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Serum ghrelin, IL-1β, and IL-6 during the postestrus period in Murrah buffaloes (Bubalus bubalis)

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ABSTRACT

The knowledge in blood constituents is important for assessing the physiological status and the health of animals. The present study was carried out to find out whether the estrus affects the concentrations of ghrelin, interleukin-1β (IL-1β), and IL-6 in Murrah buffaloes. The result indicated that serum ghrelin concentrations were higher in luteal phase compare to other phase, the highest concentrations of IL-1β and IL-6 were observed at the end of the luteal phase.

Keywords: Ghrelin, cytokine, buffalo, estrus period.

INTRODUCTION

Ghrelin is a 28-amino acid, octanoylated peptide which is secreted primarily by cells in the abomasum in ruminants. Ghrelin concentrations increase prior to scheduled meals and in response to fasting in ruminants, and feeding suppresses ghrelin secretion (Bradford and Allen, 2008). Ghrelin is expressed and produced in several tissues, but the gastric mucosa is the major source of circulating ghrelin. Ghrelin is a hormone with multiple functions and diverse biological actions. The acylated ghrelin is the only known biologically active form of ghrelin while the majority of circulating ghrelin is des-acylated ghrelin with no identified function (Alhojaily, 2014).

Ghrelin is a major hormone involved in the regulation of hunger and satiety. Growth hormone (GH) has been suggested to have an inhibitory effect on ghrelin level. Administration of a large dose of GH to normal rats resulted in a 50% decrease of ghrelin levels in the serum (Tschöp *et al.*, 2002).

Cytokines are pleiotropic glycoproteins, which orchestrate most aspects of mammalian reproductive physiology, ranging from the maternal recognition of pregnancy to embryogenesis, implantation and decidualization, trophoblast differentiation, placental growth/function, term labor/delivery and lactation (Orsi et al., 2007). While the role of cytokines is undoubtedly to coordinate the activity of immune effector cells, the identity of uterine cytokines likely to be implicated in local immunoregulation remains largely undetermined. Cytokines have also been the subject of studies investigating their embryotrophic properties in a range of mammalian embryos (Ekbote *et al.*,

2007). IL-1 is produced as a procytokine upon initial stimulation of immune cells via toll like receptors. Its release is required for the subsequent release of other inflammatoryand anti-inflammatory cytokines to remove and resolve the infection.

The aim of the present study has been to assess the changes of serum ghrelin, IL-1β, and IL-6 during the postestrus period in Murrah buffaloes.

MATERIALS AND METHODS

Animals: The study was conducted at Guangxi Buffalo Research Institute Farm, Nanning, China. Twenty-three cycling Murrah buffaloes (2^{nd} - 4^{th} parity) that had calved between 48 and 96 days ago were selected from the buffalo herd for experiments. The animals selected for the study were free from any anatomical and reproductive disorders and were not suffering from any health problems. All the selected animals were in good body condition. The animals in the dairy farm were housed in an open free-stall barn and provided ad libitum access to a balanced total mixed ration.

No treatment was given to induce or synchronize the estrus in these buffaloes. Occurrence of estrus in the animals was monitored by hourly observation of various behavioral estrus signs and by vasectomised bull (teaser) parading at 08:00, 14:00, 20:00 h for 30 minutes and further confirmed by observing uterine tone on rectal palpation. The behavioural estrus signs exhibited during spontaneous estrus by buffaloes including swollen vulva, excitement, frequent urination, bull mounting, chasing by bull, mucus discharge, tail raising, bellowing and so on.

Blood sampling: Blood samples were collected via coccygeal venipuncture into 10 mL tubes without anticoagulants (Hunan Pingan Medical Devices Technology CO., LTD) at two days interval from the day estrus confirmed by rectal palpation until the sixteenth day after the estrus confirmed. The day of the estrus confirmed was specified as d-0. All the blood samples were kept in box containing ice (4°C) and carried back to laboratory immediately. Blood samples were centrifuged immediately at 3,500 rpm for 15 min and serum was stored at -20°C until analyzed.

Analysis: Goat anti-Cattle Ghrelin, goat anti-Cattle IL-1β and goat anti-Cattle IL-6 were determined in serum samples using ELISA kits (adlitteram diagnostic laboratories, ADL, USA) and Microplate Reader (DG5032, Nanjing Huadong Electronics Group Medical Equipment Co., Ltd.) was used for the ELISA analysis.

Statistical analysis: Mean values of ghrelin, IL-1β, and IL-6 were reported throughout with the standard error of the mean (± SEM). Analysis of variance (ANOVA) test was used to compare the ghrelin between the different days postestrus of Murrah buffaloes, respectively. Differences were considered significant at $P<0.05$.

RESULT AND DISCUSSION

A total of 207 blood samples were collected from 23 cycling Murrah buffaloes. The ghrelin concentrations were higher in luteal phase (d-6 to d-8) compare to other phase. And the highest serum concentrations of IL-1β (304.6±15.17 pg/ml) and IL-6 (287.9±24.84 pg/ml) were observed at the end of the luteal phase (d-8) ($P < 0.01$ in comparison with all remaining days) (Table 1).

Table 1 The changes of serum ghrelin, IL-1β, and IL-6 during the postestrus period in Murrah buffaloes

	d-0	d-2	d-4	d-6	d-8	d-10	d-12	d-14	d-16
Ghrelin(nml)	89.57 ± 9.58^{aA}	111.9 ± 14.93^{abAB}	81.68 ± 2.51^{aA}	151.1 ± 5.61^{cB}	136.8 ± 16.02^{bcB}	84.83 ± 2.89^{aA}	131.8 ± 16.92^{bcB}	92.17 ± 2.52^{aA}	91.1 ± 3.81^{aA}
IL-1β(pg/ml)	74.31 ± 11.69^{aA}	185.8 ± 23.7^{bB}	137.6 ± 36.26^{abAB}	112.3 ± 20.18^{abAB}	304.6 ± 15.17^{cC}	129.5 ± 26.93^{abAB}	111.1 ± 14.95^{abAB}	182.2 ± 32.13^{bB}	198.5 ± 38.71^{bB}
IL-6(pg/ml)	84.8 ± 9.81^{aA}	184.4 ± 16.95^{bA}	124.1 ± 24.58^{abA}	186.2 ± 33.29^{bA}	287.9 ± 24.84^{cB}	177.1 ± 35.92^{bA}	87.3 ± 16.56^{aA}	143.5 ± 27.02^{abA}	125 ± 32.16^{abA}

a,b Different superscript lower case letters within the same row means $P < 0.05$.

A,B Different superscript capital letters within the same row means $P < 0.01$.

Discussion

The serum concentrations of ghrelin, IL-1β, and IL-6 have been investigated in some species. Ghrelin and leptin are important regulators of appetite and energy balance. Leptin is an indicator of body fat tissue. Whereas the ghrelin is initiating the action of eating by stimulating the appetite, leptin terminates eating by reducing it. Leptin and ghrelin share some relevant functional features, as both molecules are peripheral factors involved in the control of food intake and the somatotropic axis. Moreover, leptin and ghrelin have been implicated, with opposite roles, in body weight homeostasis: leptin, as satiety factor that signals for energy abundance, and ghrelin, as orexigenic factor that signals for energy insufficiency (Zigman and Elmquist, 2003). However, in contrast to the well-documented effects of leptin upon the reproductive system, analysis of the potential reproductive actions of ghrelin has received little attention. Yet, although fragmentary, several lines of evidence suggest that, indeed, ghrelin might participate in the control of gonadal axis. This phenomenon likely includes both systemic effects at different levels of the reproductive system, as well as direct gonadal actions of locally produced ghrelin (Barreiro and Tena-Sempere, 2004).

In the rat, expression of ghrelin gene was demonstrated in the ovary throughout the estrous cycle, with the lowest levels in proestrus and peak expression values in diestrous d1; i.e. during the luteal phase of the cycle. In good agreement, ghrelin immunoreactivity was predominantly located in the luteal compartment of the ovary; with intense immunostaining being detected in steroidogenic cells from corpus luteum of the current cycle as well as in regressing corpora lutea (Caminos *et al*., 2003). Likewise, strong ghrelin immunostaining was observed in young and mature corpora lutea of the human ovary, whereas it was not detected in ovarian follicles at any developmental stage (Gaytan *et al*., 2003). Worthy to note, the profile of ghrelin expression in the human corpus luteum is roughly coincident with its peak in functional activity within the ovarian cycle, suggesting a potential regulatory role of locally produced ghrelin in the control of corpus luteum function. In addition, ghrelin immunoreactivity was also demonstrated in interstitial hilus cells of the human ovary. This cell type is steroidogenically active, with ability to secrete testosterone in response to LH stimulation, and shows distinctive morphological characteristics (e.g. presence of crystals of Reinke) identical to those of differentiated testicular Leydig, i.e. the source of ghrelin expression within the testis. Concerning the functional receptor, expression of GHSR1a protein in the human ovary showed a wide pattern of tissue distribution, with detectable expression in oocytes as well as somatic follicular cells, luteal cells from young, mature, old and regressing corpora lutea, and to a lower extent, in interstitial hilus cells (Gaytan *et al*., 2003). To note, expression of GHS-R1a peptide in somatic cells from ovarian follicles roughly paralleled follicular development. This suggests a potential relationship between GHS-R expression and follicle growth, which remains to be proven. Overall, the simultaneous expression of ghrelin and its cognate receptor in several ovarian compartments is compatible with a potential action of locally produced ghrelin in the auto/paracrine regulation of human ovarian function. In addition, the wide pattern of GHS-R1a expression makes it

possible that circulating ghrelin may operate upon specific cell targets within the cyclic ovary, as has been demonstrated for other factors with key roles in body weight homeostasis, such as leptin (Barreiro and Tena-Sempere, 2004).

IL-1 is a pleiotropic cytokine that plays a critical role in the generation of inflammatory response and the initiation of many normal biological events (Islam *et al.*, 2013). After stimulation by various factors (e.g. endotoxins), IL1β is mainly secreted by mononuclear cells, including monocytes and macrophages, in response to infections (Dinarello, 2005) and acts as a potent stimulator of T- and B-lymphocytes (Islam *et al.*, 2013). Increase in the serum concentration of IL-1 during parturition and subsequent enhancement of contraction and evacuation of the debris from the uterus has been reported (Islam *et al.*, 2013). Expression level of mRNA of various cytokine genes in the endometrial tissue (Galvão *et al.*, 2011) and peripheral blood monocytes (Galvão *et al.*, 2012) has been correlated with the postpartum reproductive diseases.

The IL-1 system is a requisite for preimplantation development (Krüssel *et al.*, 2003); its role during the estrous cycle is unclear, although it has been implicated in cervical tissue remodeling (Uchiyama *et al.*, 2005). IL-1 increases the plasma calcium concentration, which stimulates myometrial contractions and the removal of debris from the uterus. The cytokine also stimulates prostaglandin synthesis, which enhances contraction and evacuation of the uterus (Singh *et al.*, 2008). The significantly lower IL-1 at estrus might indicate the better status of the body and become free from infection as also revealed in clinicogynaecological examination.

IL-6 is a pleiotropic cytokine with numerous immunologic and metabolic actions, it is generally considered to be an important cytokine in the network of cytokines that regulate immune reactions and acute phase responses. Activation of cytokine system, as observed in several chronic inflammatory conditions including liver cirrhosis, may result in increased energy expenditure and reduced nutrition intake (Ataseven *et al.*, 2006).

Mating, in particular exposure to seminal plasma, is known to induce a transient uterine inflammatory response, which dissipates by the time of blastocyst hatching and implantation, and is believed to result in the immunopermissive phenotype exhibited by resident lymphocytes. Due to paucity of literature, the IL-1 and IL-6 protein levels in the peripheral circulation during estrus period could not be compared in buffaloes.

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