull ages increasing. A significantly positive correlation was observed between FSH and P_4 at the different ages in Mithun bulls but any relationship was absence between FSH and LH. The results were not accord with the patterns in Holstein bulls (Peirce *et al.*,1987) and buffalo

bulls (Dixit *et al*., 1998) which had fully differences from secreting rules of FSH between *Bos taurus*, *Bubalus bubalis* and *Bos frontalis* to bulls. Here, positive correlation was identified between FSH and P_4 in Mithun bulls. And bulls grew up and strengthened the production of T secretion to inhibit the limited secretion of P_4, FSH and LH in some content.

The secretion pattern of LH concentration exhibited slight fluctuation at the different ages in Mithun bulls. A negative feedback mechanism was also inferred between LH and T hormone in agreement with the previous research in Swamp buffalo bulls (Chantaraprateep *et al*., 1981). There were not significant differences from plasma LH pattern associated with the different ages in Mithun bulls. There existed some marked divergences between LH and FSH to the individual bulls at the different ages which was possibly related to the body size in Mithun bulls. The variations of FSH and LH concentrations depended on species, breeds, ages and seasons (Bindon *et al*., 1976; Chantaraprateep *et al*., 1981; Peirce *et al*., 1987; Miyamoto *et al*., 1989; Jimenez-Severiano *et al*., 1996; Dixit *et al*., 1998; Li *et al*., 2006). The changes of FSH and LH levels in Mithun bulls must connect with its sexual behavior, mating frequency in the wild.

CONCLUSION

In Mithun bulls, the level of plasma T concentration significantly increased with age while the levels of FSH, LH, E_2 and P_4 concentrations decreasing. Meanwhile, plasma T level distinctly increased with body weight increasing in Mithun bulls while P_4 decreasing. According to the hormone changes, the optimal breeding ages were ranging from 4-8 years in Mithun bulls based on the profiles of E_2, LH and T administration with the important functions on sexual maturity, development and body maturation. Then, plasma T levels could offer potential information on selection and breeding in Mithun bulls.

There was a mutual effect between P_4 and FSH. But no significant correlations were found between FSH and LH and negative effects between plasma E_2 and P_4, E_2 and T, P_4 and T, respectively. It indicated that the concentrations of E_2, FSH, LH and P_4 in prepubertal bulls kept higher levels played a key action on sexual development. And T hormone did research the leading function on reproduction in adult bulls. Overall, it was newly suggested the various plasma concentrations of E_2, FSH, LH, P_4 and T and their relationships at the different ages in Mithun bulls for the first time.

ACKNOWLEDGEMENTS

Researchers wish to thank Yang, S.H. and M. Yang for the fieldwork and guide. Gratitude is also expressed to Mr. Li, Z.L., W.Z. Ma, Y.H. San, Z.Y. Ying and X.Y. Tu for extending help to this research. The research was supported by the Ecosystem Approaches to the Better Management of Zoonotic Emerging Infectious Diseases in the Southeast Asia Region, Technological Innovation and Training Innovative Talents Program, Yunnan Province (No. 2010CI082), Sci-Tech Tackle Key Project and High-Tech Development Program, Yunnan Province (No. 2006NG23), the General Program of Applied Basic Research, Yunnan Province (No. 2006C0068M) and MATS-Beef Cattle System of China (No. CARS-38).

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