Vet.Med.J.,Giza. Vol.49, No.4. (2001):609-622.

MODULATORY EFFECTS OF BUFFALO BASIC AND ACIDIC UTERINE LUMINAL PROTEINS ON PHAGOCYTIC AND BACTERICIDAL ACTIVITIES OF POLYMORPHONUCLEAR- AND MONONUCLEAR LEUKOCYTES IN VITRO

A. A. RAMADAN, A. A. ZAKI,* H. M. HASSAN and M. HASHAD**

Immunobiology and Immunopharmacology Unit; Biology of Reproduction Department *, Animal Reproduction Research Institute, P.O. Box 12556, Giza, Egypt and Microbiology Dept.**, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Received: 18. 8. 2001

Accepted: 9. 9. 2001

SUMMARY

Potential immunological effects of basic and acidic uterine luminal proteins (ULP) collected from estrus- and luteal-phase buffaloes were studied in vitro. The phagocytic and microbcidal activities of PMN and macrophages, nitric oxide production by macrophages, and superoxide anion production by PMN were elucidated. Estrus-phase basic and acidic ULP collected from buffaloes enhanced phagocytic activity of PMN at all concentrations tested, whereas basic and acidic ULP of luteal-phase buffaloes suppressed phagocytic activity in a dose-dependent manner. The bactericidal activity of buffalo PMN increased in response to basic ULP of estrus-phase buffaloes in comparison with basic luteal-phase proteins. Meanwhile, acidic ULP proteins of estrus-phase buffaloes showed significant increase in bactericidal activity only at 40, 80, and 150 µg/ml protein concentrations in contrast to acidic ULP of luteal-phase buffaloes. Nitric oxide production by macrophages increased in response to basic ULP of estrus-phase buffaloes, whereas this activity decreased when macrophages were treated with different concentrations of basic ULP of luteal-phase buffaloes. Only at high concentrations the acidic protein of estrus-phase buffaloes increased nitric oxide production by macrophages as compared with acidic proteins from luteal-phase buffaloes. Superoxide anion productions by neutrophils increased in response to basic and acidic ULP of estrus-phase buffaloes and decreased in response to acidic proteins from luteal-phase buffaloes.These data demonstrate that buffalo uterus contains immunologically active secretory proteins that can influence the natural defense barriers of the buffalo genital tract.

Key words: buffalo, uterine proteins, estrous cycle, phagocytic cells

INTRODUCTION

Being responsible for carrying allograft and subjected to many physiological changes during the reproductive life, the uterus is physiologically a unique organ. Most importantly, during the estrus phase the genital tract is prepared for matting i.e., the cervix is opened and the probability of introducing ascending infection is high likely either through semen itself or as a consequence of environmental contamination. It seems that the uterus, in itself or by the aid of ovarian hormones, can protect itself during certain times when infection can get an access into the genital tract; during the estrus phase. Recent study indicated that ewes received large numbers of pathogenic bacteria during the estrus phase did not develop any signs of infection and the bacteria were cleared from the uterus 2 days later, whereas ewes received same number of pathogenic bacteria during the luteal phase developed acute endometritis and sever fever (Ramadan et al., 1997). The uterus is known to secrete large and small molecular weight glycoproteins that demonstrated immunological activities in vitro. Uterine luminal proteins (ULP) collected from pregnant cows (Segerson et al., 1984 Segerson et al., 1986, Segerson et al., 1990) and ewes (Segerson, 1981, Segerson, 1984, Segerson, 1988, Stephenson et al., 1989) suppressed lymphocytic activity in response to different mitogens. On the other hand, ULP collected from estrus-phase ewes enhanced lymphocytic activity in response to PHA in vitro. In a previous study, we investigated the effect of two fractions of ULP collected from estrus- and luteal-phase buffaloes on phagocytic activity of neutrophils in vitro (Ramadan and Hassan, 1999) and we concluded that estrus-phase ULP contains large and small MW proteins that enhanced phagocytosis in vitro. Phagocytosis is the first step, in a complicated cascade of immunological events, in the elimination of infection but consequent killing of invading pathogen is more important. Moreover, the phagocytic cells possess many killing systems that are activated after pathogen entry inside the PMN. Therefore, current work aimed to 1- study the possible immunological activity of fractionated acidic and basic ULP collected from buffaloes during estrus and luteal phases of the estrous cycle on phagocytic and kill activities of neutrophils and 2- study whether or not killing systems of PMN will be affected by ULP and which system, nitric oxide (NO) or superoxide anion (O-2), would be more manipulative.

MATERIAL AND METHODS

Collection of Uterine Luminal Proteins

The ULPs were collected as previously described (Ramadan et al., 1997). Whole genital tracts of buffalo were collected immediately after slaughter and transferred to the laboratory on ice. The uteruses were then classified according to the

dominant ovarian structure so that genital tracts with ovaries carrying mature Grafiaan follicles (>6 mm in diameter) were classified as the follicular-phase group (F), and genital tracts with large corpora lutea (CL) on the ovaries were classified as luteal-phase group (L). In addition, gross inspection indicated that genital tracts with large Grafiaan follicles were firm, while those with large CL were flaccid. Each genital tract was kept on ice. The cervix was firmly legated, and approximately 30 ml of 0.33 M NaCl solution (pH 7.2) containing 0.02% NaN3 were injected directly into the lumen of each uterine horn. The whole uterus was massaged gently for a 10 minutes, then the tip of 1 uterine horn was removed and the flushing medium drained through the opening and transferred into large chilled flasks. Uterine luminal proteins from each group (Group F or L) were pooled, centrifuged in a refrigerated centrifuge at 10,000 xg for 30 min, lyophilized to remove moisture and kept at -20°C until further fractionation. Protein concentration was assayed (Bradford, 1976) for each pooled group.

Ion-Exchange Chromatography:

Diethylaminoethyl sephadex A-50 (Sigma Chemical Co., St. Louis, MO, USA) anion exchanger (DEAE-sephadex) was utilized to separate basic and acidic ULP. The lyophilized ULP from each group was reconstituted in 10 mM Tris-HCl buffer (pH 8.2) containing 0.02% NaN3 and extensively dialyzed against Tris-HCl buffer. Small aliquots were loaded onto 15 (1.5- cm column of DEAE-sephadex that was preequilibrated in same buffer. Basic proteins were eluted first from the column in Tris-HCl buffer, then acidic proteins were eluted with 0.5 M NaCl in Tris-HCl buffer. The eluted basic and acidic proteins from each group were pooled separately and extensively dialyzed against three changes of distilled water at 4°C to remove salts, especially sodium azide and NaCl. Dialyzed pure proteins were lyophilized.

Isolation of Polymorphonuclear Leukocytes (PMN):

Polymorphonuclear leukocytic cells were isolated as previously described (Ramadan and Hassan, 1999). Briefly, blood was obtained from one buffalo-bull in heparinized sterile tubes and centrifuged at 1200 xg for 15 min at 4°C. The leukocytic layer was harvested carefully and mixed with 1 ml HBSS (Gibco Laboratories, Grand Island, NY, USA). One ml of the mixture was laid slowly onto 5 ml Ficol-Paque solution (Gibco). The tubes were centrifuged at 400 x g for 25 min at room temperature and the pellet, which contained the PMN cells, was collected and treated with 20 ml cold (4°C) double distilled water to lyse the red blood cells and after exactly 45 seconds 10 ml of 2.7% NaCl were added to stop lyses. The collected pellet was washed once and suspended in HBSS. Cell viability (96± 2) was determined by Trypan blue dye. The PMN (92 ± 3) concentration was adjusted to 8 $X10^6$ viable cell/ml.

Preparation of Bacteria:

Heavy inoculum of freshly cultured Staphylococcus aureus on brain-heart infusion broth (Difco Laboratories, Detroit, MI, USA) was centrifuged at 2000 xg for 15 min and the pellet was washed twice with HBSS. The bacteria were opsonized with 10% homologous serum (5 different buffalo serum samples) for 30 min at 37°C and 5% CO_2 with gentle shaking. After opsonization, bacteria were centrifuged, washed once and suspended in HBSS at a concentration of 12 x 10^7 bacterial cell /ml.

Phagocytic and Intracellular Killing Assays:

Both phagocytic and intracellular killing assays were performed as described before using acridine orange stain (Hogan et al., 1990) with slight modification. When different concentrations of basic ULP isolated from L group buffaloes were incubated with the PMN for 20 min before the addition of opsonized bacteria to the mixture, no phagocytosis was observed after staining the cells with acridine orange. Therefore, uterine proteins, PMN and bacteria were added together at the same time. Briefly, 100 µl of PMN suspension in HBSS (8 x 10^5 PMN cells) were mixed with 100 µl of bacterial suspension in HBSS (12 x 10^6 bacterial cell) in 1.5-ml sterilized culture tubes. Then 100µl of basic or acidic fractions of ULP containing different concentrations of proteins (10, 20, 40, 80, 100, 200 and 300µg) were added to the mixture of PMN and bacterial cells. The test tubes were incubated for 2 h at 37°C in humidified incubator at 5% CO_2 with continuous gentle shaking. Following incubation, small aliquots were removed from each tube and diluted with mixture of acridine orange (14 mg/100 ml saline), crystal violet (50 mg/100 ml saline) at ratio of 2:1:1. Three wet mount slides were prepared from each concentration and the number of live (green) and dead (red) bacteria were counted separately per 50 neutrophils in each slide using Nikon fluorescent microscope (oil immersion lens, (1000). Phagocytic activity of PMN was calculated as average number of bacteria phagocytosed per neutrophil. Percent kill activity was determined as follow:

$$\frac{\text{number of dead phagocytosed bacteria}}{\text{number of live + number of dead phagocytosed bacteria}}$$

Bacterial sample (10^6 /ml) without treatment of PMN was prepared and incubated with other samples as control and the number of living colony forming unit (cfu) was determined by plating known volume of this sample on Brain-heart infusion agar and counting the number of live bacteria. The number of live bacteria obtained from the control was used to correct percent intracellular kill activity of PMN because dead bacteria that were phagocytosed would not be distinguished from bacteria that were killed intracellularly.

Preparation of Macrophage:

Blood was collected from single bull in hepari-

nized sterile culture tubes and centrifuged at 3000 rpm for 15 min at 4°C. The leukocytic layer was aseptically aspirated using pasture pipette and laid onto Ficol-Paque solution (Gibco) and centrifuged at 400 x g for 30 min at room temperature. The interface layer that contains mononuclear leukocytes (MNL) was aseptically aspirated and centrifuged at 1200 xg for 5 min. The residual red blood cells were lysed using cold distilled water for 45 seconds followed by addition of 2.7% NaCl solution. The MNL were washed twice with complete RPMI medium (containing 2 mM L-glutamine, 25 mM HEPES, 10-5 M mercaptoethanol and 100 U/ml penicillin and 100µg/ml streptomycin) and suspended in the same medium. The final number of MNL was adjusted to 4 x 10^6 cell/ml medium. From the MNL cell suspension, 100µl aliquots were transferred into 96-well tissue culture flat-bottom plat and incubated in humidified CO_2 incubator at 38.5°C and 5% CO_2. Three wells were prepared for each protein concentration in each treatment. Three hours later, the non-adherent cells were aspirated from the wells and 200 µl of complete RPMI medium were added to the adherent cells. The plates were incubated for three days under the same conditions.

Measurement of Nitric Oxide Production by Macrophages:

Nitric oxide was measured as described before (Rajaraman et al., 1998) with slight modification. At end of incubation, the medium was aspirated from the plates and 50µl of PBS was added to each well together with 50 µl of heat-killed bacteria (Staphylococcus aureus) and 50 µl of uterine protein. Triplicates were prepared from each concentration of the test proteins. The plates were incubated for 2 h at 38.5°C in 5% CO_2 incubator. At end of incubation, the supernatants (100µl) from each well were transferred into flat-bottom ELISA plate and 100µl of Griess reagent (0.5% sulfanilamide; Sigma Chemical Co.) in 2.5% phosphoric acid (Mereck Co.) and 0.05% N-(1-naphthyl)ethylenediamine dihydrochloride (Sigma Co.). The mixture was incubated at 21°C for 10 minutes. Absorbance of test and standards were measured at 570 nm using ELISA reader (Dynatech MR7000; Dynatech Laboratories Inc.). Absorbances of test samples were converted to micromolar (µM) of nitrite by comparison with absorbance values of sodium nitrite (Sigma Co.) standard curve within a linear curve fit.

Measurement of Superoxide Anion Production by Polymorphonuclear Leukocytes:

The assay was performed as described before (Abu El-Asrar et al., 1996) using superoxide dismutase-inhabitable cytochrom C reduction (Cohen et al., 1978) in the presence or absence of stimulus, and measured spectrophotometrically. Polymorphonuclear cells were isolated as described before. The assay was performed in triplicate at pH 7.3 and 37°C using flat-bottomed microtiter plate. Each well received 50 µl of PMN suspen-

sion in PBS (10^5). Then 400 µg of cytochrom C (Sigma, St. Louis, MO, USA) plus 100 µg opsonized zymozan (Sigma), as a stimulus, were added in 100µl to the test wells. The first 4 wells in the plate received PMN cell suspension plus cytochrom C and served as an indicator of basal activity of PMN. The second 4 wells received PMN plus cytochrom C and zymozan plus superoxide dismutase (Sigma) (4 units/well) and served as blank reference to test wells. Tested proteins were added to test wells in different concentrations. The plate was incubated at 37°C with 5% CO_2 tension for 2 hours and then read at 550 nm using ELISA reader (Dynatech MR7000). The time points at which maximum superoxide anion production was determined in preliminary experiment and the maximum production was observed at 2 hours. Basal activity of PMN in the first 4 wells was used to normalize the test and blank (reference) wells. Superoxide anion production was calculated as follow:

Λ of blank refernce wells - Λ of treated wells x 15.87

Where 15.87 is a coefficient calculated based on the quantity of solution per well and dimension of the well. Data expressed as O^{-2} nmol/10^5 PMN/ 120 minutes.

Statistical Analyses

General linear models procedure (GLM) of Statistical Analysis System (SAS) was used to test effect of treatments on phagocytic and killing activity of PMN and on nitric oxide production by macrophages as well as OFR production by PMN. Studentís t test was utilized to test the differences between treatments (estrus basic vs luteal basic proteins and estrus acidic vs luteal acidic proteins). Treatments were considered significant at $P < 0.01$. Data were presented as means (MSE. Phagocytic ability data were presented as log10 and percent intracellular killing data were transformed to arcsine.

RESULTS

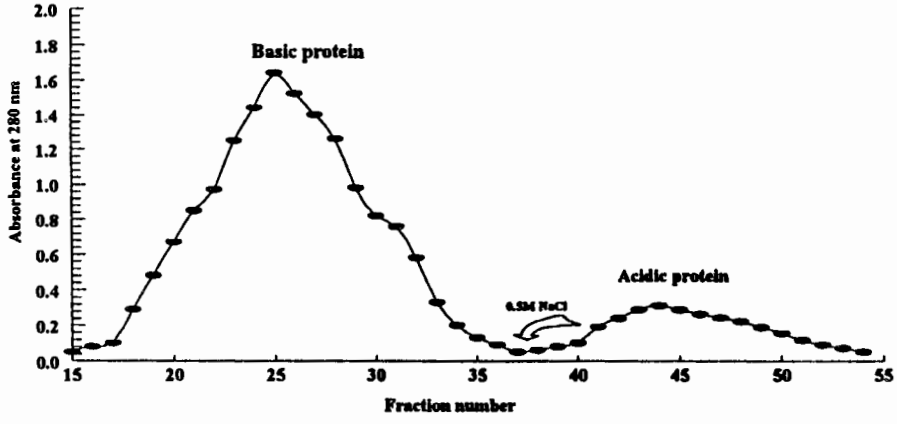

Figure 1. Chromatogram representing the elution pattern of uterine luminal proteins collected from estrus-phase buffaloes and fractionated on DEAE-sephadex ion-exchange column (15x1.5 cm). Protein was monitored at 280 nm.

Uterine luminal proteins from estrus-phase buffaloes resolved one basic and one acidic protein peak (Figure 1) that was monitored at 280 nm, whereas, ULP from luteal-phase buffaloes resolved three basic peaks and one acidic peak (Figure 2). The three basic peaks collected from luteal-phase buffaloes were pooled together in one fraction.

Basic and acidic fractions of ULP collected from uterus of buffaloes during the estrus phase significantly ($P < 0.01$) enhanced phagocytic activity of PMN at all concentrations tested (Table 1). The enhancing effect of the basic proteins were, however more significant ($P < 0.01$) than the acidic proteins of the estrous phase. On the other hand, basic and acidic fractions collected during luteal

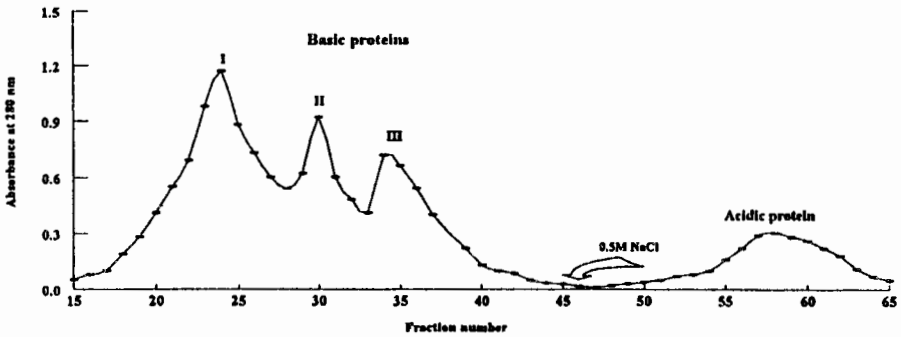

Figure 2. Chromatogram representing the elution pattern of uterine luminal proteins collected from luteal-phase buffaloes and fractionated on DEAE-sephadex ion-exchange column (15x1.5 cm). Protein was monitored at 280 nm.

TABLE 1. Phagocytic ability[a] of blood mononuclear leukocytic cells treated with basic or acidic proteins from estrus and luteal phase buffaloes.

	10µg	20µg	40µg	80µg	150µg	200µg	300µg
Estrus basic $\bar{X}$	0.91^{b}	0.9^{b}	0.87^{b}	0.89^{b}	0.89^{b}	0.94^{b}	0.92^{b}
± SE	0.039	0.041	0.039	0.046	0.043	0.034	0.02
Estrus acidic $\bar{X}$	0.68^{c}	0.68^{c}	0.67^{c}	0.66^{c}	0.62^{c}	0.58^{c}	0.55^{c}
± SE	0.032	0.031	0.029	0.026	0.025	0.023	0.025
Estrus basic $\bar{X}$	0.52^{d}	0.36^{d}	0.26^{d}	0.23^{d}	0.18^{d}	0.13^{d}	0.11^{d}
± SE	0.019	0.013	0.009	0.009	0.011	0.012	0.01
Estrus acidic $\bar{X}$	0.41^{c}	0.37^{d}	0.35^{c}	0.30^{c}	0.24^{c}	0.20^{c}	0.15^{d}
± SE	0.019	0.015	0.015	0.012	0.012	0.01	0.009

Number of bacteria phagocytosed (log10)/neutrophil.
bcdeDifferent superscripts within subcolumn significantly differ ($P < 0.01$)

phase suppressed phagocytic activity in a dose-dependent manner. At high concentrations of estrus-phase acidic proteins (200 and 300μg) phagocytic ability slightly decreased.

Killing activity PMN significantly increased ($P < 0.01$) in response to basic ULP of estrus-phase buffaloes in comparison with basic proteins of luteal-phase ones, which suppressed kill activity in dose-dependent manner (Figure 3). Meanwhile, acidic ULP proteins of estrus-phase buffaloes showed significant increase in killing activity only at 40,80, and 150 μg /mlprotein concentrations in comparison with acidic ULP of luteal-phase buffaloes (Figure 4).

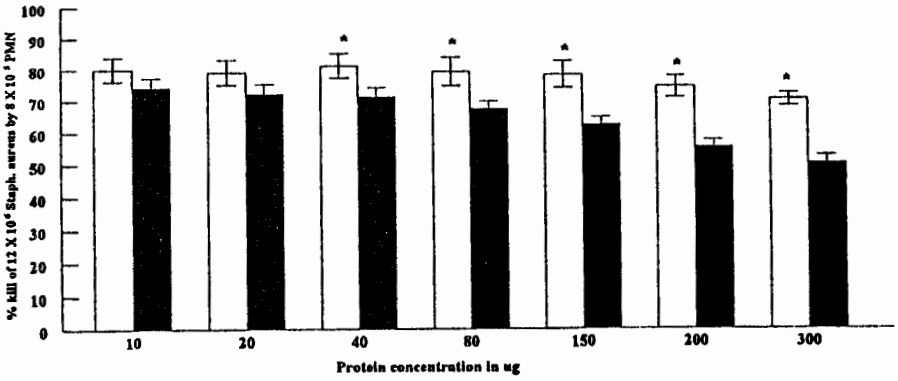

Figure 3: Percentage of killed bacteria by PMN treated, in vitro, with different concentrations of basic ULP collected from estrus- (open bars) and luteal-phase (closed bars) buffaloes (*P < 0.01).

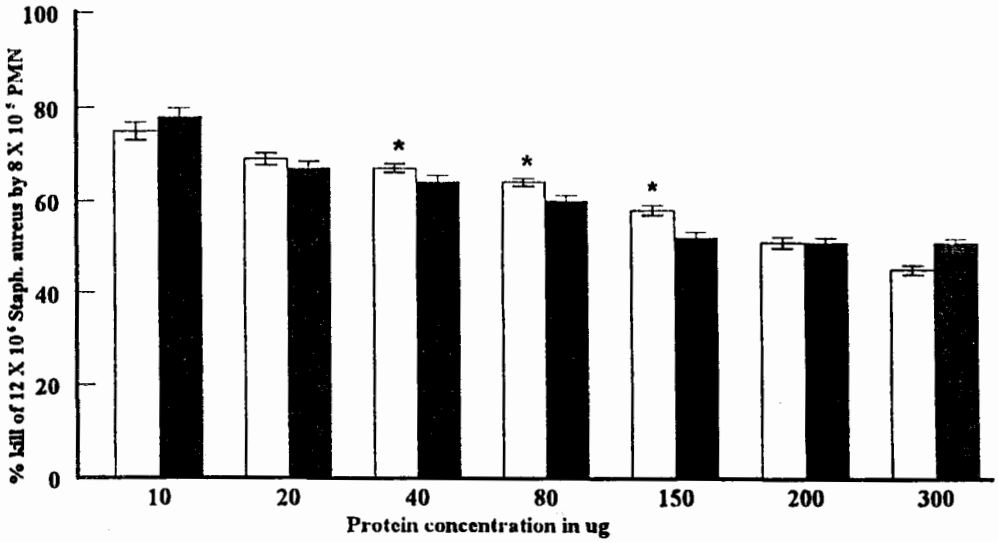

Figure 4. Percentage of killed bacteria by PMN treated, in vitro, with different concentrations of acidic ULP collected from estrus- (open bars) and luteal-phase (closed bars) buffaloes (*P < 0.01).

Macrophages treated with basic fraction of ULP collected from estrus-phase buffaloes manifested significant ($P < 0.01$) increase in the production of nitric oxide than those treated with basic proteins collected from luteal-phase buffaloes at all concentrations tested (Figure 5). Meanwhile, macrophages treated with acidic ULP of estrus-phase buffaloes showed significant ($P < 0.01$) enhancement of nitric oxide production by macrophages in comparison with acidic luteal-phase. This effect, however, was only recorded using high concentrations of tested protein (150, 200 and 300 µg/ml) (Figure 6).

The rate of OFR production by buffalo neutrophils was significantly higher ($P<0.01$) in presence of most of the tested concentrations of basic and acidic fractions of ULP collected from estrus-phase buffaloes than basic and acidic fractions collected from luteal-phase buffaloes (Figures 7 and 8).

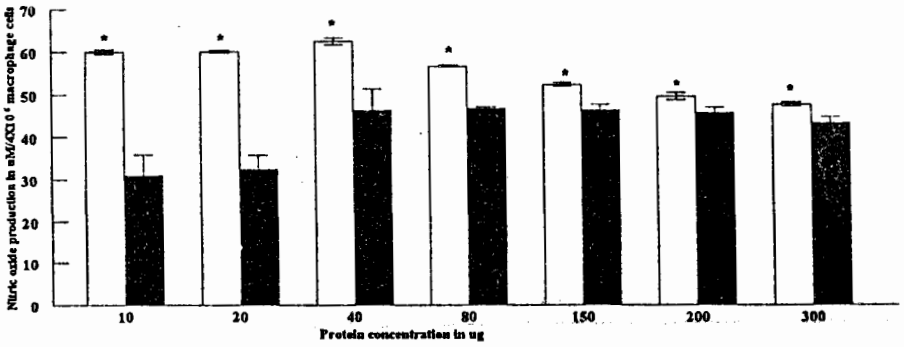

Figure 5. Nitric oxide (micromolar of nitrite) produced by buffalo blood macrophages ($4X10^6$) treated with different concentrations of basic ULP collected from buffaloes during estrus (white) and luteal (black) phases of the estrous cycle (*$P < 0.01$).

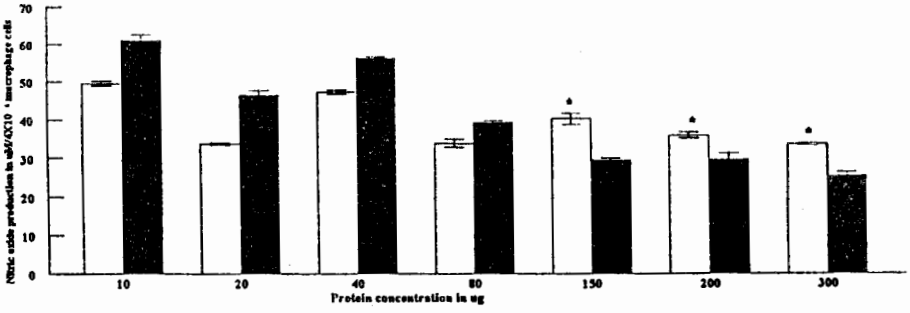

Figure 6. Nitric oxide (micromolar of nitrite) produced by buffalo blood macrophages ($4 X 10^6$) treated with different concentrations of acidic ULP collected from buffaloes during estrus (white) and luteal (black) phases of the estrous cycle (*$P < 0.01$).

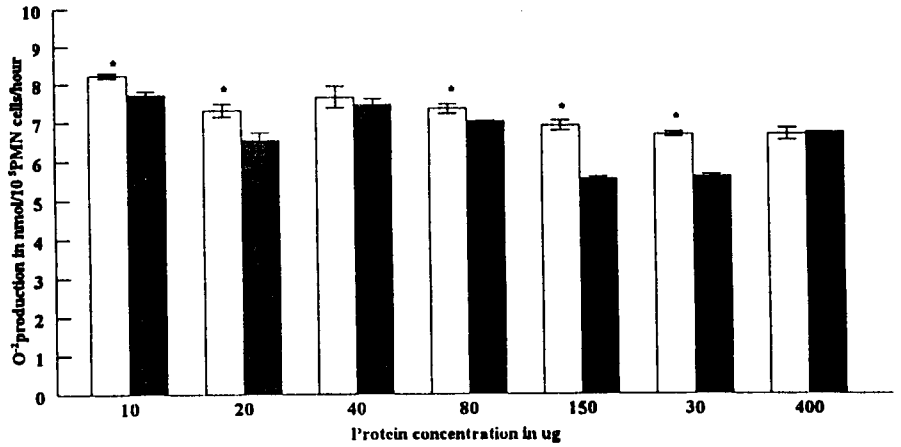

Figure 7. Oxygen-free radical (nanomols of $O^{-2}/10^5$ neutrophils/120 minutes) produced by buffalo neutrophils treated with different concentrations of basic ULP collected from buffaloes during estrus (white) and luteal (black) phases of the estrous cycle (*P < 0.01).

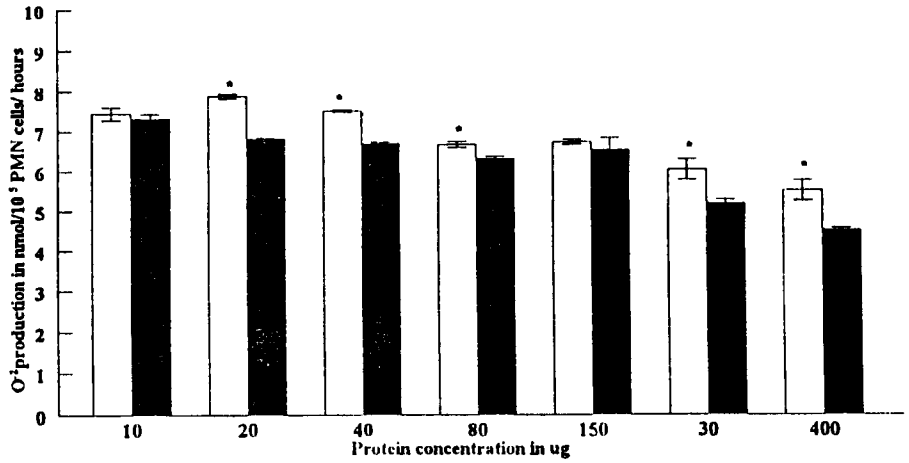

Figure 8. Oxygen-free radical (nanomols of $O^{-2}/10^5$ neutrophils/120 minutes) produced by buffalo neutrophils treated with different concentrations of acidic ULP collected from buffaloes during estrus (white) and luteal (black) phases of the estrous cycle (*P<0.01).

DISCUSSION

Comprehension of the interactions between immunological and endocrinological events of reproduction is a prerequisite for understanding the uterine defense mechanism against pathogens. Immunological adjustment of the uterine response against pathogens seems to be regulated in general by the hormonal changes during the estrous cycle and is clearly demonstrated by the competence of the uterus throughout the cycle. During the surge of estrogens, the uterus is highly competent and is able to protect itself from infection. On the contrary, during the elevated concentration of proges-

terone in the body, the uterus is incompetent against infections. Indeed, this incompetence state is necessary for the survival of the fetal allograft if pregnancy to be successful.

Current data demonstrated that basic and acidic uterine luminal proteins of buffaloes regulated PMN functions in vitro. Estrus-phase basic and acidic proteins enhanced phagocytic ability and killing activity of neutrophils in vitro. Moreover, the killing systems of PMN, represented by nitric oxide and superoxide anion productions, increased in response to different doses of basic and acidic fractions of ULP. On the other hand, basic and acidic proteins of luteal-phase buffaloes suppressed phagocytic and killing systems of PMN. It was clear that acidic fraction in estrus and luteal proteins were less effective than basic fraction and sometimes were paradoxical in effect.

Apparently, the uterine secretory proteins are induced and controlled by the reproductive hormones, especially estrogens and progesterone. Indeed, the secretory pattern of ULP varies throughout the estrous cycle of mice (Horvat et al., 1992) and guinea pig (Ogilvie et al., 1991). Moreover, the bioactivity of these proteins is associated with the secretory pattern. Lander et al. (1990) found that uterine PMN collected during the estrus phase produced more superoxide anions than those collected during diestrous phase, and it was concluded that the uterine secretory proteins altered, in situ, the activity of PMN. On the other hand, megasuppressin molecules (rich in protein and carbohydrates), isolated from the uterus of pregnant ewes and cows, suppressed lymphocyte activity in vitro (Stephenson et al., 1989, Segerson et al., 1986). Moreover, progesterone injection in ovariectomized ewes induced the uterus to secrete same immunosuppressive molecules (Stephenson and Hansen 1990, Moffatt et al., 1987). In a recent study (Ramadan, and Hassan 1999) large molecular weight protein was isolated from the uterus of buffaloes and this protein altered phagocytic activity of PMN in vitro.

Alteration of uterine cellular immune response during pregnancy is a known phenomenon that protects the fetus from being rejected by the maternal immune system. An immunosuppressive effect of ULP collected under increased concentrations of progesterone on lymphocytes has been observed in ewes (Segerson et al., 1984, Hansen et al., 1987, Ramadan et al., 1997) and cows (Segerson and Bazer 1989). Moreover, increased concentration of progesterone in the body is associated with reduced number and activity of uterine neutrophils and lymphocytes (Staples et al., 1983, Staples et al., 1983, Asbury and Hansen 1987) and increased susceptibility of the uterus to bacterial infection (Staples et al., 1983, Low and Hansen 1988, Ramadan et al., 1997). In current study, basic luteal-phase ULP downregulated phagocytic cell activities and the effect extend to the killing systems of these cells in vitro. Apparently, progesterone, during luteal phase or during pregnancy, induces the uterus to secrete immunosuppres-

sive molecules that alter the functions of lymphocytes and phagocytic cells. But how progesterone induces the secretion of these molecules?, and the events that are associated with stimulation, synthesis and secretion of such molecules are unknown.

Phagocytosis is an important event in the immune response cascade during pathogen invasion; it is a prerequisite for T and B cell response and differentiation and pathogen elimination. It seems that immunoactive secretory proteins of uterine origin acts directly or indirectly on the immune cells regardless of their type or function. A receptor model is postulated for the modulatory actions of ULP, because it is protein in nature and cannot passively pass through the cell membrane of the immune cells. The universal action of ULP may be mediated through specific or non-specific receptor. Yet it is likely high that the modulatory effect of ULP is mediated through a non-specific receptor because of the wide array of cells that are affected by these proteins. Not only phagocytic activity of PMN are modulated by ULP, but also the enzymatic systems of killing activity have been also affected. Nitric oxide (NO), a powerful killing molecule secreted by macrophages, increased in amount in response to basic proteins of estrus-phase buffaloes compared with basic fraction of luteal phase. Surprisingly, acidic fraction of luteal-phase buffaloes induced the production of NO more than that of estrus-phase buffaloes especially at low protein concentrations (10μg - 80μg/ml), but at higher concentrations, luteal-phase acidic proteins became suppressor to NO production than estrus-phase acidic one. A biphasic activity of luteal-phase ULP is proposed and it may indicate that a high concentration of this protein is required to suppress activity of PMN.

Superoxide anion (O-) production by PMN in response to basic and acidic ULP of estrus-phase buffaloes was significantly greater than that produced in response to same fractions of luteal-phase buffaloes. Also NO produced more by macrophages pulsed with basic proteins of estrus-phase buffaloes than basic fraction of luteal-phase buffaloes. Apparently, two powerful killing systems of phagocytes, namely; NO and O- are modulated by ULP in vitro, although NO may be affected more than O- in the current study.

In conclusion, uterine secretory proteins collected from buffaloes during the estrous cycle manipulated many cellular immune parameters in vitro. Phagocytic ability, bactericidal activity, NO and O- production by phagocytic cells were upregulated by estrus phase ULP of buffaloes, whereas luteal phase ULP downregulated the same parameters. A positive correlation between the secretion of these ULP and the hormonal changes in the body is proposed and a receptor-mediated action of these proteins is suggested based on the wide array of effects practiced by these proteins in vitro. The nature of suggested receptor and the mo-

lecular events accompanying actions of ULP on PMN is unknown.

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