INTERLEUKIN 1β (IL-1 β) AND PROTEIN PROFILE DURING DIFFERENT STAGES OF GESTATION IN BUFFALO

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ABSTRACT

Eighteen apparently healthy pregnant buffaloes divided into three groups according to gestational stages, i.e. early, mid and late gestation were selected and blood samples were collected. Serum total protein, albumin, globulin and A: G ratio were estimated along with IL-1β. The concentration of IL-1β increased from early gestation to mid gestation and declined towards late gestation. Concentration of serum total protein, albumin, globulin and A: G was highest during late gestation (8.23±0.26 g/dl), mid-gestation (3.95±0.10 g/dl/), late gestation (4.68±0.21 g/dl/) and early gestation (0.84±0.12). Concentration of IL-1β, serum total proteins, albumin, globulin and A: G was statistically non-significant during all three stages of gestation.

Keywords: *Bubalus bubalis*, buffalo, gestation, interleukin 1 β, protein profiles

INTRODUCTION

For establishment of pregnancy many hormones and factors play important role. But apart those hormones there are cytokines which affect reproduction. Cytokines are category of small proteins that are important in cell signalling. Cytokines are produced by a broad range of cells, including immune cells like macrophages, B lymphocytes, T lymphocytes and mast cells, as well as endothelial cells, fibroblasts, and various stromal cells. A given cytokine may be produced by more than one type of cell. (Lackie, 2010; Ibelgaufts, 2013). Cytokines are expressed in a variety of cell types of the reproductive system, although in most instances their functions are not understood. There are, however, a few instances where a role in early pregnancy has been established. (Mathialagan and Roberts, 1994).

Cytokines regulate pregnancy by both autocrine and paracrine mechanisms. The inflammatory cytokines, interleukins-1β (IL-1β), IL-6 and IL-8, help to maintain the trophoblast in early pregnancy. Serum IL-1β, IL-6 and IL-8 appear to play a major role during pregnancy (Denison *et al*., 1997; Hebisch *et al*., 2001; Laham *et al*., 1999; Stallmach *et al*., 1995), towards term and at delivery (Bahar *et al*., 2003; Makhseed *et al*., 2000; Vassiliadis *et al*., 1998), notably with increased levels in labour (Laham *et al*., 1993; Laham *et al*., 1996; Hebisch *et al*., 2001). In humans and primates, IL-1β was found to be involved in embryonic implantation and establishment of pregnancy through acting as a physiological mediator of acute phase response

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during conceptus invasion and placental formation. (Geisert *et al*., 2012). Cheng *et al*. (2016) observed higher concentration of plasma IL-1β in pregnant cows on 21st day post insemination. To study the role of IL-1β during gestation in buffaloes, level of serum IL-1β was investigated in early, mid and late stage of pregnancy along with serum total protein, albumin, globulin and A: G.

MATERIALS AND METHODS

Animals for experiment

Eighteen apparently healthy pregnant buffaloes were selected from Instructional Livestock Farm Complex, Bombay Veterinary College and some other units in Aarey milk colony, Goregaon, Mumbai - 400065. The experimental animals were divided into three groups according to the stages of gestation, each consisting of six animals. The three groups were early gestation (0 to 100 days), mid gestation (101 to 200 days) and late gestation (201 to 310 days) stages, respectively. The pregnancy diagnosis was done by per rectal examination by veterinarian on the farm. Buffaloes were maintained under uniform and standard conditions of feeding and management. The animals were housed in animal shed with asbestos cement roof, under natural daylight and temperature. The buffaloes were given maintenance ration of 15 kg of paragrass and 2.5 concentrate mixture, during morning and evening hours, daily. Paddy straw was given ad-libitum. The buffaloes were provided with clean drinking water.

Sampling

Blood samples (10 to 12 mL) were collected in vacutainers aseptically from experimental buffaloes by jugular vein puncture. Serum was separated out after centrifugation at 2000 rpm and was stored at -20°C until used for analysis.

Blood analysis

Blood samples were analysed for serum total proteins and albumin using an autoanalyzer - Prietest Touch (Robonik, India). Concentrations of serum globulin and A: G ratio were obtained from the values collected after analysis of serum total proteins and albumin. Analysis was carried out in the Deparment of Veterinary Physiology, Bombay Veterinary College, Parel, Mumbai - 400012.

Interleukin 1 β assay

Concentration of the serum Interleukin 1β was estimated by using KinesisDx Bovine Interleukin 1β (IL-1β) ELISA kit, 1179, W 29th St., Apt. 9, Los Angeles, CA 90007, USA. (www.kinesisdx.com). Each sample was run in duplicate.

Statistical analysis

Data are presented as Mean±S.E. ANOVA was used to analyze the significance of differences. Correlations were calculated and $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Serum intrleukin 1 β

The mean concentrations of serum IL-1β during early, mid and late gestation were 195.10±49.43, 233.65±51.45 and 125.90±36.18 pg/ml respectively. The serum IL-1β concentration increases from early to mid-gestation and then declines in late gestation. No work on Interleukin 1β concentration in serum during early, mid and late gestation in buffaloes is reported in available literature. The serum IL-1β in cow was elevated

around day 21 post AI (Cheng *et al*., 2016) whereas, in HF cows it was found to be decreased after parturition (Trevisi *et al*., 2015). In pigs, serum IL-1β was found to be enhanced on days 12 to 13 in pregnant sows (Franczak *et al*., 2011). In mice, it was observed that the IL-1β was low and stable throughout pregnancy and increased markedly in late pregnancy (Orsi *et al*., 2006) while in women, the serum Il-1β levels did not differed significantly between beginning and the end of gestation (Hebisch *et al*., 2004).

Obsevations from various investigations indicate that the levels of IL-1β in different animals show different trends. Contrary to the belief that IL-1β is required for embryonic implantation and establishment of pregnancy through acting as a physiological mediator of acute phase response during conceptus invasion and placental formation (Geisert *et al*., 2012), the concentration of IL-1β has increased during mid-pregnancy. This could be the trend in buffalo that can be considered as species specific (Figure 1).

Serum total proteins

The average values of serum total protein concentration in buffaloes were 7.99±0.65, 7.48±0.15, 8.23±0.26 g/dL during early, mid and late stages of gestation respectively. The concentration of serum total protein decreased gradually from early to mid-gestation and increased again during late gestation. The concentration of serum total proteins was highest in last trimester of pregnancy. This increase in the serum total protein concentration during late gestation may be due to increase in amino acids from tissues and muscles into blood and increase in feed intake (Ghadhban, 2008). This increase can also be due to increase in the levels of oestrogen with advancement of pregnancy. The secretion of glucocorticoids and thyroxin also increases sex hormone secretion which intensifies metabolic events. Increase in beta and gamma contents of serum proteins during gestation could also be the reason of increase in total proteins and this increase may be due to increased demand of proteins by growing foetus (Patel *et al*., 2016). The lower total protein concentration during early gestation than late gestation may be due to its utilization in colostrum synthesis (Pathak and Janakiraman, 1983; Singh *et al*., 1988) also, there is drain of immune fractions in the formation of colostrum (Mehta *et al*., 1989). Further, the lower concentration of total proteins during early gestation may be due to decrease in globulin concentration and increased protein breakdown required for gluconeogenesis (Quayam *et al*., 1998) (Figure 2).

Serum Albumin

The mean concentration of serum albumin in buffaloes in were 3.47±0.15, 3.95±0.10 and 3.54±0.07g/dL during early, mid and late gestation respectively. The serum albumin level increased from early to mid-gestation and decreased again from mid to late gestation. Similarly, Ghadhban (2008) reported decrease in the concentration of serum albumin in Iraqi cows in late pregnancy, which can be due to increased level of serum globulins during same period. Al-Mujalli *et al*. (2008) observed that the serum albumin concentration was low during first week before parturition in cows. In contrast, Mir *et al*. (2008) in cattle and Patel *et al*. (2016) in crossbred cows reported that the concentration of serum albumin increased throughout the gestation (Figure 3).

Similarly, according to Padodara *et al*. (2012), decrease in albumin from mid to late gestation may be due to increase in nutrient requirement of growing foetus whereas Yokus

and Cakir (2006) reported no changes in albumin concentration throughout pregnancy.

Serum globulin

The average values of serum globulin concentration recorded in present study are 4.52±0.88, 4.16±0.12 and 4.68±0.21 g/dL during early, mid and late gestation respectively. The serum globulin level is highest during late gestation and lowest in mid-gestation. The relative percentage concentration of serum globulin did not show significant variation during different stages of gestation in buffaloes (Seshagiri *et al*., 1979). Observation of this study is in contrast with the findings of Mir *et al*. (2008); Patel *et al*. (2016) in crossbred cows (Figure 4).

A: G

Concentration of Serum A: G ratio in buffaloes during early, mid and late gestation were 0.84±0.12, 0.79±0.02 and 0.75±0.02 g/dL respectively. The serum A: G ratio was highest during early gestation. Similar observations were recorded by Tekade (2009) in buffaloes and Gadhave (2000) in crossbred cows. Increased A: G ratio can be due to declined serum globulin concentration during the same period (Gadhave, 2000) (Figure 5).

Thus it can be concluded that serum IL-1β concentration increased from early to mid-gestation and declined sharply in late gestation suggesting to be a trend in buffaloes. Higher concentration of serum Interleukin 1 β during mid-gestation indicates that it may play some important role during pregnancy which needs further investigation. Similarly, the concentration of IL-1β in the buffalo conceptus also needs to be investigated to find out if it has some role during implantation. The high concentration of total proteins during late pregnancy could be for optimum secretion of gonadotropin releasing factors and number of other hormones needed for culmination of pregnancy.

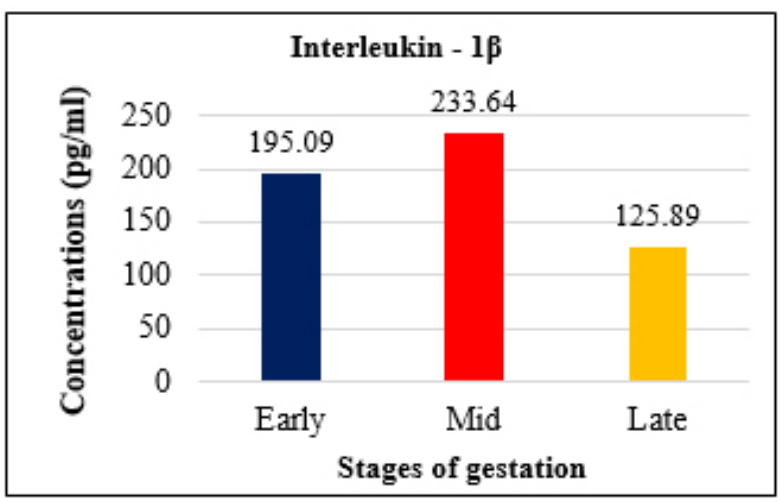

Figure 1. Serum Interleukin 1β (pg/ml) in early, mid and late gestation in buffaloes.

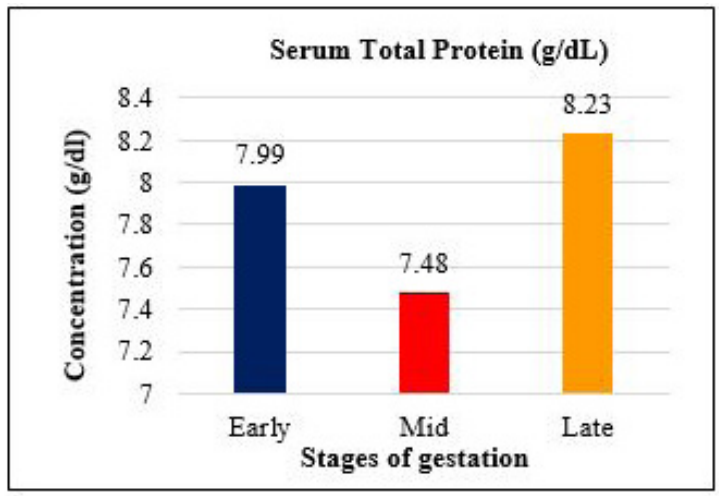

Figure 2. Serum total proteins (g/dL) in early, mid and late gestation in buffaloes.

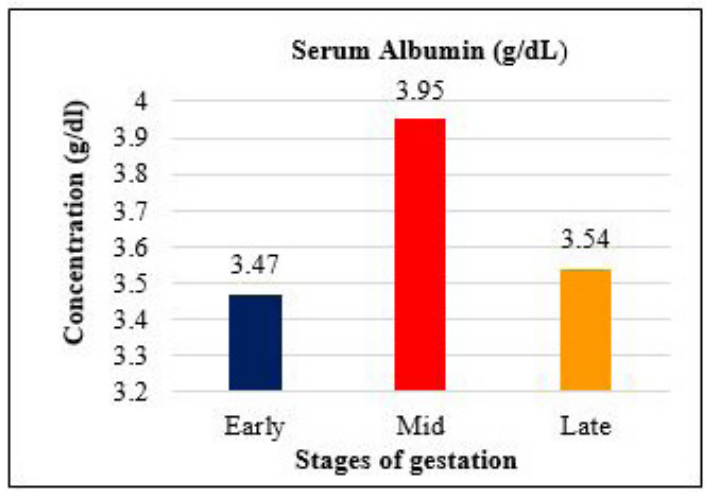

Figure 3. Serum albumin (g/dL) in early, mid and late gestation in buffaloes.

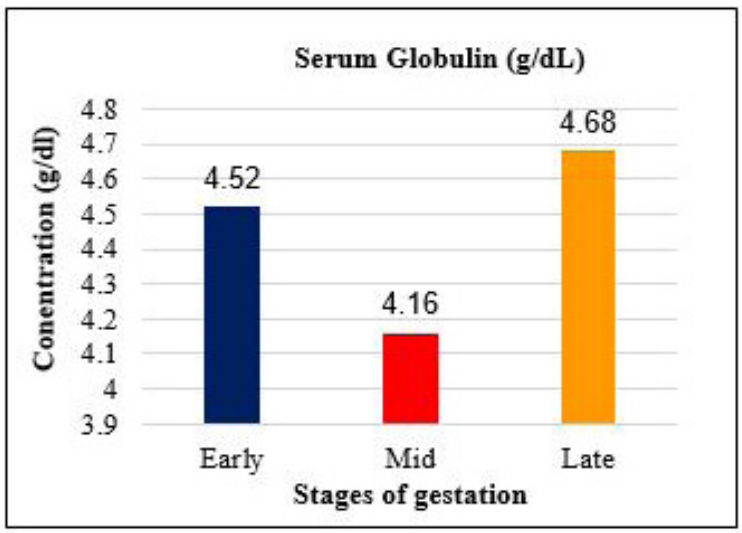

Figure 4. Serum globulin (g/dL) in early, mid and late gestation in buffaloes.

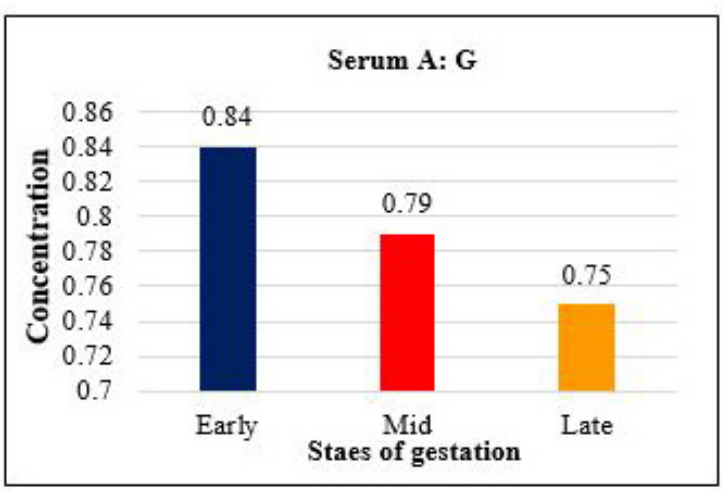

Figure 5. Serum albumin: globulin (A: G) in early, mid and late gestation in buffaloes.

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