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RELATIONSHIP BETWEEN CYTOKINE LEVELS AND POSTPARTUM REPRODUCTIVE DISORDERS IN EGYPTIAN BUFFALOES

By

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ABSTRACT

To verify the potential relevance of pro-inflammatory cytokine (PIC) with periparturient reproductive disorders, the changes of plasma IL-10 and IL-18 have been investigated in buffalo cows under Egyptian condition. A total of twenty healthy multiparous Egyptian buffaloes weighing 420–600 kg, between (2^{nd} and 6^{th} parity) in advanced pregnancy status with no history of peripartum diseases were enrolled in this study. All animals were observed carefully for incidence of any reproductive disorders such as dystocia, abortion etc. After parturition, the individual buffalo cow was critically monitored for the diagnosis of postpartum disorders like retained placenta, clinical metritis, and delayed involution of the uterus during the course of four weeks' postpartum period. Buffalo cows were divided into four groups on the basis of postpartum disorders to normal (NM), retained of fetal membrane (RFM), clinical metritis (CM) and delayed involution of the uterus (DIU) (5 animals in each). Blood samples were collected from one month before expected calving (at 30,15, 5 and 1 days), the day of calving and three months after parturition (1,7,15 and 30,45,60 and 90 days). Results showed that both of CM and RFM groups showed significantly ($P < 0.05$) higher values of IL-10 than NM group all over the experimental period. Also, group CM showed a higher value than that for group RFM in pre-calving period and vice versa for group RFM than that for group CM in post-calving period. A tendency for a gradual decrease in the level of IL-10 at the date of birth for all experimental groups were noticed and then rise again after that. Group DIU showed a less valuable of IL-10 compared to other groups through the experimental period. For IL-18 group CM showed a liner increase in IL-18 concentration from day -30 pre-calving till day 90 post-calving, whereas, group RFM showed the same pattern except for days 60 and 90 post-calving.

Group DIU showed a less valuable level of IL-18 compared to other groups through the experimental period. In the day of calving DIU group show a sharp decrease in the level of IL-18 and then rise again after that.

In conclusion, these data support the utility of PIC (IL-10 and IL-18) measurement in late pregnancy as prognostic markers to identify the buffalo cows that will develop RFM and CM.

Keywords:

Buffalo cows, interleukin10 and18, post-partum reproductive disorders.

INTRODUCTION

Fertility is one of the key determinants in the life time performance of any animal. Reproductive diseases affect adversely the reproductive efficiency of a dairy buffalo and is greatly influenced by late attainment of puberty, long calving intervals, increased number of services per conception, increased days open, uterine infections and various obstetrical problems **(Samad *et al*., 1987)**. Previous studies have shown that animal diseases have caused huge economic losses and probably still continue with more or less the same intensity. The reproductive problems may be congenital or acquired. Anestrus, repeat breeding, cystic ovarian degeneration, uterine and tubal disorders have been observed as the most common gynecological problems in buffaloes **(Agarwal *et al.*, 2005).** Peri-parturient disorders have been found to have a negative effect on metabolic status and milk yield **(Dann *et al.*, 2005**). Milk yield has been reported to decrease by 239 kg for retained fetal membrane, 181 kg for stillbirth, 173 kg for dystocia, and 98 kg for metritis **(Simerl *et al.,* 1992)**. About 18-40% of buffaloes were culled mainly due to infertility **(Sharma *et al.,* 1993)** which incriminates direct losses to the farmer as well as to the genetic resource. Parturition associated problems include dystocia, still birth, abortions, retained placenta etc. stillbirth and dystocia have been recognized as the most important factors compromising the future reproductive life of the animal. They have negative impact on the reproductive performance, increase the odds of developing metritis and retained placenta **(Correa *et al.,* 1993)**. The incidence of reproductive disorders in buffaloes is increasing over years, ranging from 4.66% to 12.66% **(Taraphder, 2002**). The extent of uterine disease within a week of parturition (metritis) was up to 40% (**Sheldon *et al.,* 2009).** Retained placenta has been found to be the single largest postpartum complication in the bovine species **(Pattabiraman and Bawa, 1977)** and the incidence varied from (3 to 7%) in buffalo cows **(El-Malky, 2007).** Postpartum uterine health is most critical component of reproductive and productive efficiency of dairy herds. During early postpartum

period uterus is highly vulnerable to even low grade injuries and infections, which may cause delayed uterine involution and further inflammation of the endometrium, depending on the nature of causative agent and its persistence. Thus, such conditions results in delay of the first ovulation postpartum reduce conception rate to first insemination and increase the risk of culling due to infertility even with best management practices **(Sheldon, 2004)**. The prevalence of clinically relevant endometritis between 15 and 60 days postpartum was 25.9%, but the risk of infertility and/or sterility increased if it occurred after 14 days of parturition **(Gautam *et al.,* 2009). Islam *et al.,* (2014)** mentioned that dairy cows undergo tremendous adaptive changes during the transition period (3 weeks before and/or after parturition), which it determining health, productivity and profitability. In this period cows are affected by different stressors including the increase of body lipid mobilization, oxidative stress, major changes in endocrine status, and altered immune function, which together result in an increased risk of diseases **(Jonsson *et al.*, 2013).** Inflammation is involved in most of these changes through several mediators, such as pro-inflammatory cytokines (PIC) **Kushibiki (2011).** The PIC are released mainly by macrophages **(Koj, 1998),** exert both paracrine and systemic effects. In the liver, PIC stimulates the synthesis of positive acute phase proteins such as heptoglobin **(Gruys *et al.*, 2005**). Concomitantly, PIC impair the synthesis of negative acute phase proteins some of them being important for normal liver metabolism **(Bertoni and Trevisi; 2013).** Indeed, the low negative acute phase proteins concentrations (*e.g.* albumin and lipoproteins) have been associated with decreased reproductive performance and increased disease incidences **(Bossaert *et al.*, 2012)**. In accordance to these results, the roles of PIC have been investigated as potential indicators of postpartum reproductive diseases **(Islam *et al.*, 2013b).** Also, animals undergo pronounced physiological changes that might cause suppression of the host defense mechanisms including both the cellular and humoral response of the immune system and an increase in susceptibility to uterine and mammary gland infection **(Tan *et al.,* 2012).** Relaxation of the vulva and cervical dilatation during and after the onset of parturition allows the entry of bacteria into the uterus, causing infection in 80-100% of cows by 14-21 days' post-partum **(Islam *et al.*, 2013a).** The improper balance between uterine infection and the intrauterine antimicrobial self-defense mechanisms often lead to the main postpartum reproductive diseases such as puerperal metritis, clinical endometritis, pyometra and subclinical endometritis **(Islam *et al.,* 2013a)**. Innate immunity of the cows might have taken the predominant role to protect them

from the development of postpartum reproductive disease **(Sheldon *et al.,* 2009)**. Therefore, it is important to predict postpartum reproductive diseases as early as possible, and to develop a prophylaxis to prevent these diseases. It has been reported that cytokine and growth factors with reproductive hormones may locally or systemically play important roles in placental development and prevention of fetal loss during pregnancy, for elimination of the fetus and fetal membrane at parturition **(Schäfer-Somi, 2003).** Local immune cells or cytokines in the uterus played roles in maintaining pregnancy, support fetal growth and preventing infection **(Galvao *et al.*, 2012). Mor *et al.* (2011)** referring to, it is especially important for elimination of the fetal membrane and phylaxis after parturition to shift from T-helper 2-type (Th2) cell dominance to T-helper 1-type (Th1) cell dominance of T-helper cells in the intrauterine immune system around parturition. **Patra *et al.* (2013)** hypothesized that if the local/systemic immune function could be monitored during pregnancy and the mechanism leading from a prepartum immune functional deficiency to the occurrence of a crisis in postpartum reproductive diseases could be clarified, then an improved method of diagnosis and a better approach to prevention of postpartum reproductive diseases in dairy animals might be devised. Research work is focused to understand the expression profile of the cytokines in the circulatory leukocytes and varying degrees of simultaneous expression of pro- and anti-inflammatory cytokines in monocytes of buffalo cows with or without uterine diseases has been reported (**Galvao *et al*.,2012).**So that, the present study was undertaken to investigate the level of pro-inflammatory cytokines(IL-10 and IL-18)in periparturient buffalocows.The determination of the level of IL-10 and 18 before parturition might aid in the screening of buffalo cows in advance that are likely to develop various postpartum reproductive disease.

MATERIAL AND METHODS

The experimental animals used in this study were selected from the buffalo herd maintained at the Mehallet Mousa station belonging to Animal Production Research Institute (APRI), Agricultural Research Center, located in the North Centre part of the Nile Delta, Kafr El-Sheikh Governorate, Egypt. A total of twenty healthy multiparous Egyptian buffaloes weighing 420-600 kg, between (2^{nd} and 6^{th} parity) in advanced pregnancy status with no history of peripartum diseases were enrolled in this study. All buffaloes were fed on equilibrium balanced ration twice a day. Water was offered freely in water troughs. On average the climatic conditions were: environmental temperature around 25°Cto 30°C, relative humidity between 43% to 73%, photoperiod with 13 h of light (from 5:00 to 17:00 h)

and 11 h of dark. All animals were housed in semi shaded open pens then they were transferred to the maternity unit before one or two days of the parturition date. On the day of calving the animals was monitored and/or observed for nature of parturition, and expulsion of placenta and lochia. The parturition was critically observed for any abnormality like dystocia, ect. After parturition, the individual buffalo cow was critically monitored for the diagnosis of postpartum disorders like retained fetal membrane (RFM), clinical metritis (CM), and delayed involution of the uterus during the course of four weeks' postpartum period (DIU). Animals which had reproductive disorder after parturition were re-evaluated 2 weeks after initial uterine disease diagnosis to determine new cases, spontaneous recovery and/or persistence of uterine inflammatory conditions. A buffalo cow diagnosed with uterine disease at both initial and follow-up examinations was considered to have persistent inflammation. Uterine involution and ovarian activity was monitored at 15, 30, and 45 days postpartum by transrectal palpation. All calves were allowed to nurse the colostrum after parturition, dams were transferred to the milking unit and milked twice daily at 7 a.m. and 5 p.m. and they were subjected to the regular managerial practices of the breeding stock. The animals did not receive any hormonal treatment during pregnancy, before or after parturition. RFM was considered to be retained, if it was not dropped naturally within 24 hours after fetal expulsion **(Laven and Peters, 1996)**. CM was diagnosed in the animals that were not systemically ill, but had an abnormally enlarged uterus and a purulent uterine discharge detectable in the vagina, within 21 days postpartum **(Chapwanya, 2008)**. Uterine involution was diagnosed by pelvic location of the uterus, with a normal consistency and tonicity, symmetric uterine horns, and absence of uterine contents at day 45 postpartum following the method described by **Custer *et al.* (1990).** DIU was diagnosed in buffalo cows that were not diagnosed with any kind of postpartum uterine disease described in this section until 45 days postpartum and then were found to have asymmetric uterine horns or partial or complete abdominal location of the uterus on transrectal examination at 45 days postpartum. The 20 buffalo cows included in this study were designated, based on initial and re-examinations, in one of the following groups: normal (NM), retained fetal membrane (RFM), clinical metritis (CM) and delayed involution of the uterus (DIU) (5 animals in each). Blood samples were obtained from each animal at 3 time points: as close as possible to one month before calving, at calving, and as close as possible to three months post-calving. Pre-calving samples were collected during 30, 15, 5 and 1 days before the expected calving. Calving samples were obtained within 24 h after

parturition. Postcalving sampling occurred between 1,7,15 and 30, 45, 60 and 90 days after parturition. Blood was collected from the jugular vein on evacuated tubes and allowed to clot and kept at -4 ^{0}C until separation of serum. Clotted blood was centrifuged at 3000 rpm for 20 minutes, the separated serum was aspirated from the supernatant gradually without disturbing the sediment. The separated serum was transferred to a sterile 2-mL plastic microfuge tube and stored at -20 ^{0}C until analysis for assay of (IL-10 and IL-18). The concentration of the interleukin-10 and 18 in blood serum was determined using commercially available kits, that is, bovine enzyme-linked immunosorbent assay (ELISA) kits for IL-10 and 18 (USCN Life Science Inc., Houston, TX, USA). The inter- and intra-assay coefficients of variation for all examined cytokine were <12% and <10%, respectively. All procedures were performed according to the guidelines provided by the manufacturers and methods available in the literature **(Kim *et al.* 2014)**. All the kit components and samples were brought to room temperature (20-25^{0}C) before start of assay and performed as per manufacturer's instructions. The optical density was measured immediately at 450 nm using a microplate reader. A standard curve was obtained by plotting the concentration of the standards (0, 50, 100, 250, 500 and 1000 pg/ml) against their optical densities. The value of unknown samples was interpolated from the standard curve and was presented in pg/ml.

Statistical analysis:

Data were analyzed using GLM procedures of the **SAS (2010)**. Means were separated by using Duncan's multiple range test **(Duncan, 1955).**

$\mathbf{Y_{ijk}=\mu+T_i+ D_j+ (TD)_{ij}+ eijk}$

Whereas: $\mathbf{Y_{ijk}}$=observation **μ**= overall mean $\mathbf{T_i}$= effect from treatment

$\mathbf{D_j}$= effect of days $\mathbf{(TD)_{ij=}}$ effect of interaction **eijk**= residual error

RESULTS AND DISCUSSION

Table (1) showed that the mean serum concentration of IL-10 (pg/mL) in buffalo cows suffering from various reproductive diseases from -30 d (pre-calving) to 90 d (post-calving). In pre -calving period tabulated data showed that a significantly ($P < 0.01$) higher for CM and RFM groups (2235.54±126.22 and 1766.03±112.53) than that NM and DIU groups (869.32 ± 60.73 and 312.11±69.55), respectively. In post-calving period a significantly higher ($P< 0.01$) for RFM and CM groups (1753.13± 20.87 and 1642.91±124.15) than that NM and DIU groups (742.73±22.25 and 347.56±43.37) were found. Also, data illustrated that a significantly ($P < 0.01$) higher for RFM and CM groups (1746.67± 21.94 and

1636.53±134.91) than that NM and DIU groups (731.69±22.22 and 331.86±40.75) from calving to 90 d post-calving. The overall mean of IL-10 concentration was significantly ($P < 0.01$) higher for CM and RFM groups (1854.35±131.75 and 1753.71±54.88) than for NM and DIU (781.74±36.22 and 324.68±51.22) groups, respectively. With reference to effect of days, IL-10 concentration was not significantly affected in pre and/or postcalving. Both CM and RFM groups showed significantly ($P < 0.05$) higher values of IL-10 than NM group all over the experimental period. Also, group CM showed a higher value than that for group RFM in pre-calving period and vice versa for group RFM than that for group CM in post-calving period. (Table 1) shows that there is a tendency for a gradual decrease in the level of IL-10 at the date of birth for all experimental groups and then rise again after that. Group DIU showed a less valuable of IL-10 compared to other groups through the experimental period.

Interleukin (IL)-10 is the most important anti-inflammatory cytokine, produced by monocytes or macrophages, and various subsets of T cells. The main biological function of IL-10 is to inhibit monocyte or macrophage-derived tumor necrosis factor (TNF)-a, IL-1, IL-6, IL-8, IL-12, and granulocyte colony-stimulating factor, the major is to compatibility complex class II expression. The rapid up regulation of the anti-inflammatory cytokine IL-10 might have contributed to the down regulation of the pro- inflammatory cytokines. The IL-10 response is thought to reflect the strength of the preceding inflammatory response, and the source and the timing for IL-10 release is an important factor in resolution of inflammation. The higher IL–10 levels in RFM and CM buffalo cows observed in the present study are well supported by **Islam *et al.* (2013a)** who recently reported a significantly higher IL-10 level in pre-partum serum samples (15 days before expected calving) of crossbred cows which subsequently developed RFM and CM. They also reported a higher serum IL-10 level on the day of calving in cows with RFM and CM as compared to normally calved cows. IL-10 being a potent anti-inflammatory cytokine is capable of suppressing pro–inflammatory cytokine production and hinders class II MHC and CD14 expression, thereby inhibiting T cell activation by macrophages **(Ouyang *et al.,* 2011).** The main biological activity of IL–10 is inhibiting the production of monocyte/macrophage derived TNF-α, IL-6, IL-1, IL-8, IL-12, Granulocyte colony stimulating factor (GCSF) and also cytokine production by neutrophils and natural killer cells **(Opal and Depalo, 2000)**. It is known that anti-inflammatory cytokines are produced in concurrence with pro-inflammatory cytokines and their levels indicate the severity of the preceding infection **(Couper *et al.,* 2008).** The higher IL-10 levels might have

suppressed the T cell activation and macrophage activity, thus inhibiting the mechanisms responsible for placental separation. The placental separation, an immune mechanism by itself, might have failed to occur in presence of a strong anti–inflammatory environment created by IL–10. Also, **Galvao *et al.* (2012)** found higher expression of IL-10 in unstimulated monocytes during culture of cells from cows with metritis compared with healthy cows. Furthermore, **Ishikawa *et al.* (2004)** reported that a lower serum level of IL-6 pre-partum was found to affect RFM and a high level at pre-partum tended to affect endometritis. They claimed that measurement of change in the IL-6 concentration during pregnancy is one useful tool for predicting crisis in postpartum reproductive diseases in dairy cattle. Likewise, the estimation of the serum level of cytokine (IL-10) pre-partum is also a good indication for the prediction of postpartum reproductive diseases. A decreased level of IL-10 on day of calving followed by an increasing trend until 90 d postpartum is in close agreement with the results of **Kim *et al.* (2005)** for TNF-a concentration in serum of dairy cows with postpartum endometritis. Also, agreement with results of **Islam, (2012)** who attributed this decreased to the immunomodulatory role of the increased cortisol level in these groups around the day of calving and/or associated with the decreased lymphocyte count leading to immune suppression in the postpartum days. This might lead to the development of postpartum reproductive disease because decreased T cells at calving were linked to the appearance of mastitis and puerperal metritis in cows **(Ohtsuka *et al.*, 2004)**. The increased IL-10 level in buffalo cows with RFM and CM before calving also indicated that a highly stimulated immune response might be because of the severity of the preceding infection **(Couper *et al.*, 2008).** The higher IL-10 level obtained in the study during the periparturient period in buffalo cows with RFM and CM might indicate that IL-10 plays a predominant role in regulating the level of pro-inflammatory cytokines that are released during inflammatory conditions. Therefore, the determination of IL-10 level has a high potential to forecast the development of postpartum inflammatory uterine disease. It clearly indicates that the level of IL-10 is dependent on the severity and time of the inflammatory conditions **(Mette *et al.*, 2010).** In another experiment, **Islam *et al.*(2013b)** claimed that increased concentration of the IL-10 at 15 d pre-partum can be a good predictor of retained placenta and metritis.

Table (1): Interleukin 10 concentration (pg/mL) in the peripheral blood of buffalo cows with or without postpartum reproductive diseases.

Groups \ days	Periparturient period													
	Pre-calving					Calving	Post-calving							Overall mean
	-30	-15	-5	-1	Ave. (-30 to -1 days)	0	+7	+15	+30	+45	+60	+90	Ave. (7 to 90 days)	
NM	976.39^{Ac} ±75.05	881.61^{Bc} ±47.90	825.93^{BCc} ±60.55	793.32^{BCc} ±59.42	869.3^{c} ±60.73	665.44^{Dc} ±22.03	644.39^{Dc} ±28.66	599.26^{Ec} ±30.64	738.60^{CDc} ±15.37	786.77^{Cc} ±9.76	798.22^{BCc} ±14.81	889.15^{ABc} ±34.28	742.73^{c} ±22.25	781.74^{c} ±36.22
RFM	1630.99^{Ab} ±134.78	1791.89^{ABb} ±112.95	1813.08^{Ab} ±103.19	1828.14^{Ab} ±99.19	1766.0^{b} ±112.5	1707.92^{Ea} ±28.35	1715.45^{Ea} ±8.84	1738.47^{Dba} ±16.03	1765.47^{Ca} ±17.72	1779.61^{BCa} ±26.24	1785.47^{BCa} ±34.51	1734.28^{CDa} ±21.89	1753.13^{a} ±20.87	1753.71^{b} ±54.88
CM	2095.90^{Ca} ±155.40	2274.37^{ABa} ±89.87	2332.44^{Aa} ±73.52	2239.42^{Ba} ±186.08	2235.5^{a} ±126.2	1598.22^{Eb} ±199.52	1593.43^{Eb} ±247.50	1619.82^{Eb} ±196.25	1649.64^{Eb} ±116.22	1682.14^{Db} ±53.71	1641.70^{Db} ±84.60	1670.75^{Db} ±46.60	1642.91^{b} ±124.15	1854.35^{a} ±131.75
DIU	376.92^{BCd} ±100.93	322.39^{Cd} ±63.47	287.55^{Bd} ±60.35	261.56^{Bd} ±53.44	312.1^{d} ±69.5	237.61^{Fd} ±25.06	251.58^{EFd} ±22.15	362.07^{BCd} ±45.40	385.65^{Bd} ±51.71	392.07^{Ad} ±50.65	364.81^{BCd} ±46.12	329.19^{Cd} ±44.17	347.56^{d} ±43.37	324.68^{d} ±51.22

Values are shown as mean _ SEM; means with different superscripts in a column (a, b, c and d) and row (A, B, C…) differ significantly ($P < 0.05$).

Abbreviations: NM, normal ;RFM, retained fetal membrane ; CM, clinical metritis and DIU, delayed involution of the uterus. * $0.05 < P < 0.10$. ** $P < 0.05$. *** $P < 0.01$.

The IL-18 level (pg/mL) in buffalo cows suffering from various reproductive diseases during periparturient period from -30 d pre-calving to 90 d post-calving is presented in (Table 2). The level of IL-18 level (pg/mL) differed significantly between the groups at all the experimental period (P<0.01). The overall mean of IL-18 concentration was significantly higher for RFM (715.91±27.55) than NM (584.58±27.79), CM (580.61±33.23) and DIU (393.74±29.91) respectively. The same pattern was found in pre-calving period and the day of calving, but in the post-calving period group RFM was (758.08±25.55) followed by groups CM (670.41±28.83), NM (604.85±22.26) and DIU (377.61±25.50), respectively and the differences were significantly higher (P<0.01). Due to the effect of days, IL-18 concentration was significantly (P<0.05) affected in pre and/or post-calving periods. Also, IL-18 concentration was highly significantly (P<0.01) affected due to the interaction between groups and days. Group CM showed a liner increase in IL-18 concentration from day -30 pre-calving till day 90 post-calving, whereas, group RFM showed the same pattern except for days 60 and 90 post-calving. Group DIU showed a less valuable of IL-18 compared to other groups through the experimental period. In the day of calving DIU group show a sharp decrease in the level of IL-18 and then rose again after that. Interleukin-18 (IL-18) is a cytokine produced by activated macrophages that belongs to the IL-1 family, known as an (IFN-γ) inducing factor (IGIF) because of its ability to induce IFN-γ production by Th1cells **(Ushio *et al.,* 1996)**, promotes the synthesis of IL-6 **(Takahata *et al.,* 2001)**, activates natural killer (NK) cells and also plays a relevant role in the protection against bacterial infections, a central role in the inflammatory cascade and in the process of innate and acquired immunity and has an immune-regulatory function **(Zhang *et al.,*2004).** Involved in reproductive immunology, such as the maternal-fetal interface **(Tokmadzic *et al.,* 2002)** pregnancy and labor **(Ida *et al.,* 2000)** and neonatal immunity **(Takahata *et al.,* 2001)**. In human pregnancy, IL-18 mRNA level was seen to be reduced during the first and second trimesters. IL-18 levels in the serum from pregnant women were gradually elevated from the first trimester until the onset of labor **(Ida *et al.,* 2000)**. Suggested that IL-18 plays an important role in the Th1/Th2 immuno-trophism of the host during pregnancy and the neonatal period **Yoshihiro *et al.* (2005)**. However, scarce studies on IL-18 related with reproductive diseases has been performed in domestic animal species so far in spite of the importance of their reproduction. During bovine pregnancy **Nakanishi *et al.* (2001)** revealed to the expression of IL-18 may be suppressed on the fetal side due to IL-18 is an IFN-γ inducing factor that is involved in the

Th1 response and inflammatory response. Also, **Yoshihiro *et al.* (2005)** detected a significant levels of IL-18 in the sera of normal pregnant cows; suggest that IL-18 may be play an important role in the maintenance of pregnancy in dairy cows. **Erminio *et al.* (2015)** pointed to immune variations and inflammatory conditions can be an indicator to improve the dairy cows status in the peripartum period. Studied the relation between pro-inflammatory cytokines (IL-1β and IL-6) and periparturient health problems of Holstein-Friesian cows before and after parturition by month. Elucidate that cows with the highest pro-inflammatory cytokine concentrations in the last month of pregnancy showed the worse health status in early lactation. Our results showed that IL-18 concentrations were low during pregnancy in all groups except DIU group because they are mainly released by Th1 cells and elevation concentrations of IFN-γ which means unbalance of Th1 and Th2 ratio. Similar results were also reported in dairy cows by **Ishikawa *et al.* (2004), Trevisi *et al.* (2012) and Jahan *et al.* (2015).** At parturition, the decrease of the Th2/Th1 ratio should occur rapidly in the uterus, switching from a condition that provide tolerance for the foetus (Th2) to a condition that offers protection against infecting agents. **Orsi *et al.* (2006)** indicated that, the Th1-Th2 dichotomy poorly describes the complex immunological process like pregnancy in mice. **Galvao *et al.* (2012)** reported that impaired monocyte function at various days of parturition from calving to 42 days post calving to be associated with the development of metritis. The results of our present study disagreement with **Erminio *et al.* (2015)** who found that concentrations of pro-inflammatory cytokines were remarkably reduced after calving due to the strong increase of 17β-estradiol **(Rogers and Eastell, 2001)** and cortisol **(Oppert *et al.*, 2005),** both markedly increase at calving time **(Goff and Horst, 1997**). Moreover, **Vitoratos *et al.* (2008)** suggested a role of placenta in the synthesis of some cytokines (*e.g.* IL-1b, adiponectin) and explained the reduced concentration of pro-inflammatory cytokines in plasma after parturition due to the removal of placenta. **Erminio *et al.* (2015)** and **Ishikawa *et al.* (2004)** were agreement on high concentrations of pro-inflammatory cytokines in pre-partum were able to predict post-partum reproductive disorders. In contrast, **Islam *et al.* (2013a)** recently suggested that low concentrations of IL-1b at 15 d prior or at calving may be used to identify the cows susceptible to develop post-partum reproductive diseases. Add to that, the higher IL-1 level in cows with postpartum reproductive disorders on day 30 is in agreement with the finding of increased expression of IL-1 on day 35 and 49 postpartum in endometrial cells from endometritie cows and on the day 0 to 7 and 21 to 28 by *E. coli*

stimulated circulating monocytes **Galvao *et al.* (2012)**, and these rises might be associated with infection and inflammation of uterus. In human pregnancy, **Reinhard *et al.*(1998)** mentioned to the maintenance of pregnancy is associated with an altered Th1/Th2 balance and cytokines produced by Th2 cells predominate over those produced by Th1 cells **(Saito,2000)**. In addition to that, during pregnancy period is useful for a successful peripartum because it optimizes the feto-maternal immune interaction and vascularization **(Reinhard *et al.*, 1998).** In human suffering from endometriosis both **Fairbanks *et al.* (2009) and Glitz *et al.* (2009)** found that there is no alteration in the concentration of IL-18 in serum or peritoneal fluid and no difference in IL-18 in patients or controls with or without infertility due to minimal or mild endometriosis. In conclusion, these data support the utility of PIC (IL-10 and IL-18) measurement in late pregnancy as prognostic markers to identify the buffalo cows that will develop RFM and CM.

Table (2): Interleukin 18 concentration (pg/mL) in the peripheral blood of buffalo cows with or without postpartum reproductive diseases.

Group sldays	Periparturient period													
	Pre-calving					Calving	Post-calving							
	-30	-15	-5	-1	Ave. (-30 to -1 days)	0	+7	+15	+30	+45	+60	+90	Ave. (7 to 90 days)	Overall men
NM	611.93^{Cb} ±43.61	577.16^{Eb} ±44.99	539.15^{Eb} ±32.54	529.15^{Cb} ±30.05	564.35^{b} ±37.80	543.89^{Fb} ±20.92	565.83^{EFb} ±11.69	575.18^{Eb} ±7.83	607.49^{CDc} ±33.34	652.45^{Ac} ±21.37	638.46^{Bc} ±22.28	589.71^{Dc} ±37.06	604.85^{c} ±22.26	584.58^{b} ±27.79
RFM	638.29^{Ea} ±36.70	653.88^{Ea} ±38.62	663.82^{DEa} ±35.21	672.53^{Da} ±14.56	657.13^{a} ±31.27	697.96^{CDa} ±24.63	703.23^{Ca} ±29.07	707.56^{Ca} ±32.40	765.91^{Ba} ±15.91	802.69^{Aa} ±28.07	800.83^{Aa} ±30.16	768.29^{Ba} ±17.73	758.08^{a} ±25.55	715.91^{a} ±27.55
CM	422.84^{Id} ±41.32	462.24^{Hc} ±43.79	480.55^{GHc} ±37.03	496.05^{Gc} ±32.06	465.42^{c} ±38.55	502.59^{Gc} ±38.34	524.88^{Fc} ±38.11	570.00^{Ec} ±18.07	614.68^{Db} ±15.90	731.53^{Cb} ±25.32	778.28^{Bb} ±39.93	803.06^{Aa} ±35.67	670.41^{b} ±28.83	580.61^{b} ±33.23
DIU	495.13^{Ac} ±58.59	450.90^{Bd} ±28.03	415.75^{Cd} ±25.44	400.47^{Dd} ±14.53	440.57^{d} ±31.65	303.25^{Gd} ±49.34	324.59^{Fd} ±46.06	342.36^{Ed} ±33.26	391.73^{DEd} ±18.36	393.71^{DEd} ±12.04	408.70^{Cd} ±19.99	404.54^{CDd} ±23.32	377.61^{d} ±25.50	393.74^{c} ±29.91

Values are shown as mean _ SEM; means with different superscripts in a column (a, b, c and d) and row (A, B, C,....) differ significantly ($P < 0.05$).

Abbreviations: NM, normal ;RFM, retained fetal membrane; CM, clinical metritis and DIU, delayed involution of the uterus. * $0.05 < P < 0.10$. ** $P < 0.05$. *** $P < 0.01$.

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