

## Release of transaminases subsequent to preservation in diluents containing EDTA and cysteine HCL of beetal and cross bred (Beetal x Black bengal) buck spermatozoa\*

KH. MANI SINGH<sup>1</sup>, M.P.SINGH<sup>2</sup>, AND A. K. SINHA<sup>3</sup>

Department of Animal Reproduction, Gynaecology & Obstetrics  
Ranchi Veterinary College, Ranchi -834006 (Jharkhand)

### ABSTRACT

The study was conducted to observe the role of EDTA and Cysteine HCL on the semen of buck spermatozoa during preservation at refrigerator temperature. Egg Yolk citrate and Tris egg yolk fructose citric acid and yolk with EDTA and Cysteine HCL added @ 1 mg/ml in each extender separately were used for preservation. The leakage of transaminases (AST and ALT) from the spermatozoa at different hours of preservation in different extenders was observed. Results obtained during the present study indicate that leakage of both AST and ALT enzymes was reduced by inclusion of Cysteine HCL and EDTA in both the diluents as compared to control (EYC and TEYFC) extender at all hours of preservation.

**Key words:** Transaminases , Buck spermatozoa, EDTA, Cysteine HCL.

Transaminases such as aspartate transaminase (AST) and alanine transaminase (ALT) located in the mid piece of sperm cells (Mann and Lutwak Mann, 1981) are essential for metabolic process which provides energy for survival, motility and fertility of spermatozoa. Any trauma to sperm membrane due to cold shock causes release of these enzymes. Hence, estimation of enzyme activity in the seminal plasma represents the extent of damage to the sperm during preservation or freezing and has been used to evaluate the quality of semen (Jani *et al.*, 1983 and Singh *et al.*, 1996). Degree of damage reflected through higher and lower values of transaminases in the seminal plasma is ostensibly due to inherent quality of sperms to withstand cooling (Singh *et al.*, 1991). The addition of chelating agents and sulphhydryl compounds was reported to improve the quality of semen after freezing in different livestock (Saxena and Tripathi, 1984; and Singh *et al.*, 1989). No report is available on the release of enzymes from buck spermatozoa after addition of EDTA and Cysteine HCL at different hours of preservation in different extenders.

A total of 12 bucks (6 Beetal and 6 Beetal X Black Bengal) belonging to Network project on goat, at Ranchi Veterinary College, Ranchi were used for the study and semen was collected twice weekly, using a sterile artificial vagina. After initial evaluation semen samples were pooled breedwise and centrifuged at 3000 rpm for 15 mts to remove enzyme lecithinase. Subsequently, the split semen sample was extended in the ratio of 1:10 in egg yolk citrate (Salisbury *et al.*, 1941) and Tris egg yolk fructose citric acid diluent (Davis *et al.*, 1963) containing different additives (Ethylene diamine tetra acetic acid and Cysteine hydrochloride). The additives were mixed @ 1 mg /ml of extender separately and one control for each extender containing no additives was also used. The extended semen samples were preserved in refrigerator at 5° C. The seminal plasma /diluting medium was separated at 0, 24, 48, 72, and 96 hours and estimation of enzymes AST and ALT was done as per the methods of Henry (1974).

\*Part of M.V.Sc. Thesis.

1. M.V.Sc. Student, 2. University Professor, 3. Dean, RVC, Ranchi.

Means and standard errors were calculated. Data were subjected to statistical analysis by F-test and means were compared through critical difference test (Snedecor and Cochran, 1967). Analysis of data for Beetal and Crossbred bucks were done separately.

Variations in leakage of enzymes (AST and ALT) of Beetal and Crossbred buck spermatozoa at different hours of preservation in different extenders have been shown in Table 1 and 2. It is evident from the table that with increasing hours of preservation there was increase in both enzyme levels in all the extenders. Effect of extenders on AST and ALT level of these enzymes was significant ( $P < 0.01$ ) at all hours of preservation. The level of both the enzymes were higher in cross-bred as compared to Beetal bucks. Results obtained during the present study indicate that leakage of both AST and ALT enzymes was reduced by inclusion of EDTA and Cysteine HCL in both the diluents as compared to control (EYC and TEYFC) extenders. The present observations are in corroboration with the findings of previous workers (Dhami and Sahni, 1993). However, Singh *et al.* (1993) reported that leakage of AST and ALT in goats was more in Tris extender than egg yolk extender which is not in agreement with the present observations. It is an established fact that loss of motility, from whatever cause, coincides with a loss of intracellular contents reflected in the outflow of small and large molecular substances, the latter including aminotransferases, lactic dehydrogenase and cytochrome C (Mann and Lutwak Mann, 1981)

On the basis of results obtained it can be concluded that Beetal spermatozoa are more resistant to cooling as compared to crossbred sperms. It can also be inferred that inclusion of Cysteine HCL & EDTA improve the keeping quality of buck semen.

**Table 1.** Mean seminal AST level of Beetal and cross bred bucks in different extenders at different hours of preservation (IU/L).

Hours of Preservation	No. of observation	Extenders					
		EYC Cyst	EYC + HCL	EYC + EDTA	TEYFC	TEYFC + HCL	TEYC + Cyst
<b>(Beetal)</b>							
0	6	69.94 <sup>a</sup> ±0.28	68.87 <sup>b</sup> ±0.11	68.46 <sup>b</sup> ±0.19	67.49 <sup>c</sup> ±0.25	66.71 <sup>d</sup> ±0.19	66.24 <sup>d</sup> ±0.22
24	6	83.02 <sup>a</sup> ±0.18	81.52 <sup>b</sup> ±0.34	79.74 <sup>c</sup> ±0.37	79.86 <sup>c</sup> ±0.28	75.93 <sup>d</sup> ±0.23	72.88 <sup>e</sup> ±0.20
48	6	97.45 <sup>a</sup> ±0.23	92.91 <sup>c</sup> ±0.20	88.95 <sup>e</sup> ±0.27	93.88 <sup>b</sup> ±0.28	89.96 <sup>d</sup> ±0.26	85.82 <sup>f</sup> ±0.15
72	6	113.71 <sup>a</sup> ±0.37	106.87 <sup>c</sup> ±0.27	99.12 <sup>e</sup> ±0.16	108.88 <sup>b</sup> ±0.13	103.89 <sup>d</sup> ±0.12	97.01 <sup>f</sup> ±0.29
96	6	132.89 <sup>a</sup> ±0.24	125.97 <sup>c</sup> ±0.24	119.85 <sup>e</sup> ±0.21	128.92 <sup>d</sup> ±0.15	122.98 <sup>d</sup> ±0.23	117.91 <sup>f</sup> ±0.23
<b>(Cross bred)</b>							
0	6	75.83 <sup>a</sup> ±0.18	74.81 <sup>b</sup> ±0.10	74.09 <sup>c</sup> ±0.21	73.93 <sup>c</sup> ±0.15	72.83 <sup>d</sup> ±0.26	72.09 <sup>e</sup> ±0.08
24	6	89.58 <sup>a</sup> ±0.23	87.71 <sup>b</sup> ±0.21	85.71 <sup>c</sup> ±0.22	87.19 <sup>b</sup> ±0.29	85.24 <sup>c</sup> ±0.25	84.02 <sup>d</sup> ±0.26
48	6	103.12 <sup>a</sup> ±0.21	100.94 <sup>c</sup> ±0.22	97.72 <sup>e</sup> ±0.21	101.89 <sup>b</sup> ±0.17	98.78 <sup>d</sup> ±0.21	95.26 <sup>f</sup> ±0.19
72	6	120.16 <sup>a</sup> ±0.32	116.82 <sup>b</sup> ±0.23	112.96 <sup>d</sup> ±0.28	117.51 <sup>b</sup> ±0.22	113.86 <sup>c</sup> ±0.16	109.15 <sup>e</sup> ±0.40
96	6	138.04 <sup>a</sup> ±0.22	132.97 <sup>c</sup> ±0.21	126.71 <sup>e</sup> ±0.27	133.98 <sup>b</sup> ±0.19	127.82 <sup>d</sup> ±0.25	122.84 <sup>f</sup> ±0.18

Values bearing same superscripts in a row did not differ significantly.



**Table 2:** Mean seminal ALT level of Beetal and cross bred bucks in different extender at different hours of preservation (IU/L).

Hours of Preservation	No. of observation EDTA	Extenders					
		EYC cyst	EYC + HCL	EYC + EDTA	TEYFC	TEYFC + HCL	TEYC + Cyst.
<b>(Beetal)</b>							
0	6	54.10 <sup>a</sup> ±0.21	53.09 <sup>b</sup> ±0.37	52.81 <sup>c</sup> ±0.05	50.91 <sup>d</sup> ±0.20	49.30 <sup>e</sup> ±0.22	49.09 <sup>e</sup> ±0.15
24	6	62.30 <sup>a</sup> ± 0.18	60.25 <sup>b</sup> ± 0.30	58.97 <sup>c</sup> ± 0.16	59.94 <sup>b</sup> ± 0.26	57.13 <sup>d</sup> ± 0.22	55.21 <sup>e</sup> ± 0.24
48	6	74.89 <sup>a</sup> ± 0.16	71.91 <sup>b</sup> ± 0.14	68.76 <sup>c</sup> ± 0.24	71.96 <sup>b</sup> ± 0.18	68.88 <sup>c</sup> ± 0.25	65.74 <sup>d</sup> ± 0.14
72	6	90.25 <sup>a</sup> ± 0.16	86.96 <sup>b</sup> ± 0.14	83.95 <sup>d</sup> ± 0.24	87.47 <sup>b</sup> ± 0.21	83.69 <sup>c</sup> ± 0.25	79.3 <sup>e</sup> ± 0.24
96	6	111.89 <sup>a</sup> ± 0.20	105.93 <sup>c</sup> ± 0.21	100.83 <sup>e</sup> ± 0.14	106.75 <sup>b</sup> ± 0.18	101.93 <sup>d</sup> ± 0.24	97.72 <sup>f</sup> ± 0.21
<b>(Cross bred)</b>							
0	6	56.83 <sup>a</sup> ± 0.12	55.62 <sup>b</sup> ±0.17	55.04 <sup>c</sup> ± 0.12	52.80 <sup>d</sup> ± 0.21	51.79 <sup>e</sup> ± 0.17	51.05 <sup>f</sup> ± 0.13
24	6	67.79 <sup>a</sup> ± 0.24	65.82 <sup>b</sup> ± 0.25	62.79 <sup>d</sup> ± 0.20	63.80 <sup>c</sup> ± 0.25	61.97 <sup>e</sup> ±0.21	58.87 <sup>f</sup> ± 0.23
48	6	79.87 <sup>a</sup> ±0.18	76.47 <sup>b</sup> ± 0.31	73.84 <sup>d</sup> ±0.16	75.19 <sup>c</sup> ±0.16	72.75 <sup>e</sup> ± 0.27	69.79 <sup>f</sup> ± 0.25
72	6	93.98 <sup>a</sup> ± 0.21	89.04 <sup>c</sup> ±0.16	85.26 <sup>e</sup> ± 0.20	90.37 <sup>b</sup> ±0.21	86.97 <sup>d</sup> ±0.14	82.97 <sup>f</sup> ± 0.13
96	6	122.85 <sup>a</sup> ± 0.16	106.85 <sup>c</sup> ± 0.19	101.23 <sup>e</sup> ± 0.17	109.22 <sup>b</sup> ± 0.18	103.30 <sup>d</sup> ± 0.15	98.70 <sup>f</sup> ± 0.17

Values bearing same superscripts in columns did not differ significantly.

## REFERENCES

- Davis, L.S., Bratton, R. W. and Foote, R. H.(1963) Livability of bovine spermatozoa at 5°C Tris buffered and citrate buffered yolk glycerol extender . J. Dairy Sci., 46: 57-60.
- Dhami, A. J. and Sahni, K.L. (1993). Effect of extenders additives and sheep freezing on the leakage of transaminases from murrh buffalo spermatozoa. Buffalo Journal, 9: 55-64.
- Jani , V. R. , Prabhu, G. A. , Derashri . H.J., Patel, M. M. and Kodagali, S. B. (1983). Release of transaminase from buffalo spermatozoa during deep freezing. Indian J. Anim. Reprod., 4: 30-32.
- Henry J. B.(1974) Clinical diagnosis and management by laboratory methods. W. B. Saunders Co., Philadelphia, PA, Page 361.
- Mann, T. and Lutwak, Mann , C. (1981). Male reproduction function and semen. Ist Ed. Springer – Verlag berlin , Heidelberg, New York.
- Salisbury, G . W., Fuller, H. K. and Willet, E. L. (1941). Preservation of bovine spermatozoa in yolk citrate diluent and field results from its use J. Dairy Sci., 24: 905-910.
- Saxena, V.B. and Tripathi, S.S.(1984). Preservation of ram semen at 3<sup>o</sup>-5<sup>o</sup> C. Indian J. Anim. Sci., 54: 813- 815.
- Singh, J., Pangawkar, G. R., Biswas, R. K. and Shrivastava. A. K. (1991). Studies on leakage of trasaminases from buffalo spermatozoa in relation to deep freezing in certain extenders. Indian J. Anim. Reprod., 12: 135- 140.
- Singh , L. P., Meur, S.K. and Purbey , L. N. (1993). Leakage of transaminases during preservation of buck semen. Indian J. Anim. Sci., 63: 963-965.

Singh, M.P., Sinha, A. K., Singh, B. K. and Prasad, R. L. (1996). Effect of cryoprotectants on release of various enzymes from buck spermatozoa during freezing. *Theriogenology*, 45: 405-416.

Singh, N.P., Manik, R.S. and Raina, V.S. (1989). Effect of Cysteine fortification on preservability of buffalo semen in milk whey extender. *Theriogenology*, 32: 979-986.

Snedecor, G. W. and Cochran, W. G. (1967). *Statistical Methods*. 6<sup>th</sup> Ed. Oxford and IBH Publishing Co., Calcutta.

**State Agricultural Management Extension Training Institute (SAMETI),  
SKUAST-K, Shalimar-191 121**



**SAMETI is an autonomous centrally sponsored Institution with the following key functions :**

- ✓ To improve capacity building support in Extension Management related areas to the extension functionaries both from public and Private sector.
- ✓ To provide consultancy in the areas like project planning, appraisal, implementation etc.
- ✓ Develop and promote the application of Management tools for improving the effectiveness of Agriculture Extension service through better management of human and material resources.
- ✓ Organize need based training programme of middle level agricultural Extension functionaries.
- ✓ Develop modules on Management, Communication, Participatory Methodologies etc. as a sequel to the feed back from training programme.

Contact us

**Prof. G. M. Wani**  
Director SAMETI

SK University of Agricultural Sciences & Technology (K),

Post Box 461 G. P. O. Srinagar, Kashmir 190 001

Phone no. 0194-2463459 (O) 2431508 (R) 2461317 (Fax)

Cell: 09419095342

<http://www.kameti.org>

at different

buffered yolk

s from murrah

from buffalo

ge 361.

delberg, New

d field results

spermatozoa

dian J. Anim.

June, 2007