

SOME BIOCHEMICAL STUDIES ON BUFFALO SEMINAL PLASMA

BY

G.W. YOUSSEF, ELS. T. AWAD, G. ABD-ELMALAK AND K.A. KAHILLO

Biochemistry Department, Faculty of Vet. Med. Cairo University, Giza-Egypt.

(Received: 18.11.1992).

SUMMARY

*The present work was devoted to study the activities of certain seminal plasma enzymes of the Egyptian buffalo (*Bos bubalis*); Lactic dehydrogenase (LDH), alkaline phosphatase (ALP), acid phosphatase (ACP) and prostatic acid phosphatase (P_r ACP). Some lipid constituents (Total lipids, phospholipids as well as total cholesterol) were also studied. Parallelly, some physical characteristics of semen were recorded. Furthermore, the correlation between the different items studied was calculated. We obtained significant positive correlations between total cholesterol and LDH activity, alkaline and acid phosphatases, acid phosphatase and prostatic acid phosphatase, prostatic acid phosphatase and phospholipids, total lipids and sperm concentration in semen.*

INTRODUCTION

The activities of lactic dehydrogenase and phosphatases in semen were investigated by Melampy et al., (1952); Hlipse, (1960) Hartree and Srivastava, (1973); Abdulla et al., (1973); Abdou et al., (1974 & 1978); Buruiana, (1976) and Dhimi & Kodagali, (1987). These enzymes are concerned with metabolic pathways of sperm cell (Salisbury et al., 1978). Little information is available on the lipid composition of the seminal plasma of buffalo bulls. The present study was planned to illustrate the activities of certain enzymes and the utilization of some lipid constituents in seminal plasma of Egyptian buffalo bulls (*Bos bubalis*).

MATERIAL AND METHODS

Semen samples were collected from buffalo bulls (*Bos. bubalis*) raised in the Animal Reproduction research Institute, Giza (a governmental center of ministry of agriculture in Pyramid district). All animals were under the same conditions of vaccination, management and nutrition. They were all free of venereal diseases. They appeared healthy and nearly of the same age (about 8 years). For

the purpose of artificial insemination in the above mentioned center, bulls were grouped into 5 groups, each of 5 animals. A total of 25 samples were collected from these bulls by artificial vagina. Immediately after collection of semen, the physical characters of each semen sample (volume, motility percentage and concentration of live sperms) were recorded. To obtain the seminal plasma, the samples were centrifuged at 300 rpm for 20 minutes. The plasma was then preserved at -20°C till the time of assay. Lactic dehydrogenase and alkaline phosphatase activities were assayed after Emffehlungens der Deutschaft fur Klinische Chemie (1970 and 1970 and 1970). The methods used to estimate acid phosphatase and prostatic acid phosphatase activities were these described by Fishman and Lerner (1953). Total lipids were estimated by the method of Frings et al. (1970). Phospholipids & Cholesterol measured after Youngburg and Youngburg (1930), and Trindler (1969), respectively.

The obtained results were analysed by the Student "t" test, analysis of variance and correlation coefficient (Snedecor and Cochran, 1967 and Schwartz, 1977).

RESULTS AND DISCUSSION

Table (1): Serum enzyme activities and certain lipids in seminal plasma of butchle bulls (Means \pm S.R.M.).

Butchle bulls	LDH IU/L	ALP IU/L	ACP IU/L	Prostatic ACP IU/L	Total lipids mg%	Phospho-lipids mg%	Cholesterol mg%
Group I	307.89 \pm 67.94	2221.29 \pm 442.48	2286.64 \pm 367.02	1660.44 \pm 271.79	153.87 \pm 11.07	89.41 \pm 3.72	21.98 \pm 3.53
Group II	444.09 \pm 3.81	2051.50 \pm 270.21	1940.24 \pm 234.56	1027.70 \pm 139.89	159.53 \pm 8.75	50.86 \pm 6.81	21.98 \pm 8.24
Group III	303.19 \pm 50.58	647.10 \pm 73.18	1369.58 \pm 110.99	995.92 \pm 113.53	151.70 \pm 13.12	24.28 \pm 10.88	16.62 \pm 0.25
Group IV	305.15 \pm 72.99	1410.82 \pm 106.74	2003.84 \pm 202.84	1276.64 \pm 200.52	116.23 \pm 14.88	61.66 \pm 12.89	15.20 \pm 8.40
Group V	170.75 \pm 10.81	1726.23 \pm 78.78	2500.00 \pm 344.41	2288.69 \pm 340.81	139.68 \pm 16.19	60.37 \pm 9.32	14.61 \pm 1.77
Overall means \pm S.R.M.	306.20 \pm 36.52	1611.34 \pm 146.36	2000.06 \pm 131.38	1449.64 \pm 135.77	143.80 \pm 6.18	69.32 \pm 5.14	18.09 \pm 2.44

Table (2): Coefficient of correlations between enzymes, lipids estimated in seminal plasma of buffalo bulls and sperm output character.

Parameters	LDH	ALP	ACP	Pr.ACP	Total lipids	Phospholipids	Cholesterol
ALP(IU/L)		1					
ACP(IU/L)		$r=0.52$ $P < 0.01$	1				
Prostatic ACP(IU/L)		$r=0.36$ N.S.	$r=0.65$ $P < 0.01$	1			
LDH(IU/L)	1	$r=0.24$ N.S.	$r=0.20$ N.S.	$r=-0.31$ N.S.			
Cholesterol-ol(μg^2)	$r=0.60$ $P < 0.01$	$r=0.13$ N.S.	$r=0.24$ N.S.	$r=-0.06$ N.S.			1
Phospholipids(μg^2)	$r=0.03$ N.S.	$r=0.37$ N.S.	$r=0.38$ N.S.	$r=+0.40$ $P < 0.05$		1	$r=0.05$ N.S.
Total lipids(μg^2)	$r=-0.19$ N.S.	$r=0.10$ N.S.	$r=0.002$ N.S.	$r=+0.08$ N.S.	1	$r=-0.05$ N.S.	$r=0.01$ N.S.
Fertility (%)	$r=0.21$ N.S.	$r=0.22$ N.S.	$r=0.07$ N.S.	$r=-0.30$ N.S.	$r=0.09$ N.S.	$r=-0.13$ N.S.	$r=0.22$ N.S.
Density (10 sperm/ml)	$r=0.16$ N.S.	$r=0.21$ N.S.	$r=0.19$ N.S.	$r=+0.28$ N.S.	$r=0.48$ $P < 0.05$	$r=-0.01$ N.S.	$r=-0.20$ N.S.
Volume(ml)	$r=0.25$ N.S.	$r=0.22$ N.S.	$r=0.09$ N.S.	$r=+0.14$ N.S.	$r=0.23$ N.S.	$r=-0.07$ N.S.	$r=-0.25$ N.S.

1. Enzyme activities:

Studies on the activity of LDH in seminal plasma showed great degree of variability. Stallcup et al., (1968) found LDH activity of the epididymal fluid of bulls to be 3860 U/ml. Tuli and Singh (1982) recorded 1836.2 IU/L in the semen of bulls. These results are too high compared with the present results (306.20 IU/L). This may be due to the place in the genital organs of bulls from which semen was collected or due to feeding status and species difference. Our results are early the same as those given by Dharmi and Kodagali, (1987) as 387.05 IU/L.

In this the activity of acid phosphatase (ACP) was found to be higher than that of alkaline phosphatase (ALP). This finding is in agreement with that formerly given by Naformita et al. (1977). They recorded 2002.2 and 1508.04 IU/L, for acid and alkaline phosphatases, respectively being close to 2020.06 and 16.11.34 IU/L obtained in this present work.

Other authors (Abdou et al., 1974) gave higher results of 8087.18 and 3945.83% IU/L, for acid and alkaline phosphatases, respectively. The great difference in enzyme levels given by different authors is probably due to the effect of the feeding status of animals, condition of testes as testicular degeneration and age of animals (Roussel and Stallcup, 1965). The prostatic acid phosphatase activity of seminal plasma (Pr ACP) has been found to be 1449.88 IU/L. This value amounts to 70.8% of total acid phosphatases and coincides with the record of Bell and Lake (1962). It is clear that in seminal plasma of buffalo bulls, the acid phosphatase is higher than alkaline and prostatic acid phosphatase. This fact agrees with the statement of Abdulla et al. (1973) and Kavanagh and Bardsley (1979). Table (2) shows a significant positive correlation between ACP and ALP ($r = 0.52$), and between ACP and prostatic acid phosphatase ($r = 0.65$).

2. Lipids content in seminal plasma:

The phospholipids are important structural components of spermatozoal membrane (Jain and Anand, 1976). They can play a role especially in

chemical changes associated with sperm maturation in epididymis (Poulos et al., 1974). In the present study, the total lipids (Table 1) were found to be 143.84 mg% which is early the same as given by Jain and Anand (1976) who recorded 150 mg%. The total phospholipids obtained in this study amounted to 69.32mg%. This agrees with the values given by different authors (Komarek et al., 1964; Pursell and Graham, 1967; Clegg and Foote, 1973 and Jain and Anand, 1976). These authors worked on seminal plasma of different breeds of buffalo bulls and gave values for phospholipids as 89.0, 31.3, 96.0 and 59.4 mg%, respectively. In the present study, the average value of total cholesterol was 18.09 mg% in buffalo seminal plasma and this result is nearly the same as given by Pursell and Graham, (1967) who recorded 19.8 mg%.

The correlations between the different lipids concentrations in seminal plasma and sperm characters on one hand and previous enzymes studied on other hand were studied in details (Table 2). This analysis demonstrated that the total lipids were significantly and directly correlated ($P < 0.05$ with the concentration of sperm ejaculates ($r = 0.48$). A positive correlation has been found between phospholipids and prostatic acid phosphatase ($r = 0.40$) and between total cholesterol and LDH ($r = 0.60$) estimated in the same sample of seminal plasma.

REFERENCES

- Abdou, M.S.S.; El-Guindi, M.M.; El-Menoufy, A.A. and Zaki, K. (1978): Enzymatic profile of the semen of bovines. (*Bubalus bubalis* and *Bos taurus*) Zbl. Vet. Med., A, 25; 222-230.
- Abdou, M.S.S.; El-Guindi, M.M.; Mostafa, M.A.; El-Wishy, A.B. and Farhat, A.A. (1974): Comparative study of the phosphatase activity in the semen of bovines (*Bos bubalis* and *Bos taurus*) in Egypt. Zbl. Vet. Med., A, 21; 759-767.
- Abdulla, A.; El-Guindi, M.M. and Mostafa, M.S. (1973): Alkaline and acid phosphatase activity in buffalo semen. Zbl. Vet. Med., A, 20; 567-570.
- Bell, D.J. and Lake, P.E. (1962): A comparison of phosphoesterase activities in the seminal plasma of domestic cock, turkey tom, boar, bull, buck rabbit and man. J. Reprod. Fert., 3; 363-366.
- Buruiana, L.M. (1976): The disc electrophoresis pattern of spermatozoa proteins of some species and their phosphatase activity. Proceed. VIII Intern. Cong. (on Animal Reproduction and Artificial Insemination), 4; 880 - 883.
- Clegg, E.D. and Foote, R.H. (1973): Phospholipids compo-

- sitions of bovine sperm fraction, seminal plasma and cytoplasmic droplets. *J. Reprod. Fert.*, 38: 375-383.
- Dhank, A.J. and Kothigal, S.B. (1977): Correlation between biochemical and enzymatic constituents of semen of Surti buffalo bulls. *Ind. J. Anim.*, 27 (12): 1223-1228.
- Empfehlungen der Deutschen Gesellschaft für Klinische Chemie (1970): Kinetic determination of LDH and-DH and-GH butyrate dehydrogenase activity as recommended by the German Clinical Chemistry Society (DGKC). Z. Klin. Chem. u. Klin. Biochem.**, 18:658.
- Empfehlungen der Deutschen Gesellschaft für Klinische Chemie (1972): Kinetic determination of ALP activity as recommended by the German Clinical Chemistry Society (DGKC). Z. Klin. Chem. u. Klin. Biochem.**, 10:191.
- Fahman, W.H. and Lerner, E. (1953): A method for estimation serum acid phosphatase of prostatic origin. *J. Biol. Chem.*, 201: 89-97.
- Fligg, R.L. (1968): Metabolism of bovine serum. IX: Glutamic oxaloacetic and glutamic pyruvic transaminase activities. *J. Dairy Sci.*, 45: 773.
- Frings, L., Christopher, E. and Dun, R. (1970): Colorimetric method for determination of total serum lipids based on the sulphophosphovaniline reaction. *Am. J. Clin. Path.*, 55: 89-91.
- Hartree, E.F. and Srivastava, P.N. (1965): The chemical composition of the ram spermatozoa. *J. Reprod. Fert.*, 9:47.
- John, Y.C. and Sand, I.R. (1975): The lipids of buffalo spermatozoa and seminal plasma. *J. Reprod. Fert.*, 47:255-261.
- Kavonagh, J.P. and Barrisley, W.G. (1979): The identity of the acid and alkaline phosphatases of human seminal plasma. *J. Reprod. Fert.*, 57 (2): 45.
- Komarov, R.I.; Pietiet, E.W.; Lanz, R.W. and Jansen, R.G. (1964): Lipid composition of bovine spermatozoa and seminal plasma. *J. Dairy Sci.*, 47:531.
- Witkamp, I.M.; Cavazos, L.F. and Porter, J.C. (1952): Cytocemical reactions of bovine spermatozoa and seminal plasma. *J. Dairy Sci.*, 35: 140-148.
- Naloratta, M.; Posa, M.; Dypre, L.; Negro, M. and Nicolet, W. (1977): Some physical and chemical values and the activity of some enzymes in bull semen in winter. *Lavori Scientifici Istituto Agronomico Timisoara Medicina Veterinara*, 14: 227-231.
- Podus, A.; Brown, F.D.; Cox, R. and White, I.G. (1974): Changes in the phospholipid compositions of spermatozoa in reproductive tract of the ram. *J. Reprod. Fert.*, 36 (2): 442-443.
- Puzell, V.G. and Graissan, E.F. (1967): Phospholipids of bovine spermatozoa and seminal plasma. *J. Reprod. Fert.*, 14: 205-211.
- Rousseau, J.J. and Stalcup, O.T. (1965): Influence of age on transaminase and phosphatase in male Holstein blood serum. *J. Dairy Sci.*, 48:841.
- Salisbury, G.W.; Van-Demark, N.L. and Lodge, J.R. (1978): *Physiology of Reproduction and Artificial Insemination of Cattle*. Freeman Co., San Francisco.
- Schwarz, B. (1977): *Methodes statistiques a l'usage des medecins et des biologistes, collection statistiques. e biologic et en medecine*, PP. 318.
- Svedekor, G.W. and Cochran, W.G. (1967): 6th Ed., Iowa State University Press, Ames, USA.
- Stalcup, O.T.; Brown, C.J. and Cole, W.T. (1968): Lactic dehydrogenase and transaminase in bovine epididymal fluid. *J. Reprod. Fert.*, 15: 317-319.
- Trinder, P. (1969): Enzymatic colourimetric method for determination of cholesterol. *Ann. Clin. Biochem.* 6: 24-27.
- Tull, R.K. and Singh, H. (1962): Lactic dehydrogenase activity in exotic bull semen and seminal plasma. *Ind. J. Dairy Sci.*, 25 (3): 249-251.
- Youngburg, G.E. and Youngburg, M.V. (1930): *J. Lab. Clin. Med.*, 41: 486.
- Cited by Varley, H. (1976): *Practical Clinical Biochemistry*. 4th Ed., Gulab Vazirani Arnold-Heinemann (India), PP: 317.