Reproductive Hormone Concentrations and Their Correlation of Different Physiological Stages in Dulong Cows (*Bos frontalis*)

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Abstract: Plasma hormone levels were conducted through radioimmunoassay from grazing Dulong cows. Two surges of E_2 profile were found on standing estrous (18.56±7.87 pg mL^{-1}) and on days 240-270 of pregnancy (11.85±3.45 pg mL^{-1}), the lowest on days 5-10 of post-copulation ($p<0.05$). P_4 concentration was found the highest on days 5-10 of post-copulation (9.35±2.84 ng mL^{-1}) ($p<0.01$) and the lowest on standing heat (0.29±0.13 pg mL^{-1}), significantly lower than that during gestation ($p<0.05$). A biphasic FSH surges were observed during estrous forepart (2.03±0.54 mIU mL^{-1}) and on days 2-30 of postpartum (2.23±0.61 mIU mL^{-1}). Surge LH concentration reachedon days 5-10 of gestation (10.21±9.04 mIU mL^{-1}). Surge T level was found on days 240-270 of pregnancy, significantly higher than those in other PPSs ($p<0.01$). The significant positive correlations were found between PPSs and plasma FSH ($r = 0.34$, $p<0.05$), P_4 ($r = 0.31$, $p<0.05$), T ($r = 0.48$, $p<0.01$) concentrations, respectively, also between P_4 and LH ($r = 0.31$, $p<0.05$), T and FSH ($r = 0.40$, $p<0.01$) while a negative correlation was between plasma E_2 and P_4 ($r = -0.29$, $p<0.05$). In conclusion, Dulong cows were found with two surges of hormone E_2 and FSH and a single surge of hormone P_4 and T. There were increasing at levels of FSH, P_4 and T, the synergistic relationships between P_4 and LH, T and FSH, the antagonistic relationship between E_2 and P_4, were firstly reported in Dulong cows during PPSs.

INTRODUCTION

Mithun or gayal (*Bos frontalis*), is prized as a semi-wild ruminant species[1, 2], domesticated >8,000 year and is presumed to be the domestic form of wild gaur (*Bosgaurus*)[3]. They are docile and have massive body size[4], a potential source of meat[5]. The population is mainly spread in the northeast hill region of India and Myanmar, Bhutan, Bangladesh, China and Malaysia[1, 3, 6, 7].

In China, mithun was named as “Dulong cattle”, due to inhabited Dulong River valley and firstly domesticated by Dulong people, also called “Da’e cattle” which possess a broader frontal and flattened forehead. The extant Dulong cattle inhabit in the remote and dense forests along Dulong River and Nujiang River in the Northwestern Yunnan, China. The chromosome number of Dulong cattle is 2n = 58, more than those of Yunnan bioson (*Bos gaurus* readel, 2n = 56) but fewer than those of zebu (*Bos indicus*, 2n = 60) and yellow cattle (*Bos taurus*, 2n = 60)[8, 9]. The population size was once <100 heads in 1987[10] and reached 3,950 heads in 2016. It was listed in the “World Watch List for Domestic Animal Diversity”[11].

Hormones play a major role in the control of reproductive events in animals[12]. The reproductive hormone in a normal reproductive cycle or during gestation in bovine species cows have been clarified in Holstein (*Bos taurus*)[13-17], zebu (*Bos indicus*)[18], yak (*Bos grunniens*)[19, 20], buffalo (*Bubalus bubalis*)[12, 21, 22] and Brown Swiss cows[23]. Some works had been investigated in reproductive hormone including oestradiol-17β (E_2), Progesterone (P_4), Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) of mithun cows in estrus cycle[1, 5, 24-27] and in mithun bull[28]. However, there are no available information regarding to the role of E_2, P_4, FSH, LH and Testosterone (T) in pregnant Dulong or mithun cows. Dulong cow possesses well reproductive performance under a semi-wild condition where the remoteness of their habitats and ecological and other factors and the endocrine regulations on reproductive function has not been sufficiently documented or Dulong cattle remain one of the least-studied ungulates.

The objective of study was to investigate the patterns of plasma E_2, P_4, FSH, LH and T during estrus cycle, gestation and postpartum and the relationship between hormone concentrations and reproductive behavior in Dulong cows. The findings from this study might be useful for understanding the fundamental regulation of those reproductive hormones and for further reproductive management in Dulong cattle.

MATERIALS AND METHODS

Experimental animals and herd management: The study was performed at the Dulong Cattle Conservation Farm located on the Phoenix Mountains in Lushui County, Yunnan Province. Dulong cattle were maintained in semi-intensive condition, allowing for free grazing and natural service. All animals fed on bamboo, edible shrub and locally available green grasses and leaves. Mineral blocks were available during the experimental period. Forty-six healthy Dulong cows with ages and body weights ranging from 2.5-13 year and 218-424 kg, respectively were used in the experiment. The estrous signs and mating behaviors were daily observed and recorded throughout the study.

Blood sampling: According to the Progenitive Physiological Stages (PPSs) including non-pregnacy (3 groups), gestation (5 groups) and postpartum (1 group), 46 Dulong cows were divided into 9 groups, viz. anestrus (n = 7), preoestrus (n = 3), standing heat (n = 8), 5-10 days of post-copulation (n = 4), 60-90 days (n = 9), 120-150 days (n = 4), 180-210 days (n = 4) and 240-270 days (n = 4) of pregnancy and postpartum 2-30 days (n = 3).

Blood samples were collected by jugular venipuncture and kept in the heparinized tubes (20 IU of heparin/mL of blood) for further determination of plasma E_2, P_4, FSH, LH and T concentrations. Blood samples collected were centrifuged at 3,500 rpm for 10 min. The plasma samples were rapidly labeled, frozen and stored at -20°C pending analysis.

Hormonal assay: Plasma oestradiol-17β (E_2), Progesterone (P_4), Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Testosterone (T) concentrations were analyzed in this experiment. According to the kit statements and previous methods[19, 22, 29], the levels of plasma E_2, P_4, LH, FSH and T were assessed by GC-1200 Gamma Radioimmunoassay Counter (USTC Chuanxin Co. Ltd.) and commercially available Radioimmunoassay (RIA) kit (Shenzhen Larvwen 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 (CV), the sensitivity and range of hormone kit were showed as the previous work[28].

Statistical analysis: The data were expressed as mean±Standard Error (SE). The E_2, P_4, FSH, LH and T concentrations were analyzed by the means of repeated measure ANOVA in SPSS 19.0 procedure. The Duncan’s multiple-range test was used to confirm the significant differences. To assess the correlation between the parameters presented in Table 1, the partial correlation analysis was performed (SPSS, 2010). All results were considered significant differences at the levels of 5% ($p<0.05$) and 1% ($p<0.01$).

Table 1: Simple relationship of hormone E_2, P_4, FSH, LH,T of PPSs in Dulong cows

Relationship	PPSs	E_2	P_4	FSH	LH
E_2	0.02				
P_4	0.31*	-0.29*			
FSH	0.34*	0.11	-0.17		
LH	-0.03	-0.14	0.31*	-0.08	
T	0.48**	0.16	0.08	0.40**	-0.02

*, ** indicated significant differences at the levels of 5% (p<0.05) and 1% (p<0.01), respectively

RESULTS

The alteration of plasma E_2 concentration in the different PPSs: The plasma E_2 concentrations in the different PPSs were shown in Fig. 1a. Two surges of E_2 were observed during PPSs. The first surge was found in standing heat (undertaking bull mounting) (18.56±7.87 pg mL^{-1}) which was significantly higher than those in anestrus and on days 5-10 of post-copulation ($p<0.05$). The second surge showed on days 240-270 of pregnancy (11.85±3.45 pg mL^{-1}). E_2 concentration during the pregnancy slightly tended to increase, then slowly decreased on parturition until days 2-30 of postpartum periods ($p>0.05$). There was no significant difference of E_2 concentration during days 60-270 of the pregnancy in Dulong cows. A negative correlation was found between the concentrations of plasma E_2 and P_4 ($r = -0.29$, $p<0.05$) (Table 1).

The alteration pattern of plasma P_4 concentration in the different PPSs: The plasma P_4 concentrations on different PPSs were described in Fig. 1b. Surge P_4 concentration was found on day 5-10 of post-copulation (9.35±2.84 pg mL^{-1}) which was significantly higher than those of the other PPSs ($p<0.01$). The lowest P_4 concentration fell during the period of standing heat (0.29±0.13 pg mL^{-1}) which was significantly lower than those of day 5-10 of post-copulation, days 60-90 and 180-270 of gestation ($p<0.05$). The P_4 concentration during the gestation (except days 120-150 of gestation) was significantly higher than those in anestrus, preoestrus and standing heat ($p<0.05$). The plasma P_4 concentration showed a positive correlation with the different PPSs ($r = 0.31$, $p<0.05$) and provided the significant positive correlation with plasma LH ($r = 0.31$, $p<0.05$) (Table 2).

Changes of plasma FSH concentration in the different PPSs: The plasma FSH concentrations in the different PPSs of Dulong cows were depicted in Fig. 1c. A biphasic FSH surges were observed during the periods of preoestrus (2.03±0.54 mIU mL^{-1}) and days 2-30 of postpartum (2.23±0.61 mIU mL^{-1}). The maximum concentration of plasma FSH was kept during days 2-30 of postpartum which was significantly higher than those in anestrus and standing heat ($p<0.05$). In Table 2, there was a significantly positive correlation between the plasma FSH concentration and PPSs ($r = 0.34$, $p<0.05$). Plasma FSH level had also a markedly positive correlation with T ($r = 0.40$, $p<0.01$).

The alterations of plasma LH concentration in the different PPSs: The plasma LH concentrations in the different PPSs were shown in Fig. 1d. The surge of LH concentration was observed during days 5-10 of gestation (10.21±9.04 mIU mL^{-1}) followed by gradually decreasing on the late-gestation. In Table 1, there was a distinctly positive relationships between the LH concentration and P_4 concentration ($r = 0.31$, $p<0.05$).

The alterations of plasma T concentration in the different PPSs: The plasma T concentrations in the different PPSs were shown in Fig. 1e. Three surges of T were observed on the preoestrus (0.11±0.04 pg mL^{-1}), days 120-150 (0.17±0.04 pg mL^{-1}) and 240-270 (0.30±0.03 pg mL^{-1}) of gestation. The plasma T concentration during days 240-270 of gestation was significantly higher than those of the other stages ($p<0.01$) and the plasma T level on days 120-150 of pregnancy was significantly shown higher than those of anestrus, standing heat, days 5-10 of post-copulation, days 60-90 of gestation and days 2-30 of postpartum ($p<0.01$). The plasma T concentration on days 180-210 of gestation was significantly higher than those on preoestrus, days 5-10 of pregnancy and days 2-30 of postpartum ($p<0.05$). For Dulong cows in the different gestation periods, the plasma T concentration increased from days 5-10 to 60-90 followed by sharply increasing on days 120-150 and decreasing on days 180-210 then sharply increasing to the surge on days 240-270 of gestation. In Table 1, plasma T had a significantly positive correlation with different PPSs of cows ($r = 0.48$, $p<0.01$) and FSH ($r = 0.40$, $p<0.01$) and E_2 ($r = 0.16$, $p>0.05$), respectively.

The alterations of plasma P_4 and T ratio in the different PPSs: In different stages of reproductive physiology, ratio values of Progesterone (P_4) and Testosterone (T) (P_4/T) were shown 28.50, 27.91, 9.67, 467.50, 109.75, 18.00, 40.55, 15.13 and 150.00, respectively for stages of 1-9 which were obvious changes and the lowest values in stage 3, the highest value in stage 4 and began to decline from stage 5 and dropped to the lowest level in stage 8. The results also showed that the ratio of P_4 and T played an important regulative role in different reproductive physiological stage of Dulong cows.

Effect of hormone concentrations on expression of reproductive behavior in Dulong cows: The relationship between sexual behavior and hormone levels were shown as follows:

- The estrous behaviors in cows were characterized as frequently urination, tension and mounting each other when plasma FSH and T levels were becoming higher with stage 2 or preoestrus
- The sexual behaviors of cows such as: approaching bulls, raising tail, undertaking bull mounting and mating were shown when plasma E_2 level was the highest while plasma P_4, LH, FSH and T levels were lower with stage 3 or standing heat

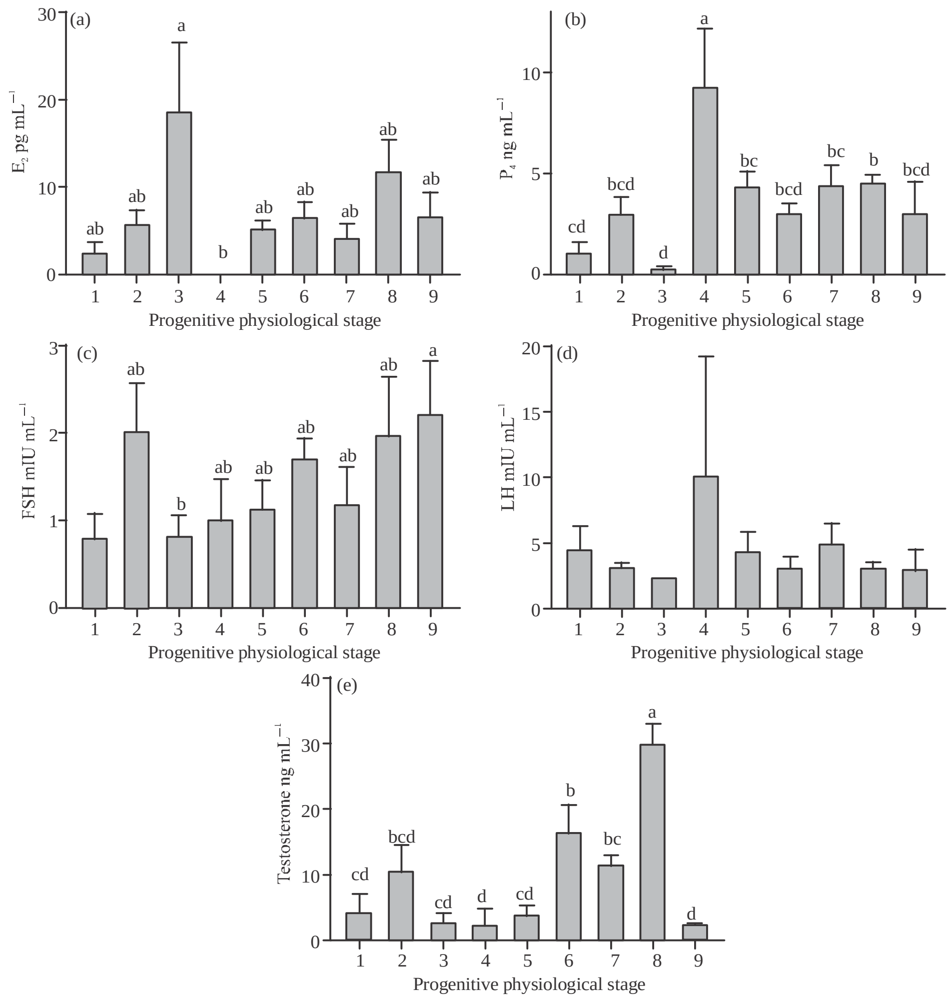

Fig. 1(a-e): Hormone patterns of nine PPSs in Dulong cows. The number 1-9 on the abscissa were listed for the stages: 1. anestrus, 2. preoestrus, 3. standing heat, 4. 5-10 days of post-copulation; 5. 60-90 days of pregnancy, 6. 120-150 days of pregnancy, 7. 180-210 days of pregnancy, 8.240-270 days of gestation; 9.2-30 days of postpartum, respectively. The different lowercases in the same figure represented significant at the level of 5% ($p<0.05$) and no significant differences with unsigned letters ($p>0.05$)

- The false heat could be found when plasma E_2 and T concentrations were higher during late-pregnancy (240-270 days of gestation)

DISCUSSION

During the estrous cycle, a surge of plasma E_2 concentration was found in Dulong cows in this study, like Brahman cows[18] and swamp buffalo[22]. Herein, the E_2 level of Dulong cows at preoestrus was higher than those of anestrus and standing heat with an E_2surge of 18.56 pg mL^{-1}. A significant negative relationship was found between E_2 and P_4, in agreement with those in mithuncows[25, 26]. E_2 surge on the onset of standing heat in Dulong cows differentiated from those of buffalo and yellow cattle[22] which were similar to Holstein[14], Brahman[18], yak[19] and mithun[25]. The plasma E_2 levels in Dulong cows at standing heat was significantly higher than in Brahman (0.09 pg mL^{-1})[6] and yellow cattle[22], indicating that the E_2 changes related to the different species in estrous cycle. The plasma E_2 in Dulong cows remained the lowest concentration during the early pregnancy (days 5-10 of post-copulation), similar to Holstein of ET during the first 20-day of gestation[30] and Brahman at day 7 after AI[6]. E_2 level in Dulong cows was tended to increase throughout the mid-to late-gestation, coincided with the cases in Holstein[15, 30] and buffalo[21] and the change of E_2 levels did not significantly during the pregnancy period. In this experiment, lower E_2 level was found on initial pregnancy period which might be related to pregnant luteinisation, high LH secretion and improving P_4 from luteal secretion, limiting follicle development and E_2 secretion. It indicated endocrine condition of low E_2 level, high LH and P_4 levels in pregnant forepart maybe was an important assurance to normal development of embryos and implantation. The secretion pattern of E_2 was similar to T, especially, both reached to the highest surge during the last gestation.

Plasma P_4 concentration in Dulong cows was higher on preoestrus than that of anestrus. The lowest P_4 level occurred on standing heat and the plasma P_4 patterns with distinctly fluctuation in Dulong were similar to other findings in dairy cows[14, 31], yak[19], Brahman cow[6] and mithun cows[5, 25, 26]. Plasma P_4 level of Dulong cows in preoestrus showed an increase and higher than in anoestrus which was maybe related to lower E_2 level and relatively high FSH, LH and T concentrations. P_4 level of Dulong cows in preoestrus was close to buffalo, lower than in yellow cattle[22], indicating estrous and ovulation of Dulong cows was mainly decided by increasing E_2 concentration, decreasing P_4 concentration with LH and E_2 balancing. But, it is not very clear the mechanism of T hormone, especially its vital functions on estrous cows. Progesterone action was via the endometrium of the uterusis critical for concept us growth and elongation[32-34]. The concentration of P_4 in Dulong cows was the highest on days 5-10 after mating (9.35 pg mL^{-1}), matching the models in buffalo[21] and Holstein[16] which showed significantly higher than those of during days 60-270 of gestation and post-parturition. Whereas, plasma P_4 levels were slightly fluctuating, ranged from 3.06-4.54 pg mL^{-1} during gestation in Dulong cows while there were like the situations in the lactation period of dairy cows in milk[35] or in plasma[16, 36] but differentiated from crossbred zebu[37]. Previous report showed that P_4 secretion in bovine could limit the LH release[38]. There was a cooperation relationship between P_4 and LH in Dulong cows. Ratio of E_2 and P_4 was a value of 64 which was the highest at natural service in Dulong cows, kept 0.90-2.60 pg mL^{-1} on days 60-270 of pregnancy and 2.18 pg mL^{-1} on postpartum. It displayed normal sexuality and round pregnancy in Dulong cows depended on holding a proper ratio of E_2 and P_4. The findings supported that high P_4 constant during gestation be responsible for meeting pregnancy in Dulong cows.

FSH and LH promote follicular development, follicular maturation and ovulation[25]. In this study, FSH surge in Dulong cows appeared on preoestrus and then declined to the basal level of anestrus. And FSH levels during estrous cycle in Dulong cows did not differ significantly, which conformed to yellow cattle and swamp buffalo[22]. LH concentration of standing estrous was lower than those on preoestrus and anestrus ($p>0.05$), differed from the previous findings[14, 22, 25]. And LH levels during estrous cycle in Dulong cows had no significant difference, in accordance with the cases in yellow cattle and swamp buffalo, respectively[22]. However, plasma E_2, LH and P_4 kept high levels during preoestrus in accordance with the cases of Indian mithun[25], yellow cattle and buffalo[22]. And FSH and LH levels in Dulong cows were lower than those in buffalo and yellow cattle[22]. FSH levels were distinctly changing or had differences from various species or breeds in estrous cycles, especially visibly changing in dairy cows and Dulong cows, slight fluctuation in yellow cattle and buffalo[22]. Although, Dhali *et al*.[25] reported biphasic FSH and LH surges in mithun cows showed on from day 5 to day 3 of the cycle, biphasic FSH and LH surges in Dulong cows did whether or not show out during the cycle will be done later.

Plasma FSH concentrations of Dulong cows during the gestation were relatively slowly increasing from days 5-10 of post-copulate to the late-gestation (range 1.02-1.98 mIU mL^{-1}) and gradually reached to a surge on days 2-30 of postpartum which was slightly higher than other pregnant stages. The pattern of FSH secretion during the gestation in Dulong cows was increasing, differed from buffalo[39]. During the gestation and days 2-30 of postpartum in Dulong cows, positive correlations were between FSH and E_2 ($r = 0.35$, $p>0.05$) and T ($r = 0.41$, $p<0.05$) and negative correlations between FSH and P_4 ($r = -0.52$, $p<0.01$). LH level appeared surge (10.21 mIU mL^{-1}) on days 5-10 of post-copulation in Dulong cows, differentiated from dairy cows on days 0-30 of post-mating[16]. LH concentration of Dulong cow was not evidently showed changes through the gestation (3.11-5.03 mIU mL^{-1}) and was slightly increasing on days 2-30 of postpartum, coincided with buffalo[21]. A significant positive correlation between LH and P_4 was found in present study. The ratio of LH and FSH was showing three surges from mating to late pregnancy during the whole gestation period.

Testosterone (T) as one of important reproductive hormones in mammals to maintain the normal physiological function of male and female is of great significance. For females, in addition to have a function to promote the mature follicle development and ovulation and to maintain the endometrial tissue environment stability and mammary gland development[40-43]. But, T levels would lead to female reproductive disease, such as polycystic ovary syndrome[44, 45]. In this study, plasma T levels of preoestrus (0.11 pg mL^{-1}) was significantly higher than that of standing heat (0.03 pg mL^{-1}) which increased and promoted development and maturity of dominant follicle, ensured secretion E_2 from follicle during the preoestrus. And plasma T concentrations were observed to be significantly positive relationship with individual PPS in Dulong cows. The changes of plasma T level during estrous cycle were in accordance with mare[46]. And ovulatory follicle continued to grow and mature, then increased at function of secretion E_2 from follicle which induced cows the onset of oestrum and reached surge E_2 levels on standing heat, meanwhile T level decreased to lower. And a positive effect was found between T and E_2during estrous cycle ($r = 0.160$, $p>0.05$), indicating that T hormone had a similar action on boosting E_2 level. However, the profiles of T level in Dulong cows were significantly lower than those in mare[46] and in Holstein cows[17].

During the pregnancy, the secretion patterns of plasma T in Dulong cows were significantly increasing during the pregnancy, which differed from those in Norwegian Red cattle[13] and buffalo[12] in the same stage. T levels (0.11-0.30 pg mL^{-1}) on mid- and last-pregnant had significantly higher than those on days 5-10 of post-mating and postpartum (0.023-0.024 pg mL^{-1}) in Dulong cows which were similar to those of buffalo[12]. At the same time, T levels in Dulong cows on days 5-210 of gestation were found slightly lower than those in cattle and buffalo[12, 13] and the highest T levels in Dulong cows on days 240-270 of pregnancy, in accordance with those in buffalo on days 270-315 of gestation[12]. During the gestation, plasma T level was found to be significantly positive correlation with different pregnant stages ($r = 0.85$, $p<0.01$), E_2 ($r = 0.63$, $p<0.01$) and FSH ($r = 0.51$, $p<0.01$).

P_4/T value of Dulong cow was the lowest on standing estrus, of which P_4 and T levels were lower than other PPSs. And P_4/T value was the highest on day 5-10 of post copulate, at the moment P_4 content was the highest and T content was the lowest. Then on day 60 of pregnancy to before delivery, pregnancy in P_4 value content was relatively stable and P_4/T value was also higher and fluctuated from 109.75 to 15.13, showing that high P_4/T value to ensure the normal development of embryos and pregnancy after mating early fetal development was very important.

Especially, LH level was found to be decreasing from dioestruos to standing heat, the occurrence of LH pulses during pre-ovulatory LH surges which was required for mammal ovulation. P_4 and T concentrations in preoestrus were higher than that in anestrus and the lowest concentration at standing heat and with E_2 secretion increasing, controlling P_4 and T secretion from diosetrum to standing heat.

In present study, the highest E_2 concentration and the lowest concentrations of P_4 and LH in Dulong cows were found when standing heat, in agreement with Mondal *et al.*[24] findings. However, lower concentrations of FSH and T were also found on the standing heat in Dulong cows. Especially, the occurrence of the highest LH surges within a narrow interval from 2-5 h post-oestrus onset in mithun could have contributed to animals' ovulation in a short time[1]. The lowest levels of E_2 and T, the highest levels of LH and P_4 were found on days 5-10 after mating, then relatively stable concentrations of E_2, LH, FSH, P_4 and T were maintained throughout pregnancy of which Dulong cows become quiet. These results were very promising of a practical standpoint of potential success when AI program in the animal was implemented in a big way.

CONCLUSION

The first report is involving the variations in plasma profiles of E_2, FSH, LH, P_4 and T and their interrelationship in Dulong cows for the PPSs in this study. It showed that:

- Higher LH and FSH levels while lower E_2, P_4 and T levels to diestrum
- Slightly increasing at E_2, FSH, P_4 and T concentrations, while decreasing at LH topreoestrum
- Distinctly increasing at E_2, decreasing to the lowest level at P_4 and slightly decreasing at T, FSH and LH level to standing heat in Dulong cows

During the process of pregnancy, normally developing zygote and embryonic implantation were assured by increasing at LH and P_4 secretion, decreasing at E_2 and T levels on days 5-10 of post-copulate in Dulong cows. In the initial pregnant stage, there were a decrease for LH and P_4, an increase E_2 levels close to proestrum levels, as FSH and T levels kept to forepart stage during days 60-90 of pregnancy; on mid-pregnancy from days 120-210, the levels of E_2, LH, FSH and P_4 kept stabilization and significantly increasing for T levels. On late pregnancy stage (range days 240-270), there were significantly increasing at T levels and keeping relatively balancing levels on E_2, FSH and LH.

The concentrations of T, P_4 and FSH were visibly changing with PPS process in Dulong cows. Concomitant relationships were found between P_4 and LH, FSH and T, respectively. In the different PPSs, the hormone levels of LH and FSH remained relatively stable which were regulated by the changing levels of E_2, P_4 and T. Especially, T was carrying the same function as E_2 and P_4 on normal estrum and gestation in Dulong cows.

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REFERENCES

01. Mondal, M., C. Rajkhowa and B.S. Prakash, 2006. Oestrous behaviour and timing of ovulation in relation to onset of oestrus and LH peak in Mithun (*Bos frontalis*) cows. Reprod. Domestic Anim., 41: 479-484.
02. Mondal, M., C. Rajkhowa and B.S. Prakash, 2007. Development and validation of a highly sensitive economic enzymeimmunoassay for prolactin determination in blood plasma of mithun (*Bos frontalis*) and its application during milk let down and cyclicity. Anim. Reprod. Sci., 99: 182-195.
03. Mason, I.L., 1984. Evolution of Domesticated Animals. Prentice Hall Press, New York, USA., ISBN-13: 978-0582460461, Pages: 468.
04. Bhusan, S., C. Rajkhowa and K.B. Ujarbaruah, 2005. Body weight and growth pattern in Mithun (*Bos frontalis*). Indian J. Anim. Sci., 75: 1186-1188.
05. Mondal, M., C. Rajkhowa and B.S. Prakash, 2006. Relationship between plasma growth hormone concentrations and temperament in mithuns (*Bos frontalis*). Hormones Behav., 49: 190-196.
06. Zhanxing, H., H. Xiechao and Z. Jicai, 2007. Comparison of superovulation effect and hormone change of Brahman with BMY cattle in whole year grazing. J. Northwest Sci-Tech Univ. Agric. For., 35: 39-43.
07. Mondal, M., M. Karunakaran, K.B. Lee and C. Rajkhowa, 2010. Characterization of mithun (*Bos frontalis*) ejaculates and fertility of cryopreserved sperm. Anim. Reprod. Sci., 118: 210-216.
08. Shan, X.N., Y.F. Chen, L.H. Luo, X.M. Cao, J.Z. Song and Y.Z. Zeng, 1980. Comparative studies on the chromosomes of five species of cattle of the genus Bos in China. Zool. Res., 1: 75-81.
09. Qu, K.X., Z.X. He, W.H. Nie, J.C. Zhang and X.D. Jin *et al*., 2012. Karyotype analysis of mithun (*Bos frontalis*) and mithun bull × Brahman cow hybrids. Genet. Mol. Res., 11: 131-140.
10. Editorial Committee of the Breeds of Domestic Animal and Poultry in Yunnan, 1987. Breeds of Domestic Animal and Poultry in Yunnan. Yunnan Sci-Tech Press, Kunming, China, pp: 77-87 (In Chinese).
11. Scherf, B.D., 2000. World Watch List for Domestic Animal Diversity. 3rd Edn., Food and Agriculture Organization of the United Nations, Rome, Italy, ISBN-13: 978-9251045114, pp: 549-552.
12. Nanda, A.S. and R.D. Sharma, 1985. Serum testosterone concentrations in advanced pregnancy in water buffaloes (*Bubalus bubalis*). Anim. Reprod. Sci., 9: 383-385.
13. Sundby, A. and O. Joakimsen, 1982. Plasma testosterone in cows related to day of gestation and sex of the foetus and plasma testosterone levels around parturition. Acta Vet. Scan., 23: 438-445.
14. Zhi, X., J. Zhenguo, X. Yishu and Z. Dongmei, 1996. Concentration and its variational regularity of Follicule-Stimulating Hormone (FSH), Luteinizing Hormone (LH), prolactin hormone (PRL), Progesterone (P4), 17beta-oestradiol in the peripheral blood of dairy cows during oestrous cycle. Acta Agric. Boreali-Sinica, 11: 121-127.
15. Hirako, M., T. Takahashi and I. Domeki, 2002. Peripheral changes in estrone sulfate concentration during the first trimester of gestation in cattle: Comparison with unconjugated estrogens and relationship to fetal number. Theriogenology, 57: 1939-1947.
16. Ayad, A., N.M. Sousa, J. Sulon, J.L. Hornick and J. Watts *et al*., 2007. Influence of progesterone concentrations on secretory functions of trophoblast and pituitary during the first trimester of pregnancy in dairy cattle. Theriogenology, 67: 1503-1511.
17. Wei, S.C., W.J. Tong and F.L. Tian, 2009. Detection of serum reproductive hormones and elements concentration and correlation analyses in Holstein cow. J. Cent. Univ. Nationalities (Nat. Sci. Edition), 18: 5-9.
18. Lamothe-Zavaleta, C., G. Fredriksson and A. Madej, 1991. Reproductive performance of zebu cattle in Mexico: Seasonal influence on the levels of progesterone, estradiol-17β, cortisol and LH during the estrous cycle. Theriogenology, 36: 897-912.
19. Yu, S.J. and B.H. Chen, 1997. Characteristics of reproduction and related hormone changes around oestrus in yak. Acta Zoologica Sinica, 43: 178-183.
20. Zhang, S., Y.A. Wang, H.Z. Shang and W.C. Li, 2003. Analysis of correlation between follicle development and concentration of Oestradiol-17β in plasma during anestrus in yak. J. Qinghai Univ., 21: 1-3.
21. Arora, R.C. and R.S. Pandey, 1982. Changes in peripheral plasma concentrations of progesterone, estradiol-17β and luteinizing hormone during pregnancy and around parturition in the buffalo (*Bubalus bubalis*). Gen. Com. Endocrinol, 48: 403-410.
22. Xie, B.K., 2005. Study on changes of serum concentrations of reproductive hormones during the estrous cycle in cattle and buffaloes in Guangxi. Master Thesis, Guangxi University, Nanning, China.
23. Pancarci, S.M., U.C. Ari, O. Atakisi, O. Gungor, Y. Cigremis and H. Bollwein, 2012. Nitric oxide concentrations, estradiol-17β progesterone ratio in follicular fluid and COC quality with respect to perifollicular blood flow in cows. Anim Repro. Sci., 130: 9-15.

24. Mondal, M., C. Rajkhowa and B.S. Prakash, 2006. Relationship of plasma estradiol-17β, total estrogen and progesterone to estrus behavior in Mithun (*Bos frontalis*) cows. Hormones Behav., 49: 626-633.
25. Dhali, A., D.P. Mishra, A. Mech, M. Karunakaran and C. Rajkhowa, 2005. Endocrine control of estrous cycle in mithun (*Bos frontalis*). Theriogenology, 64: 2010-2021.
26. Dhali, A., D.P. Mishra, A. Mech, M. Karunakaran, H. Choudhury, K. Khate and C. Rajkhowa, 2006. Changes in plasma concentrations of LH, FSH, estradiol 17-β and progesterone during oestrus in mithun (*Bos frontalis*). Reprod. Domestic Anim., 41: 162-168.
27. Dhali, A., D.P. Mishra, A. Mech, B. Prakash and C. Rajkhowa, 2007. Evidence of multiple preovulatory LH and FSH surges in mithun (*Bos frontalis*). J. Cell Anim. Biol., 1: 1-6.
28. He, Z., M. Huang, A. Wang, J. Zhang, G. Zhao, X. Yuan and B. Huang, 2014. Patterns of plasma hormone concentrations in mithun bulls under a semi-management. J. Anim. Vet. Adv., 13: 732-739.
29. Dixit, V.D., B. Singh, P. Singh, G.C. Georgie, M.M. Galhotra and V.P. Dixit, 1998. Circadian and pulsatile variations in plasma levels of inhibin, FSH, LH and testosterone in adult murrah buffalo bulls. Theriogenology, 50: 283-292.
30. Patel, O.V., N. Takenouchi, T. Takahashi, M. Hirako, N. Sasaki and I. Domeki, 1999. Plasma oestrone and oestradiol concentrations throughout gestation in cattle: Relationship to stage of gestation and fetal number. Res. Vet. Sci., 66: 129-133.
31. Peters, A.R. and P.J.H. Ball, 1995. Reproduction in Cattle. Blackwell Science, Oxford, England, UK., Pages: 234.
32. Spencer, T.E., O. Sandra and E. Wolf, 2008. Genes involved in conceptus-endometrial interactions in ruminants: Insights from reductionism and thoughts on holistic approaches. Reprod. (Cambridge, Engl.), 135: 165-179.
33. Lonergan, P., 2011. Influence of progesterone on oocyte quality and embryo development in cows. Theriogenology, 76: 1594-1601.
34. Forde, N. and P. Lonergan, 2012. Transcriptomic analysis of the bovine endometrium: What is required to establish uterine receptivity to implantation in cattle?. J. Reprod. Deve., 58: 189-195.
35. Chen, H. and Y. Fu, 2008. Research of diagnosis of early pregnancy of Chinese Holstein cows by measuring the Continent of progesterone and oestraniol in milk. Acta Agric. Nucleatae Sin., 22: 93-96.
36. Thompson, J.A., D.W. Forrest, T.L. Blanchard, A.R. Bronson and N.L. Lowes, 1994. Ratios of serum concentrations of testosterone and progesterone from yearling bulls with small testes. Theriogenology, 41: 1045-1052.
37. Garcia, M. and L.E. Edqvist, 1990. Progesterone determinations and clinical examinations of reproductive organs in purebred and crossbred female Zebu cattle. Theriogenology, 33: 1091-1103.
38. Walters, D.L., R.E. Short, E.M. Convey, R.B. Staigmiller, T.G. Dunn and C.C. Kaltenbach, 1982. Pituitary and ovarian function in postpartum beef cows. II. Endocrine changes prior to ovulation in suckled and nonsuckled postpartum cows compared to cycling cows. Biol. Reprod., 26: 647-654.
39. Palta, P. and M.L. Madan, 1996. Effect of gestation on GnRH-induced LH and FSH release in buffalo (*Bubalus bubalis*). Theriogenology, 46: 993-998.
40. Knapczyk-Stwora, K., M. Grzesiak, M. Duda, M. Koziorowski and M. Slomczynska, 2013. Effect of flutamide on folliculogenesis in the fetal porcine ovary-regulation by Kit ligand/c-Kit and IGF1/IGF1R systems. Anim. Reprod. Sci., 142: 160-167.
41. Du, X., Q. Li, Z. Pan and Q. Li, 2016. Androgen receptor and miRNA-126* axis controls follicle-stimulating hormone receptor expression in porcine ovarian granulosa cells. Reproduction, 152: 161-169.
42. Gervasio, C.G., M.P. Bernuci, M.F. Silva-de-Sa and A.C.J.D.S. Rosa-e-Silva, 2014. The role of androgen hormones in early follicular development. Int. Scholarly Res. Notices, Vol. 2014, 10.1155/2014/818010
43. Davey, R.A. and M. Grossmann, 2016. Androgen receptor structure, function and biology: From bench to bedside. Clin. Biochem. Rev., 37: 3-5.
44. Dumesic, D.A. and J.S. Richards, 2013. Ontogeny of the ovary in polycystic ovary syndrome. Fertili. Sterility, 100: 23-38.
45. Sen, A., H., Prizant, A. Light, A. Biswas, E. Hayes, H.J. Lee and S.R. Hammes, 2014. Androgens regulate ovarian follicular development by increasing follicle stimulating hormone receptor and microRNA-125b expression. Proce. National Acad. Sci., 111: 3008-3013.
46. Gastal, M.O., E.L. Gastal, M.A. Beg and O.J. Ginther, 2007. Elevated plasma testosterone concentrations during stallion-like sexual behavior in mares (*Equus caballus*). Hormones Behav., 52: 205-210.