The Indian Journal of Animal Reproduction; 25(1): 25-28; June 2004

Alkaline phosphatase activity and immunoglobin concentration in *in-vitro* uterine flushings of buffaloes (*Bubalus bubalis*)

K. PROMOD[1], S. MAHMOOD[2†], A.G. TELANG[3], R. SINGH[4] AND G.S. BISHT[5]

Livestock Production and Management
Indian Veterinary Research Institute, Izatnagar - 243 122 (UP)

Received : April 15, 2002
Accepted : February 5, 2003

ABSTRACT

Alkaline phosphatase activity and immunoglobulin concentration during follicular phase, luteal phase (early, mid and late), inactive ovarian stage and infected uterine flushing *in-vitro* was studied in buffalo uteri (n=55) collected from local abattoir. The alkaline phosphatase activity varied significantly (P<0.05) between two uterine horns during early and late-luteal phase, however, the difference was non-significant during other stages. On the other hand, immunoglobulin concentration was non-significant between two uterine horns at all the stages. Both alkaline phosphatase activity and immunoglobulin concentration were significantly (P<0.01) higher in infected compared to other stage uterine flushings. Thus, in conclusion, these two parameters simultaneously could be used as an indicator of sub-clinical inflammatory conditions.

Key Words - Buffaloes, uterine flushing, immunoglobulins, alkaline phosphatase

Endometritis due to uterine infection and secondary inflammation has been reported to cause infertility. In bovines, alkaline phosphatase is reported to be regulated by ovarian steroids (bovine : Larson *et al.*, 1970) and activity increases in inflammed uterus (mare : Katila *et al.*, 1990). Uterine reaction against pathogenic organism has both cellular and humoral components which act as opsonin to enhance phagocytosis. Uterine immunoglobulin has been reported to increase during endometritis (cow : Brochart and Mascarenhas, 1990, buffaloes : Ahmed *et al.*, 1993).

Therefore, the present study was aimed to evaluate the alkaline phosphatase activity and immunoglobulin concentration simultaneously during various stages of oestrous cycle including infected in *in-vitro* uterine flushings of buffaloes.

MATERIALS AND METHODS

Fifty-five genital tracts were collected from non-descript buffaloes irrespective of their age, breed, parity and body weight within 30-60 minutes after slaughter. The genital organs, ligated both at cervix and utero-tubal junction, kept in a separate polythene bags, were transported to the laboratory on ice in a thermosflask.

In the laboratory, the genitalia were examined grossly for any apparent abnormalities and were categorised into follicular phase (Luktuke and Rao, 1962; Dobson and Kamonpatana, 1986) and luteal phases (Ireland *et al.*, 1980), inactive ovarian stage and infected uteri (not classified as ipsilateral and contra-lateral to assess the role of infection in this study). Each uterine horn was flushed with 10 ml of sterile normal saline (0.9% W/V) using Foleys catheters (INMED Corporation, USA) as per the method of Boos *et. al.* (1988) within 4-5 hours of collection after cleaning with normal saline and then with 70% ethyl alcohal. Equal volumes of flushed fluid recovered from each horn were centrifuged at 1500 rpm for 15 min. The supernatant part was decanted and stored in plastic vials (in duplicate) at -20°C until analysis. In supernatants, alkaline phosphatase activity was estimated as per the protocols provided with diagnostic kits (Span diagnostic, India) and immunoglobulin (Ig) concentration was estimated as per the method of Mc Ewan *et al.* (1970) with slight modification as 5 ml of Zinc Sulphate solution (250 mg/1) and distilled water (boiled and cooled) at same temperature (37±1°C) were taken in culture tubes (30 ml, Borosil, India) for test and control samples respectively. To those sets of tubes, 1 ml of uterine flushings (supernatant) was added by auto-pipette (finnpipette) to each test and control tubes. Finally, 0.1 ml of distilled water (boiled and kept similar to Zinc sulphate) was also added to make the final volume of 6.1 ml, mixed and incubated at 37±1°C for 60 min. Turbidity so developed was

[1]Kanjira Kuzhiyil, Vadayampady, Putheneruze, Ernakulum, Kerala.
[2]Sr. Scientist
[3,4]Sr. Scientist, CADRAD
[5]Scientist, Computer Centre

[†]Corresponding author

Table 1. Mean(±SE) of alkaline phosphatase activity (U/l) in uterine flushings during follicular phase, luteal phases, inactive ovarian stage and infected uteri of buffaloes

Parameters	Follicular	Luteal phase				Parameters	Inactive ovarian stage	Infected uteri
		Early	Mid	Late	Pooled			
Ipsi-lateral	130.88±20.73^{c}	376.78±36.46*^{d}	536.56±36.70^{b}	610.80±54.68*^{b}	507.06±30.81	Left	43.10±12.22^{c}	806.49±82.53^{a}
Contra-lateral	128.76±20.39^{c}	335.55±31.22^{d}	521.11±31.88^{b}	555.90±60.02^{b}	469.12±30.66	Right	38.98±8.69^{c}	823.62±82.09^{a}
Pooled	129.82±20.08^{c}	368.60±29.99^{d}	528.83±29.57^{b}	583.35±56.64^{b}	492.38±28.95	Pooled	41.04±10.13^{c}	815.06±81.26^{a}
No.of observations(n)	10	10	9	10	29		8	8

In row, figures with different superscripts differ significantly ($P < 0.01$)
In column differ significatnly ($P < 0.05$)

Table 2. Mean(±SE) immunoglobin concentration (mg/dl) in uterine flushings of follicular phase, luteal phase, inactive ovarian stage and infected uteri of buffaloes

Parameters	Follicular	Luteal phase				Parameters	Inactive ovarian stage	Infected uteri
		Early	Mid	Late	Pooled			
Ipsi-lateral	6.39±0.69^{b}	5.31±0.29^{b}	5.04±0.42^{b}	5.21±0.55^{b}	5.19±0.24	Left	2.42±0.21^{b}	16.60±3.68^{a}
Contra-lateral	6.22±0.63^{b}	5.37±0.45^{b}	5.07±0.49^{b}	5.63±0.60^{b}	5.36±0.31	Right	2.69±0.33^{b}	14.99±3.87^{a}
Pooled	6.29±0.59^{b}	5.34±0.33^{b}	5.05±0.43^{b}	5.42±0.60^{b}	5.28±0.26	Pooled	2.55±0.24^{b}	15.79±3.69^{a}
No.of observations(n)	10	10	9	10	29		8	8

In row, figures with different superscripts differ significantly ($P < 0.01$)

Table 3. Mean(±SE) alkaline phoshatase and immunoglobulin concentration in flushings, during follicular phase, luteal phase, inactive ovarian stage and infected uteri of buffaloes

Parameterss	Follicular phase	Luteal phase	Inactive ovarian stage	Infected uteri
Alkaline phosphatase (μ/l)	129.82±20.08^{c}	492.38±28.95^{b}	41.04±10.13^{c}	815.06±81.26^{a}
Total Immunoglobulin (mg/dl)	6.29±0.59^{b}	5.28±0.26^{b}	2.55±0.25^{b}	15.79±3.69^{a}
No. of observations (n)	10	29	8	8

In row, figures with different superscripts differ significantly ($P < 0.01$)

read at 580 nm in a spectrophotometer (Beckman D and 640 B, USA), values were calculated against the turbidity of standard immunoglobulins (Ig). Data were analysed as per the standard statistical procedure (Snedecor and Cochran, 1994). Intra and inter-assay coefficient of variation were 3.03 and 8.37 per cent respectively for alkaline phosphatase activity. The immunoglobulin Intra and inter-assay coefficient of variation was 15.50 and 13.70 per cent respectively.

RESULTS AND DISCUSSION

Perusal of the table 1 showed non-significantly higher alkaline phosphatase activity in ipsi-lateral compared to contra-lateral uterine horn flushing during follicular and mid-luteal phase. However, in early and late-luteal phases the difference was significant ($P<0.05$). This could possibly be due to the influence of hormone secreted by ovarian structures. Boos *et al.* (1988) in cows and Jain *et al.* (1995) in buffaloes also reported significantly higher activity in ipsi-lateral horn. Furthermore, alkaline phosphatase activity was significantly ($P<0.01$) higher in late-luteal phase compared to early-luteal phase, follicular phase and inactive ovarian stage. Larson *et al.* (1970) reported it to be due to stimulatory effect of progesterone during luteal phase. In buffaloes, similar to present observation, Pahwa *et al.* (1980), Bugalia and Sharma (1990) and Jain *et al.* (1995) also reported higher activity during luteal phase which promote glucose secretion needed for blastocyst implantation. On the other hand, the difference was non-significant between two horns during inactive ovarian stage.

In infected uteri, alkaline phosphatase activitywas significantly ($P<0.01$) higher compared to luteal phase follicular phase and inactive ovarian stage (Table-3). Similar to our study, Rao and Seshagiri (1998) also reported significantly ($P<0.01$) higher activity during endometritis in cows which could possibly be due to leakage of enzymes from necrotic or damaged cells and endotoxin.

Perusal of the table 2 showed non-significant variation of immunoglobulin between two uterine horn flushings. Finally, the overall mean values were non-significantly higher during follicular phase compared to luteal and inactive ovarian stage uteri. Similar to present study, Whitmore and Archbald (1977) in bovines, also reported higher Ig concentration during oestrus. However, in buffaloes, Ahmed *et al.* (1993) also reported higher values at follicular phase compared to other groups but the difference were significant ($P<0.05$).

Interestingly, the mean immunoglobulin concentration was significantly ($P<0.01$) higher in both the infected uterine horn compared to other reproductive stage uteri (Table-3). Similar to present finding in buffaloes, Ahmed *et al.* (1993) also reported significantly ($P<0.01$) higher values during metritis indicating probably acute nature of inflammation. However, in bovines, Brochart and Mascarenhas (1990) described Ig G_1 and Ig G_2 as an indicators of sub-clinical endometritis.

Thus, alkaline phosphatase activity and immunoglobulin concentration simultaneously in *in-vivo* uterine secretion could be used to diagnose endometritis. However, to evolve a certain marker level indicating sub-clinical endometritis in animals is warranted.

ACKNOWLEDGEMENT

Authors are grateful to the Director, Indian Veterinary Research Institute, Izatnagar for providing the facilities.

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