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ACTIVITY OF ALKALINE PHOSPHATASE (ALP) AND ASPARTATE AMINOTRANSFERASE (AST) IN UTERINE FLUSHING AND UTERUS OF BUFFALO COWS DURING THE ESTROUS CYCLE

(With 2 Tables & 3 Fig.)

By

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مستوى انزيم ALP وانزيم AST في رحم الجاموس خلال دورة الشبـــق

علاء الدين زين العابدين ، عرفات سيد
علي صديـــق

تمت هذه الدراسه على ٣٦ جهاز تناسلى لأناث الجاموس فى مجازر أسيوط . وأجرى لهم عملية غسيل فى المعمل بدفع ٣٠ سم محلول PBS لكل من قرنى الرحم وذلك باستخدام قسطرة . ثم تم تجميع محلول الغسيل لكل قرن على حده . ثم تعيين مستوى الانزيمات التاليه ALP , AST .

وقد أظهرت النتائج ان هناك علاقه معنويه ($P < 0.001$) بين انزيم ALP الموجود فى محلول الغسيل والنشاط المبيضى . كما وجد ان مستوى انزيم ALP يتأثر بالزياده فى المحلول المجمع من القرن المقابل للمبيض الذى يحمل جسم اصفر .

وايضا اظهرت النتائج عدم وجود أى اختلاف معنوى فى مستوى انزيم AST بالنسبه للنشاط المبيض خلال دورة الشبق فى الجاموس .

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SUMMARY

The flushing of both uterine horns of 36 uterine specimens of buffalo cows free from any pathological lesions via catheter using 30 ml of phosphate buffer solution (PBS) were collected. Alkaline phosphatase (ALP) and Aspartate aminotransferase (AST) were evaluated. Significant cyclic variations ($P < 0.001$) of ALP activity was found in uterine flushing media. The ALP activity was significantly higher ($P < 0.001$) in flushing media obtained from ipsilateral horn to corpus luteum than from contralateral horn. No significant cyclic variations of AST activity was found in uterine flushing media. The results revealed that ALP activity in flushing media of buffalo cows is very low when compared with cattle and causes of this variations were discussed.

Keywords: Activity, ALP, AST, uterine flushing, uterus, buffalo, Estrus cycle.

INTRODUCTION

Alkaline poshatase (ALP) is an enzyme exciting in the tissues and body fluids of all vertebrates that hydrolyse phosphomonoesters *(FERNLEY, 1971)*. A raised alkaline phosphatase activity is a common finding in the clinical chemistry laboratory *(PEAKE, et al., 1988)*. In a proportion of such cases either the tissue source of enzyme activity which is not identified by the clinician, or the contribution from physiological or pathological processes is masked *(MOSS, 1982)*. Most of the work done in cattle and buffaloes is not sufficient considering the alkaline phosphatase activity in uterine horns at different stages of estrous cycle. Recently, *HYTTEL (1985) and BOOS et al. (!986)* subjected uterine flushing taken from alive and slaughtered cows for hormonal and biochemical analysis. Large variations in ALP activity was found in flushing of uterine horns taken in vivo from estrous synchronized animals or postmortem during different stages of estrous cycle *(WITTKOWSKI and BOOS, 1986 BOOS, et al., 1988)*.

Alkaline phosphatase activity in bovine uterus is regulated by ovarian steroids *(KENNEY, 1964 and LARSON, et al., 1970)*. Also, the enzyme activity pattern more or less reflects the morphological aspects of the uterine secretion *(HYTTEL, 1985)*. Moreover, it is known that ALP activities are controlled by genetic and non genetic factors *(KUNKEL, et al., 1953 and*

ASHA MAZUMDER and MAZUMDER, 1985). On account of these findings, the biochemical tests of Alkaline phosphatase (ALP) and Aspartate aminotransferase (AST) were used to determine the ALP and AST activities in uterine flushing and its relation to ovarian structures in buffalo cows.

MATERIAL AND METHODS

The genital organs of thirty-six buffalo cows were obtained from a local abattoir, within about 30 min after slaughtering, bleeding and evisceration of the carcasses. Their age randged from 4-11 years. Uterine flushing was done by using the technique described by *BOOS* et al *(1988).* Following transcornual incision close to the uterine corpus, the uterine horns were flushed via rubber catheter using 30 ml of phosphate buffer solution (PBS) supplemented with 1% fetal calf serum. Immediately following collection, the uterine flushing was examined macroscopically and centrifuged at 3000 r.p.m for 10 min to remove cellular debris. The supernatent was transferred for storage at low temperature (6 °C) until analysis.

The genital organ of each animal was examined macroscopically and the ovarian structures were recorded. The specimens were classified according to the ovarian structures. The first one was specimen in follicular phase (Follicles 5 to 10 mm in diameter) and the second was specimen in luteal phase (developmental or mature corpus luteum). While, the third group was specimens with follicle less than 5 mm in diameter or no structure).

The estimation of ALP and AST activities were determined spectrophotomterically by means of a test kits supplied by BioMerieux (France) and Sclavo S.P.A. (Italia), respectively. The samples were evaluated in duplicate in supernatent fluid of centrifuged flushing media taken from both uterine horns.

Samples for histopathological examination were taken in 10% neutral buffered formalin, dehydrated embedded in parablast and sectioned at 7 μ. Tissue sections were then stained with hematoxlin and eosin for light microscopy. For detection of alkaline phosphatae activity, tissue samples were fixed in co/d acetone and stained by the method of GOMORI (1952).

Statisical analysis of data was done by using one way analysis of variance*.

*: Microstat/Pcstat Computer prog. Inco. Co. USA.

RESULTS

According to the macroscopic examination of the genital organs, nine specimens have mature follicles and twenty have a full growing corpus luteum. In addition, seven specimens have small follicles or no structures (Table 1).

The averages and ranges ALP and AST values in relation to ovarian activity are given in Table 1. The average ALP activity for specimens in luteal phase (410.46 ± 220.65 U/L) was significantly higher ($P< 0.001$) than for specimens in follicular phase (79.19 ± 28.18 U/L) and for specimens with small follicles or no structures (30.37 ± 13.60 U/L). Moreover, the specimens in follicular phase had significantly higher ($P< 0.001$) ALP value than for specimens with small follicles or no structures.

The mean ALP and AST values during estrous cycle in relation to uterine horn flushing are presented in Table 2. The uterine flushing of specimens with corpus luteum exhibited strong AlP activity that was signififcantly higher ($P< 0.001$) in the ipsilateral samples (247.13 ± 116.36 U/L) than in the contralateral samples (60.50 ± 20.60 U/L).

No significant cyclic variations of AST activity was found in uterine flushing of buffalo cows (Table 1,2).

Histopathological examination revealed that the uterine samples (endometrium) were of normal structure (Fig. 1). An intense alkaline phosphatase activity was detected in the epithelial cells of uterine mucosa of the ipsilateral horn to the corpus luteum. The reaction was in the form of black granules in the cytoplasm of epithelial cells of the uterine mucosa (Fig. 2). A moderate activity was detected in the uterine horns ipsilateral to the graffain follicle (Fig. 3).

DISCUSSION

Knowledge of the biochemical nature of intraluminal environment of the bovine female reproductive tract is of vital impotance for improving the reproductive efficiency.

The results indicated that ALP activity in uterine Flushing during estrous cycle of buffalo cows is very low when compared with cattle. *BOOS et al. (1988)* reported that the estrus limit of ALP activity in uterine Flushing of cows was 278 U/L and during luteal phase the average ALP activity was 2006 ± 2756 U/L. This variation may be attributed to the effect of genetic and non-genetic factors which controlled the enzyme activities (*ASHA MAZUMDER AND MAZUMDER, 1985*). The effects of

the breed and age of animal on ALP activity were reported by *SLNGH et al. (1972) and Asha Mazumder and Mazumder (1985).* The ALP activity in bovine uterus is regulated by ovarian steroids *(KENNEY, 1964 and LARSON, et al., 1970).* In contrast to this, the follicular population (number of follicles in each class) in buffalo is of much lower order *(MANIK, et al., 1992)* and the average size of ovulatory follicle was smaller *(SINGH, et al., 1984)* Than in cattle. In addition the progesterone levels during the luteal phase were relatively low in buffaloes *(KANAI and SHIMIZU, 1984).*

In this study, great variations in ALP activity (133.90-880.20 U/l during luteal phase are recorded in uterine flushing of buffalo cows. This is in agreement with that reported by *WITTKOWSKI and BOOS (1986)and BOOS et al. (1986)* who reported a great variations in ALP activity in uterine flushing of normal cyclic cows and of cows with cystic ovaries. This is because of indivdiual differences in ALP activity of endometrium *(WITTKOWSKI and BOOS, 1986 and BOOS, et al., 1988),* but it may be in part due to functional activity of corpus luteum. Previous works by *SCHULTZ et al. (1971), WITTKWAWSKI and BOOS (1986) and BOOS et al. (1988)* found that the metabolic capacity of ALP activity is correlated with the morphological state of corpus luteum periodicum or the amount and the morphlogical integrity of the luteal tissue in cows with cystic ovaries.

In the present study, The ALP activity was highest in uterine flushing of buffalo cows during luteal phase (810.46 ± 220.65 U/L) Than follicular phase (79.19 ±28.18 U/L) and anestrous period (30.37 ± 13.60 U/L). This is in agreement with *ABLE et al. (1976), BOITOR et al. (1986), wittkwaski AND BOOS (1986) and BOOS et al(1988)* who found a positive correlation between the activity of ALP in the uterine wall or in the uterine flushing and a luteal phase of sexual cycle. Moreover, histochemical investigations of the endometrial tissue of cow have indicated that ALP activity vary with stage of estrous cycle *(MOSS, et al., 1954 and SKJERVEN, 1956).* Especially the epithelial cells lining the uterine lumen and, to a lesser extent, the glandular epithelial cells exhibited distinct changes in ALP activity during estrous cycle *(RADMAN, 1967 and MARINOV and LOVELL, 1968).*

The results of this study, also indicated, that there was significant difference between the average ALP activity in uterine flushing obtained form horns situated ipsilateral to the corpus luteum and from horns situated contralateral. These results were coincided with findings in cows during luteal phase of estrous cycle *(WITTKWASKI and BOOS, 1986 and BOOS, et al., 1988).* The authors suggested a local relation between the uterine horn and the ovary that carries distinct luteal

structures. This effect was also found in overiectomized and hormonal treated cows *(LARSON, et al., 1970).*

The present results revealed that, there is no significant difference for AST activity in uterine flushing of buffalo cows. Earlier investigators *(WITTKWASKI and BOOS, 1986 and BOOS, et al., 1988)* also reported that AST lacked a cyclic pattern in flushing media of dairy cows.

Finally, this experiment corporates the conclusion presented in previous work *(BOOS et al., 1988)*, where it was stated that the increase of the activity of ALP is probably connected with the function of the ovarian luteal structures as well as a local relation between the uterine horn and the ovary that carried distinct luteal structures. Detailed research is required to investigate the repeat breeder buffalo cows to see what levels of ALP are present and to see if these differ from buffalo cows with good fertility.

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REFERENCES

Able, b.V, Dutt, R.H, Thrift, F.A, and bradley, N.w, (1976): Association between endometrial phophatase activity and fertility in beef cattle. J. Anim. sci. 42: 400-404.

Asha Mazumder and Mazumder, N.K. (1985): Serum alkaline phosphatase activity and some factors influencing the enzyme in crossbred cattle (Bos Tourus x Bos Indicus). Indian J. Anim. Sci. 55(7): 520-523.

Boitor, L., Kadar, L., Ghitulescu, C. and Radulescu, E. (1986): Biochimsche und histochemische Aspekte des uterus beim Rind unter physioloigischen und pathologischen Bedingungen Tierarztl. Umschau. 41:493-497.

Boos, A., Ehlers, J., Roming, L.G.P. and schwarz, R. (1986): Progesteronkonzentration in der spulflussigkeit aus dem ipsi-und Kantralateral zum corpus luteum periodicum gelegenen utershorn sowie im Bluutserum beim Rind. Dtsch. Tierarztl. Wschr. 93; 243-245.

Boos, A., wittkowski, G. and schwarz, R. (1988): Activity of alkaline phosphatase in uterine flushings of dairy cows affected with ovarian cysts or endometrities. Theriogenology 30; 217-225.

Fernley, H.N. (1971): Mammalian alkaline phosphatase. pp. 417-477. In; The enzmes Vol. 3 rd ed. (Boyer. F.S. ed.), Academic press, Newyork and london.

Gomori, G. (1952): In theory and practice of histologic technigues (1977) by: Banchroft, j.D. and stevens, A.S. foreward by Dawson, I.M.P. Churchill Livingstone Edinburg, London and new work, pp, 290, 1st ed.

Hyttel, p. (1985): The epithelium of uterine biopsies from cyclic dairy cattle: ultrastructure and endocytotic activity. Acta Anat. 123:93-100.

Kanai, Y. and Shimizu, H. (1984): Plasma concentrations of LH, progesterone and oestradiol during the oestrous cycle in swamp buffaloes (Bubalus bubalis). J. Reprod. Fer. 70: 505-507.

Kenney, R.M. (1964): Histochemical and biochemical correllates of estrous cycle in the normal cyclic uterus and ovary of the cow. phD. thesis, cornell University.

Kunkel, H.O., Stokers, p.K., Anthony, w.B, and Andfutrell, M.F. (1953): serum alkaline phosphatase activity in European and Brahman breeds of cattle and their crossbred types. J. Anim. Sci. 12: 765-770.

Larson, L.l., Marion, G.B. and Gier, H.T. (1970): Glycogen metabolism in bovine endmetrium. Am. J. Vet. Res. 31:1929-1935.

Manik, R.S., Madan, M.L., Ambrose, J.D., Singia, S.k. and Chauhan, M.S. (1992): Real time ultrasound scanning for study of follicular population on the day of estrus and ovulation in buffaloes. 12 th Inter. Anim. Reprod., Netherland Vol. 1: 234-236.

Marinov, U. and Lovell, J.E. (1968): Cytology of the bovine uterine epithelium during the estrous cycle. Am. J. Vet. Res. 29: 13-30.

Moss, D.w. (1982): Alkaline phophatase isoenzymes. clin. Chem. 28: 2007-2016.

Moss, D.w., Wrenn, T.R. and Sykes, F.F. (1954): Alkaline phophatase, glycogen, and periodic acid-schiff positive substances in bovine uterus during estrous cycle. *Endocrinology 55: P. 261.*

Peake, M.j., Pejakavic, M. and white, G.H (1988): Quantitative method for determining serum alkaline phophatase isoenzyme activity: estimation of intestinal component. J. Clin. Pathol. 41: 202-206

Radman, Z. (1967): The histologic and some histochemical variations of the bovine endometrium in course of sexual cycle. Vet Arh. 37: 250-255.

Singh, a.P., Joshi, H.c. and Singh, R. (1972): Studies on certain blood constituents in cattle and buffaloes. Indian vet. J. 49: 473-477.

Singh, A.P., Singh, G.H. and Sharma, S.S. (1984): Studies on follicular patterns in buffalo-heifers. Theriogenology 22 (5): 453-462.

Skjerven, o. (1956): Endometrial biopsy studies in reproductively normal cattle. Act Endocr. Copenh. suppl. 26: P.134.

wittkowski, G. and Boos, A. (1986): zur endometrialen funkiton des ipsi-und kantralateralen uterushornes beim rind. Zucthyiene. 21: 156 abstr.

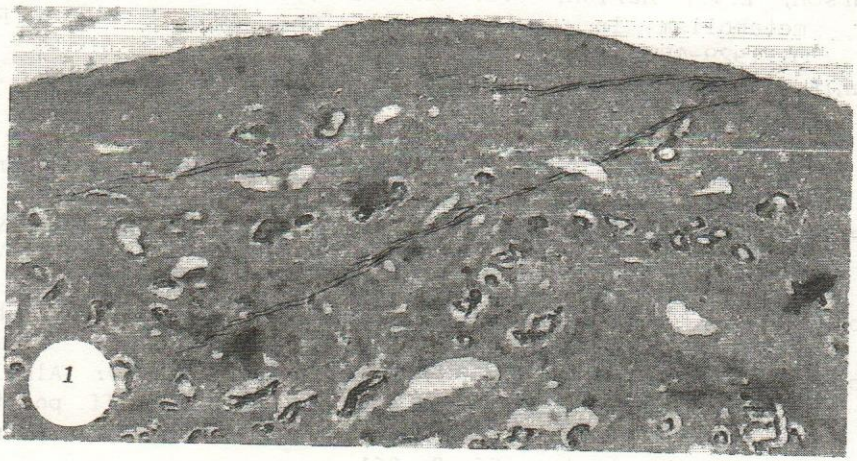

Fig. 1: Light micrograph showing normal structure of uterus (X 56, H & E).

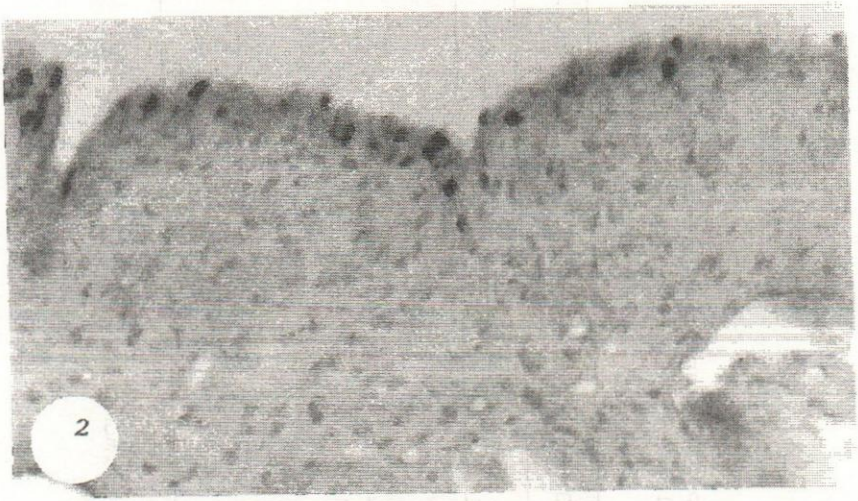

Fig. 2: Light micrograph showing intense ALP activity in the uterine mucosa (X 560, Gomori).

Fig. 3: Light micrograph showing moderate ALP activity in theuterine mucosa (X 560, Gomori).

Taole (1): Alkaline phosphatase (ALP) and aspartate aminotransferase (AST) activity (mean ± SD) in uterine flushing[1] in relation to ovarian structures.

ITEMS	Ovarian structures		
	Follicles	Corpus luteum	No structures
No. Observations	9	20	7
Alkaline Phosphatase (ALP)	79.19 ± 28.18[a] (51.30 -130.30)	410.46 ± 220.65[b] (133.90 - 830.20)	30.37 ± 13.80[c] (17.50 - 55.20)
Aspartate amino-transferase(AST)	6.10 ± 2.80 (01.30 - 20.00)	5.17 ± 3.90 (2.50 - 11.90)	5.54 ± 2.91 (2.50 - 10.50)

[1] = Both uterine horns a, b, c = P<0.001

Figures in parentheses indicate minimum and maximum levels (U/L).

Table 2: Alkaline phosphatase (ALP) and Aspartate aminotransferase (AST) activity in uterine horn flushing in relation to ovarian structures.

	Ovarian structures in relation to uterine horn			
	Follicles		corpus luteum	
	Ipsilateral	contralateral	Ipsilateral	contralateral
No. uterine horns	11	7	31	9
Alkaline phosphatase (ALP)	51.23 ± 18.98 (6.81 - 81.80)	21.34 ± 15.91 (6.00 - 50.00)	247.13 ± 116.36[a] (101.50 - 542.90)	60.50 ± 20.60[b] (23.50 - 85.40)
Aspartate aminotransferase (AST)	3.37 ± 1.95 (1.00 - 6.00)	2.54 ± 0.96 (1.30 - 3.60)	2.40 ± 1.71 (0.40 - 8.50)	3.66 ± 3.70 (0.40 - 11.50)

a,b = P<0.001

Figures in parentheses indicate minimum and maximum levels (U/L).