HARVESTING UTERINE NEUTROPHILS IN BUFFALOES BY ASPIRATIOIN AND LAVAGE TECHNIQUES

K.RAMESH BABU[1], K.MOULI KRISHNA[2] AND K.PADMAJA

Dept. of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science: Tirupati -517 502

Received : 18.10.2010 Accepted : 08.06.2011

ABSTRACT

Sixty five graded Murrah buffaloes were divided into group I (control) and group II (Infertile) and subjected to aspiration technique at day 0 and lavage at day 4 of the cycle for harvesting uterine neutrophils. Sampling at day 0 of the cycle by aspiration technique yielded cells in 92.3% cases (60/65). Whereas, 96.93% (63/65) samples provided cellular fraction, when buffaloes were lavaged at day 4 of the cycle. The mean neutrophils counts in smears obtained at day 0 were 4.6 ± 0.64 and 45.69 ± 3.88% in group I and II, respectively. Where as at day 4 of the cycle, the counts were 5.6 ± 0.78 and 57.82 ± 3.50% in group I and II, respectively.

Keywords: Endometritis, Endometrial cytology, Uterus, Neutrophils.

In the recent past endometrial cytology, which is based on the migration of leucocytes to the site of infection, has been described to rapidly diagnose endometritis in buffaloes. For harvesting leucocytes from uterine secretions different methods *viz.* direct swab, cytobrush, aspiration and lavage were described in buffaloes (Kasimanickam *et al.*, 2005). The objective of the present work was to develop a simple method of harvesting uterine neutrophils for cytological studies in buffaloes.

A total of sixty five apparently healthy cyclic buffaloes were selected for this study. The lactation length ranged from 4 months to > 1 year. Twenty five buffaloes at their first postpartum estrus with normal discharge and reproductive organs on rectal examination constituted group I (control) and remaining forty buffaloes, which had the history of infertility ranging from abnormal discharge to repeat breeding, constituted group II. At day 0 and 4 of the estrous cycle every buffalo in both the groups was subjected to aspiration and lavage techniques, respectively for harvesting neutrophils. In aspiration technique, estrual secretions from cervical region were aspirated in to a sterile A.I. sheath connected to a 20 ml disposable syringe and smeared on clean glass slide.

Initially on trial basis uterine lavage technique was tried in few animals at day 0, but results were inconsistent, which made to neglect lavage at day 0 of the cycle. On day 4 of the cycle 20 ml normal saline solution was infused into uterine lumen of each buffalo through a sterile A.I sheath connected to a 20 ml syringe and allowed to remain *insitu* for few seconds and then the fluid was drawn. The recovered fluid was centrifuged at 1000 rpm for 15 minutes. After discarding the supernatant, smears were prepared from the sediment. Smears from both the techniques were fixed in methanol, stained with Hematoxillin and eosin (Singh and Sulochana, 1996) and examined under oil immersion lens. Statistical analysis of the data was done by adopting computer soft ware programmed for windows XP (Version 9.0, spss Inc. Munich) and Excel (Version 2003, Microsoft) and also the methods described by Snedecor and Cochran (1967).

The sampling at day 0 of the cycle by aspiration technique yielded cells in 92.3% cases (60/65). Lavage at day 4 of the cycle yielded cells in 96.93% (63/65) samples. Kasimanickam *et al.* (2005) failed to obtain samples in 17% attempts of lavage, when animals were sampled in early post partum period. Whereas Gilbert *et al.* (2005) and Barlund *et al.* (2008) did not report failure of sampling with lavage technique in early postpartum cows. The lone publication, Azawi *et al.* (2008) successfully harvested neutrophils at day 0 of the cycle in heifers, normal parous buffaloes and

repeaters. In the present study, the mean neutrophils counts in cytological smears at day 0 were 4.6 ± 0.64 and 45.69 ± 3.88%; and at day 4 of the cycle the counts were 5.6 ± 0.78 and 57.82 ± 3.50 %, in-group I and II, respectively. The neutrophils count significantly differed (P d" 0.05) between groups at two different sampling intervals. In Iraqi buffaloes at estrus, Azawi *et al.* (2008) reported that the neutrophils counts were < 7% in heifers, d"14.0 ± 2.02% in control and 41.1 ± 11.91% in repeaters. This study confirmed that neutrophils harvested for cytological studies from cervix yielding an admixture of uterine and cervical secretions could represent the uterine environment. Based on the present findings it was felt that aspiration and lavage techniques may be easily and conveniently adopted for harvesting uterine neutrophils in buffaloes on day 0 and 4 of the cycle, respectively.

ACKNOWLEDGEMENT

We are thankful to the Director of Animal Husbandry, Government of A.P. for deputing the first author for M.V.Sc and authorities of S.V.Veterinary University for providing facilities.

REFFERENCES

Azawi, O. I., Omran, S. N. and Hadad, J. J. (2008). A study on repeat breeding of Iraqi buffalo cows. *Buff. Bull.* **27**: 274-283.

Barlund, C. S., Carruthers, T. D., Waldner, C. L. and Palmer, C. W. (2008). A comparison of diagnostic techniques for postpartum endometritis in dairy cattle. *Theriogenology* **69**: 714-723.

Gilbert, R. O., Sang, T. S., Charles, L. G., Hollis, N. E. and Marcel, F. (2005). Prevalence of endometritis and its effects on reproductive performance of dairy cows. *Theriogenology* **64**: 1879-1888.

Kasimanickam ,R., Duffield, T. F., Foster, R. A., Gartley, C. J., Leslie, K. E., Walton, J. S. and Johnson, W. H. (2005). A comparison of the cytobrush and uterine lavage techniques to evaluate endometrial cytology in clinically normal postpartum dairy cows. *Can. Vet. J.* **46**: 255-259.

Snedecor, G. W. and Cochran, W. G. (1967). Statistical methods 6th edition. The Iowa University Press, Ames State University, U.S.A.

Singh, U. B. and Sulochana, S. (1996). Hand book of histological techniques 2nd edition, Premier publishing house 1st floor Koti, Hyderabad, PP.37-41.