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Total protein and albumin concentration in *in-vitro* uterine flushings of buffaloes (*Bubalus bubalis*)

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ABSTRACT

Present study was carried out on fifty-five (n=55) buffalo during follicular phase, luteal phases, inactive ovarian and infected uteri stage collected from local abattoir. Pooled total protein and albumin concentration in uterine flushings varied non-significantly during first three stages, however, the values were non-significantly higher during luteal phase compared to inactive ovarian stage and follicular phase. On the other hand, concentration was significantly ($P<0.01$) higher in infected uteri compared to other stages indicating possibility of inflammatory changes in the uterus. Thus, total protein and albumin concentration in *in-vivo* uterine secretion could be used as an indicator of sub-clinical inflammatory condition along with other parameters simultaneously. However, studies to evolve certain marker level of proteins and albumin in various inflammatory conditions in animal is warranted.

Key Words: Buffalo, Uterus, Flushing, Total protein, Albumin.

Uterine fluid composition changes constantly under the influence of circulating steroids and thus provide nourishment to developing conceptus (Ashworth *et. al.*, 1989). Further the uterine proteins serve as enzymes and growth hormones (Beato and Baier, 1975). Although, precise function of many proteins within the uterine lumen are not known. However, Hafez (1980) reported that during luteal phase, amino acids and protein content of the uterine fluid provide embryo nutrition. Also, in inflammatory conditions of uterus, the protein and albumin concentration vary in cows (Rao and Seshagiri, 1998; Brochart and Mascarenhas, 1990). The present study is an attempt to elucidate the concentration of total protein and albumin in *in-vitro* uterine flushings in buffaloes.

MATERIALS AND METHODS

Fifty-five (n=55) genital tract of buffaloes collected within 30-60 minutes of slaughter irrespective of their age, breed, parity and body weight at local abattoir were ligated 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 (Luktuke and Rao, 1962; Dobson and Kamonpatana, 1986), luteal (Ireland *et. al.*, 1980), inactive ovarian and infected uteri stages. Each horn was flushed with 10 ml of sterile normal saline using Folleys catheters (INMED Corporation, USA) as per the method of Boos et.al., (1988) within 4-5 hours of collection after cleaning the genitalia with normal saline and then with 70% ethyl alcohol. Equal volumes of fluids recovered from each horn were centrifuged at 1500 rpm for 15 min. The supernatant was decanted and stored in cryovials (in duplicate) at –20°C until analysis. In supernatants, total protein and albumin concentration was estimated as per protocols provided with the diagnostic kits (Span diagnostic, Bombay). Data were analysed using standard statistical methods (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Results are summarized in Table 1 and 2. The intra and inter-assay coefficient of variation of total protein and albumin were 4.27, 3.97 and 4.86, 8.61 per cent respectively. Perusal of reports indicated non-significant variation in total protein and albumin concentrations during

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Table 1. Mean (±Se) total protein concentration (mg/dl) in uterine flushings of follicular phase, luteal phases, inactive ovarian stage and infected uteri of buffaloes

Parameters	Follicular Phase	Luteal Phase			Luteal Phase	Parameters	Inactive stage uteri	Infected Uteri
		Early	Mid	Late				
Ipsilateral	89.06±6.26^{b}	105.41±3.43^{b}	103.16±9.23^{b}	106.99±5.15^{b}	105.16±3.45	Left horn	82.16±10.67^{b}	213.40±42.41^{a}
Contralateral	87.32±7.45^{b}	105.84±7.16^{b}	99.79±9.48^{b}	106.99±7.49^{b}	104.36±4.49	Right horn	81.20±10.69^{b}	169.03±14.26^{a}
Pooled	88.19±6.71^{b}	105.62±4.78^{b}	100.66±9.05^{b}	106.85±5.70^{b}	104.51±3.69	Pooled	81.68±10.21^{b}	191.22±23.94^{a}
No. of observation (n)	10	10	9	10	29		8	8

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

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

Parameters	Follicular Phase	Luteal Phase			Luteal Phase	Parameters	Inactive stage uteri	Infected Uteri
		Early	Mid	Late				
Ipsilateral	57.29±3.71^{b}	71.60±2.99^{b}	73.07±7.82^{b}	73.39±4.99^{b}	72.70±3.04	Left horn	59.00±9.82^{b}	131.52±25.47^{a}
Contralateral	54.24±2.45^{c}	71.88±5.78^{b}	72.12±6.84^{bc}	73.19±5.77^{b}	72.41±3.39	Right horn	60.65±9.30^{bc}	104.23±6.42^{a}
Pooled	55.75±2.84^{b}	71.71±4.08^{b}	72.59±7.19^{b}	73.29±5.34^{b}	72.53±3.10	Pooled	59.83±9.47^{b}	117.87±11.73^{a}
No. of observation (n)	10	10	9	10	29		8	8

In rows, figures with different superscripts differ significantly (P<0.01).

follicular phase, luteal phases (early, mid and late) and inactive ovarian stage in uterine flushings of buffalo uterus. However, it was non-significantly higher in luteal phases compared to inactive ovarian stage and follicular phase uterine flushings. On the other hand, albumin concentration was significantly ($P<0.01$) higher in contra lateral uterine horn during luteal phases. Also, there was non-significant change in the concentration between uterine horns. Higher protein concentration during luteal phases could be due to the effect of circulating blood progesterone. Similarly, Devanathan and Pattabiraman (1996) in crossbred cows and Pahwa *et.al.* (1980) in buffaloes reported a higher uterine protein concentrations during luteal phase with comparatively lower values. In Porcine, Knight *et al.* (1973) demonstrated the role of progesterone in its secretion. Lamothe *et al.* (1972), also reported higher uterine albumin concentration during dioestrus in cows similar to our findings in buffaloes.

Interestingly, the total protein and albumin concentrations were significantly ($P<0.01$) higher in infected uteri compared to other stages in both the uterine horn flushings. In cow, Rao and Seshagiri (1998) also reported higher total protein concentration in uterine flushings during endometritis. This could possibly be due to the cellular debris, damaged tissues and lysed micro-organisms. In cows, Brochart and Mascarenhas (1990) have opined that increased albumin concentration in uterine flushings may act as an indicator of endometritis in cows.

Thus, total protein and albumin concentration in *in-vivo* uterine secretion could be used as an indicator of sub-clinical inflammatory condition along with other parameters simultaneously. However, studies to evolve certain marker level of proteins and albumin in various inflammatory conditions in animals is warranted.

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