

## BIOCHEMICAL STUDY OF SEMEN OF BUFFALOES. I. DETERMINATIONS OF FRUCTOSE, CALCIUM, GOT AND GPT\*

### ESTUDO BIOQUÍMICO DO SÊMEN DE BÚFALOS. I. DETERMINAÇÕES DE FRUTOSE, CÁLCIO, GOT E GPT

Renato Campanarut BARNABE<sup>1</sup>; Valquiria Hyppolito BARNABE<sup>2</sup>; Claudio Alvarenga de OLIVEIRA<sup>3</sup>; Rubens Paes de ARRUDA<sup>3</sup>; Renato VALENTIM<sup>4</sup>; Maria Amélia ZOGNO<sup>5</sup>

#### SUMMARY

There were made 16 semen collections from 6 Jaffarabadi x Mediterranean buffaloes bulls, aged approximately 36 months, every fortnight from may to august 1990, either by artificial vagina or by electroejaculation. Fructose and calcium in mg/ml were determined in total semen, while GOT and GPT in u/ml were determined in the fraction of semen rich in spermatozoa. It was possible to detect that values of fructose are higher in samples collected by artificial vagina, while determinations of GOT and GPT showed, higher values in semen obtained by electroejaculation. In respect to Ca<sup>++</sup> there was not significant difference between methods of collection.

**UNITERMS:** Semen; Biochemical; Buffaloes

#### INTRODUCTION AND LITERATURE

Although artificial insemination is now accepted as an essential tool for extensive use of the good germ plasma of selected males, in buffaloes it has not been a success as it is with cattle. It is therefore, essential to obtain precise information regarding buffalo semen in order to improve the effectiveness of artificial insemination.

The composition of seminal plasma has been analysed in many species including the water buffalo for electrolytes, sugars, amino acids, phospholipids and related compounds, total proteins, and certain enzymes occurring in semen, such as GOT (glutamic oxaloacetic transaminase) and GPT (glutamic pyruvic transaminase)<sup>2,8</sup>.

Among the sugars, fructose plays an important role on spermatozoa nutrition. Fructose metabolism in semen has close relation with calcium concentration, which has been found to depress sperm viability in bovine semen<sup>6</sup>. As earlier reported<sup>4,9</sup> calcium is present in relatively higher concentration in buffalo semen, than in bull semen. RAWAT<sup>10</sup> (1979) has demonstrated that the effect of calcium concentration showed that fructose utilization increased with the increase in the concentration of Ca<sup>++</sup> in the medium from 25 mg% up to 45 mg%.

According to previous reports<sup>1,11</sup> it is more convenient that levels of fructose and calcium be made in whole semen.

Many enzymes have been found in the seminal plasma. Although most of them have their origin in the accessory glands, at least some might be the result of leakage from spermatozoa. In general, when enzymes occur in higher concentrations in the spermatozoa, they are drained off from the cells upon cold-

shock or deep-freezing. Loss of such important enzymes as GOT and GPT might be a factor in explaining the depressed metabolism of cold-shocked or deep-frozen spermatozoa<sup>4</sup>. GPT value was comparable to that in bull semen whereas, GOT was less than half of similar value reported in case of bull semen. Besides, GOT/GPT ratio was relatively narrow (4:1) as compared to that in bull seminal plasma (11:1)<sup>10</sup>.

Apparently, the cell membrane of spermatozoa is more permeable to GOT than the membrane of many cells, for seminal plasma GOT activity is nearly 20 times that of blood serum GOT<sup>3</sup>.

In this trial, fructose and calcium were studied in whole semen, while the extracellular release of GOT and GPT was determined in sperm rich fraction. Concomitantly, it was studied a possible difference between methods of semen collection (artificial vagina or electroejaculation), related to fructose, calcium, GOT and GPT concentrations in semen, as a suspected factor observed in a former pilot study.

#### MATERIAL AND METHOD

Semen for biochemical analysis was collected from 6 Jaffarabadi x Mediterranean healthy buffalo bulls, aged approximately 36 months, reared in the Pirassununga "campus" of the University of São Paulo. Semen was collected every fortnight from may to August, 1990. On first collection day, 3 bulls were submitted to electroejaculation and the other 3 to artificial vagina method. On next collection, 15 days later, this situation was alternated and so on up to the end of the experimental period.

The concentration of fructose was determined according to

1 - Professor - Faculty of Veterinary Medicine, University of São Paulo

2 - Associate Professor - Faculty of Veterinary Medicine, University of São Paulo

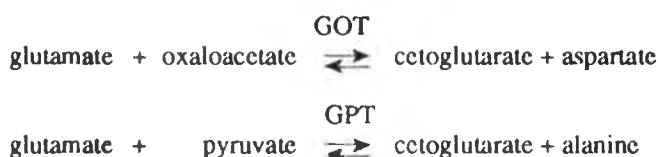
3 - DVM - Faculty of Veterinary Medicine, University of São Paulo

4 - Graduate Student - Faculty of Veterinary Medicine, University of São Paulo

5 - Biologist - Faculty of Veterinary Medicine, University of São Paulo

\* Presented at the 12<sup>th</sup> INTERNATIONAL CONGRESS ON ANIMAL REPRODUCTION, The Hague, The Netherlands

MANN<sup>8</sup>. Calcium was determined by spectrophotometry in a coloured complex resulting from cresolphthalein and calcium in alkaline solution. For GOT and GPT determinations, semen was centrifuged to 1500 rpm at 4 °C during 20 minutes. Seminal plasma was carefully separated through a fine pipette. Spermatozoa were then washed thrice in saline at 4° each washing being followed by a new centrifugation during 15 minutes 2000 rpm. This procedure aims at the cleaning off possible residues of seminal plasma. Then, centrifuged spermatozoa were frozen in liquid nitrogen and thawed in water-bath at 37 °C, also for 3 times. This process makes possible the releasing of intracellular enzymes, among which the glutamic and piruvic transaminases. GOT and GPT analysis were established by a colorimetric method\* according to the following reaction:



Statistical analysis was performed on Student's "t" test<sup>9</sup>, fixed the rejection level on 0.05.

## RESULTS AND DISCUSSION

Biochemical studies on the semen of farm animals have assumed much importance in evolving suitable semen dilutors for use in artificial insemination. There is not much information in this respect in the case of buffalo bull, whose semen does not keep well in dilutors suitable for bull semen<sup>1</sup>. In contrast to bull spermatozoa, buffalo spermatozoa have low survival and thus poor fertilizing ability when semen is either refrigerated or deep frozen<sup>6</sup>.

Under anaerobic condition, fructose appears to be the only sperm nutrient of significance, being the concentration of this sugar in buffalo semens less than in bull semen<sup>11</sup>. Notwithstanding, quantitatively fructose constitutes the major part of reducing substances present in bovine semen<sup>10</sup>. The values of fructose obtained in this experiment were equivalent to those pointed out before<sup>9</sup>. It is inexplicable that values of fructose found in samples obtained by artificial vagina were significantly higher ( $p > 0.05$ ) than by electroejaculation method.

Calcium, contrary to fructose, is more in buffalo spermatozoa as compared with bull sperm<sup>4,9</sup>. Calcium has been found to depress sperm viability<sup>6</sup> in bovine semen what could possibly also explain the higher fragility of buffalo semen. The results of calcium here obtained were much lower than those earlier reported<sup>10</sup>. However, it must be argued that our analysis were made in whole semen, while this author studied calcium concentration in seminal plasma. It has been demonstrated<sup>10</sup>, that utilization of fructose by spermatozoa increases with the increase in the concentration of calcium in semen. Statistically, there was no difference between methods of semen collection concerning to calcium concentration.

The higher permeability of cell membrane of spermatozoa to

GOT<sup>1</sup> could explain the significantly higher values obtained of this enzyme, as can be observed in Tab.1. Of course, this fact must be taken into consideration when processing deep freezing of buffalo semen. On the contrary, it was found<sup>4</sup> in extended semen stored in liquid nitrogen, less release of GOT, unlike GPT release. It is possible that this difference could be due to dilution and freezing of the semen studied<sup>4</sup> since our results were obtained from fresh semen. However, the significance of such observation, as it pertains to metabolism of spermatozoa and the formulation of semen diluents, remains to be elucidated.

The release of enzymes GOT and GPT was lower in semen collected by artificial vagina. Taking into consideration that the epididymis and seminal vesicles also are sources of these enzymes in the ram and bull<sup>6</sup> it is possible the same occurs in buffaloes. Electroejaculation could then increase the volume of semen by exhausting epididimal and seminal vesicles contents, stimulating higher release of such enzymes.

TABLE 1  
Average results of fructose, calcium, GOT, and GPT, in semen of buffaloes, according to semen collection method Pirassununga-SP, 1990.

	COLLECTION METHOD							
	A.V. Elec.		A.V. Elec.		A.V. Elec.		A.V. Elec.	
	FRUCTOSE		CALCIUM		GPT		GOT	
Month								
May	817.54	514.71	7.93	12.30	2.89	10.12	85.79	228.90
June	687.33	570.83	10.45	15.64	7.23	20.24	204.85	457.80
July	750.44	602.33	16.52	10.06	12.04	13.01	210.32	560.20
August	805.63	547.87	6.26	10.46	8.45	18.29	85.79	409.60
	a = mg/100 ml				b = µ/ml			
	A.V. = Artificial Vagina				Elec. = Electroejaculation			

The values for GPT were extremely lower in both methods of collection than those obtained for GOT, in agreement with that previously reported<sup>10</sup>.

## CONCLUSIONS

From this partial biochemical study of semen of buffaloes the following conclusions may be drawn.

1. The method of semen collection can interfere in some relative amounts of some biochemical elements.
2. Values for fructose were significantly higher in samples obtained by artificial vagina, while values for enzymes GOT and GPT were significantly higher by electroejaculation.
3. The method of semen collection did not interfere with calcium values.
4. The values obtained for the analyzed biochemical compounds of semen of buffaloes can be utilized as parameters for future determinations, as well as should be taken into consideration when processing semen dilutors for this species.

## RESUMO

Seis búfalos provenientes de cruzamentos entre as raças

\* Kit Boehringer Mannheim Bioquímica S.A.

Jaffarabadi e Mediterrâneo, com aproximadamente 36 meses de idade, foram submetidos a 16 colheitas de sêmen, quinzenalmente, entre os meses de maio a agosto de 1990, por meio de eletroejaculação ou vagina artificial. No sêmen total foram determinadas, em mg/ml, as concentrações de frutose e cálcio, enquanto que, na fração rica em espermatozoides, fizeram-se dosagens de GOT e GPT, em µg/ml. Verificou-se que nas amostras colhidas com vagina artificial os valores de frutose foram significativamente maiores, ao passo que, naquelas obtidas por eletroejaculação, predominaram valores mais altos de GOT e GPT. Relativamente ao cálcio, não houve diferença significativa quanto aos métodos de colheita.

UNITERMOS: Sêmen; Bioquímica; Búfalos

#### REFERENCES

- 01-BATTACHARYA, M.K.; KING, C.J. Buffalo semen quality in various seasons. *Indian vet. J.*, v.55, p.591-4, 1978.
- 02-BATTACHARYA, P. Reproduction. The husbandry and health of the domestic buffalo. Roma, FAO, 1974.
- 03-FLIPSE, R.J. Metabolism of bovine semen. IX. Glutamic-oxaloacetic and glutamic-pyruvic transaminase activities. *J. dairy Sci.*, v.43, p.773-6, 1960.
- 04-GANGULI, N.C. Biochemistry of semen processing. In: BUFFALO REPRODUCTION AND ARTIFICIAL INSEMINATION. Indias, Proceedings Seminar FAO/SIDA, 1979. p. 292-303.
- 05-GOMES, F.P. *Curso de estatística experimental*. 6. ed. Piracicaba, ESALQ, 1976.
- 06-HAFEZ, E.S.E. *Reprodução animal*. 4. ed., São Paulo, Manole, 1982.
- 07-LARDY, H.A.; PHILLIPS, P.H. Effect of pH and certain electrolytes on the metabolism of ejaculated spermatozoa. *Amer. J. Physiol.*, v.138, p. 741-6, 1943.
- 08-MANN, T. *The biochemistry of semen and of male reproductive tract*. London, Methuen & Co. Ltd., 1964.
- 09-PAL, K. Biochemical studies on buffalo-bull semen. *Curr. Sci.*, v.26, p.212-3, 1957.
- 10-RAWAT, J.S. Physico-chemical factors in buffalo spermatozoal activity. In: BUFFALO REPRODUCTION AND ARTIFICIAL INSEMINATION. Indias, Proceedings Seminar FAO/SIDA, 1979. p. 272-83.
- 11-ROY, A.; PANDEY, M.D.; RAWAT, J.S. Composition of bovine semen. *Indian J. Dairy Sci.*, v.13, p.112-6, 1960.

Recebido para publicação em 22/07/92  
Aprovado para publicação em 05/02/93