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Studies on certain seminal plasma enzymes of crossbred bulls in relation to freezability of semen*

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ABSTRACT

Semen from four bulls of poor freezability (<50% Post thaw motility) and four bulls of good freezability (>50 % Post thaw motility) were analyzed for lactic dehyrogenase (LDH), reduced glutathione (GSH), alkaline phosphatase (ALP), acid phosphatase (ACP) and aspartate amino transferase (AST) in neat and post-thaw semen samples. The mean value of the enzymes and substrate in the neat and post-thaw semen samples of good freezable bulls were estimated to be 111.19±3.54, 176.67±3.68 IU/L, 1.85±0.21, 0.57±0.05 mmol/L, 241.67±7.65, 285.21±4.56 IU/L, 1043.63±11.05, 782.00±21.85 IU/L and 43.73±1.15 and 52.11±1.88 IU/L. The corresponding values for poor freezable bull semen were, 76.85±4.84, 175.07±3.27 IU/L, 0.91±0.15, 1.17± 0.12 mmol/L, 264.38±6.78, 324.38±7.45 IU/L, 948.75±9.30, 979.23±16.50 IU/L and 43.79± 1.30 and 53.11±1.79 IU/L, respectively. Significant differences ($P<0.01$) were marked between the neat semen as well as with the post-thaw semen samples of good freezable and poor freezable bull semen. The relationships of these enzymes and substrate with the pre freeze and post-thaw seminal characteristics have been discussed.

Key words: Post thaw, enzymes, substrate, bull semen

The improvement in livestock to a great extent depends on superior sire and the ability of breeding bulls to reproduce superior germ plasm. Although cryopreserved semen is used routinely for breeding dairy cattle, critical examination reveals that the proportion of fully functional sperm in frozen thawed samples is quite low. Moreover, the availability of superior quality crossbred bull semen is an enigma due to problems of its freezability and fertility. Therefore, the ability to predict post-thaw sperm quality and fertility from a routine sperm function assay has been proved to be beneficial, considering the extended period of progeny (Garner *et al.*, 1994). Hence, the present investigation was undertaken to see the effect of freezing on certain post-thaw biochemical constituents and fertility status of the semen of crossbred bulls of good and poor freezability.

MATERIALS AND METHODS

The present study was carried out on twenty crossbred bulls (Red Sindhi × Jersey) of 50% exotic inheritance maintained at Frozen semen bank, Cuttack under identical nutritional and managerial conditions. The routine examination of semen was done immediately after collection. The freezability of semen samples was adjusted from the post thaw motility of spermatozoa of each ejaculate of individual bulls. This criterion was employed in the subsequent part of the investigation of select four bulls of poor freezability (<50% Post thaw motility) and four bulls of good freezability (>50% Post thaw motility). Semen was collected from each of the four poor and good freezable bulls with six ejaculates from each of the eight bulls were considered for this investigation. After routine semen evaluation each semen samples were split into two parts. One part was centrifuged at ±4°C to remove the seminal plasma and stored at -20°C for estimation of biochemical constituents. The other part was immediately processed for freezing in French straws and stored in liquid nitrogen. After storage in liquid nitrogen for a period of 1 month the straws were thawed at 37°C for 30 sec and assessment of biochemical characteristics were done. The neat semen and the post-thaw semen samples were analyzed for lactic dehydrogenase (Varley *et al.*, 1980), Reduced glutathione (Beutler *et al.*, 1963), alkaline phosphatase (King and Armstrong, 1934), acid phosphatase (Walter and Schutte, 1974) and aspartate amino transferase (Reitman and Frankel, 1957). The statistical analysis of the data was done according to Steel and Torrie (1981) on Wipro Pentium computer.

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RESULTS AND DISCUSSION

The enzymatic profile and substrate in semen of good and poor freezable cross-bred bulls before freezing and post thaw samples are presented in Table 1.

Lactic dehydrogenase (LDH) : The mean seminal plasma LDH activities observed in bulls donating good freezable semen (111.19±3.54 IU/L) differ significantly with the bulls donating poor freezable semen (76.85±4.84 IU/L). This is in agreement with the observation made by Tuli and Singh (1982), but higher values have been reported by Zvereva *et al.* (1977) and Dhami *et al.* (1990). The lower values in the might be due to the effect of season which is known to influence the seminal plasma LDH activity in cattle (Singh and Sadhu, 1972).

Reduced glutathione (GSH) : The mean values of reduced glutathione (GSH) in the seminal plasma of good freezable and poor freezable bull were found to be 1.85±0.21 and 0.91±0.15 mmol/l, which was found to have statistically significant difference. The present values is in confirmative with the findings of Slaweta *et al.* (1988), but is lower than those reported by Jain *et al.* (1990) and higher than the findings of Jain and Arora (1988).

The utility of GSH in the maintenance of spermatozoan integrity has been well advocated by Maxwell and Stojanov (1966). So the higher GSH value in the good freezable semen might be a factor in making the sperm membrane more resistant to the spontaneous lipid peroxidation that destroys the structure of the lipid matrix and is associated with loss of motility.

Alkaline phosphatase (ALP) : In the present study the mean alkaline phosphatase in the seminal plasma of good freezable and poor freezable bulls were 241.67±7.65 and 264.38±6.78 IU/L, respectively, which did not differ significantly. The present observation is in confirmative with the findings of Nafornita *et al.* (1977). However, higher values have been reported by Pangawkar *et al.* (1988) and lower values have been found by Stojanov *et al.* (1976). The Present finding of low level of ALP in bulls donating good freezable semen contradicts with the findings of Abdou *et al.* (1978) who observed that ALP was found to increase with improved libido but is affirmative with the observation made by Dubiel and Jasinski (1966) that concentration of ALP was lower in lower in young bulls than the older ones which might have occurred in the present investigation due to inclusion of some young bulls in good freezable group of bulls. The higher level of ALP in poor freezable bull semen might be due to high percentage of abnormal sperms in the ejaculate and is a fair indicator of phosphatase activities in these group of bulls reflecting initial

Table 1. Enzymes and substrate in semen (Mean±SE) of good and poor freezable crossbred bulls before freezing and in post thaw samples

Bull No.	LDH (IU/L)		GSH (µmol/L)		ALP (IU/L)		ACP (IU/L)		AST (IU/L)	
	Neat semen	Post thaw	Neat semen	Post thaw	Neat semen	Post thaw	Neat semen	Post thaw	Neat semen	Post thaw
Good freezable										
X-145	122.39±8.35	171.91±6.37	1.61±0.33	0.67±0.17	253.33±11.45	292.50±8.04	1037.30±17.24	765.90±30.81	45.67±2.05	55.57±4.03
C-73	109.29±8.48	192.63±10.44	2.56±0.40	0.56±0.07	246.67±25.22	293.33±6.15	1041.90±27.31	839.50±69.04	50.30±1.36	51.73±4.18
IX-59	112.63±4.23	176.43±3.19	1.45±0.21	0.50±0.08	224.17±11.21	285.83±8.31	1081.00±14.55	814.20±38.04	38.80±1.01	53.67±4.82
C-3627	100.48±7.43	165.72±3.11	1.78±0.47	0.56±0.07	242.50±9.46	269.17±11.79	1014.30±23.02	708.40±28.65	40.37±0.65	47.47±0.88
Overall	**111.19±3.54^{Aa}**	**176.67±3.68^{b}**	**1.85±0.21^{Aa}**	**0.57±0.05^{Ab}**	**241.67±7.65^{a}**	**285.21±4.56^{Ab}**	**1043.63±11.05^{Aa}**	**782.00±21.85^{Ab}**	**43.73±1.151^{a}**	**52.11±1.88^{b}**
Poor freezable										
X-208	73.34±9.60	192.63±3.41	0.56±0.18	1.22±0.38	265.83±13.63	346.67±18.29	947.60±17.33	982.10±24.54	35.07±2.17	48.17±0.76
X-180	72.86±7.76	163.81±6.27	1.39±0.47	1.22±0.27	255.83±12.21	330.83±12.61	966.00±14.69	984.40±39.46	45.10±2.17	53.13±4.57
X-183	84.05±14.51	175.24±4.99	0.83±0.14	1.17±0.22	256.67±10.87	311.67±15.37	947.60±21.28	998.20±30.79	54.80±0.86	51.00±3.07
C-193	77.15±7.23	168.58±5.08	0.89±0.31	1.06±0.06	279.17±17.95	308.33±9.97	933.80±22.72	952.20±40.94	49.20±1.64	60.13±3.53
Overall	**76.85±4.84^{Ba}**	**175.07±3.27^{b}**	**0.91±0.15^{a}**	**1.1±0.12^{a}**	**264.38±6.78^{a}**	**324.38±7.45^{Bb}**	**948.75±9.30^{B}**	**979.23±16.50^{B}**	**43.79±1.30^{a}**	**53.11±1.79^{b}**

damage of the sperm membrane with subsequent increase in the permeability, resulting in the leakage of the enzyme into the seminal plasma (Pangawkar *et al.*, 1988). This is further substantiated by Tamba-Berehoru *et al.* (1988) who observed in bulls which were categorized according to the percentage of ejaculates suitable for freezing revealed that the concentration of ALP as significantly lower in groups in which 82.3% ejaculates were suitable than in groups in which 41.2% ejaculates were found suitable.

Acid phosphatase (ACP) : The mean acid phosphates level (ACP) in the semen of good freezable bulls (1048.63±11.05 IU/L) and poor freezable bulls (948.75±9.30 IU/L) is higher than those reported by Uniformity *et al.* (1977) but lower than the observation made by Aguirre *et al.* (1988). The discrepancy in the average values may be due to the methods employed for its determination and the activity of the accessory sex glands of the animals under investigation. Moreover, inclusion of varied age of the animals and season might have led to this discrepancy as bulls of younger age had lower ACP vales than in the older bulls and it was reported to be higher in summer than in winter (Mann and Lutwak-Mann, 1981).

The mean value of ACP in good freezable bull seminal plasma was higher than the poor freezable bull with the difference being significant. This might be due to improved libido in good freezable bulls (Abdou *et al.*, 1978) or due to certain inflammatory process that have decreased the ACP activity by 70 per cent (Glogowski and Strzezek, 1981).

Aspartate amino transferase (AST): The mean values of AST in good freezable and poor freezable bull seminal plasma were estimated to be 43.73±1.79 and 43.79±1.30 IU/L, respectively, which finds the support of Buruiana *et al.* (1978). However, higher and lower values have been reported by Khokhar *et al.* (1987) and Verma *et al.* (1988). The variation in the AST level might be attributed to the methods employed for its determination, age of the bull, presence of abnormal sperm in the ejaculate, percentage of exotic blood and season of study.

The activity of AST had no significant difference between the two groups of bulls, which is in consonance with the findings of Mohan *et al.* (1992), but Belorkar *et al.* (1988) and Tamba - Berehoiu *et al.* (1988) observed that AST concentration was higher in bulls donating higher percentage of freezable ejaculates. This might explain that in poor freezable bulls the negative correlation of AST with seminal parameters is indicative of the damage to the sperm membrane, thereby rendering it to further damage and leakage of enzymes during the process of cryopreservation.

The process of cryopreservation in good freezable bull semen brought statistically significant difference in the level of all the above five enzymes. However, in the poor freezable bull semen the level of LDH, ALP and AST only showed significant difference. In fresh good freezable semen the level of LDH, GSH and ACP were recorded highly significant than poor freezable semen. Where as in frozen thawed semen GSH and ACP levels were only found significantly higher in good freezable semen. The good freezable bulls semen have significantly higher levels of GSH and ACP out of 5 enzymes studied in both neat and post thawed semen than poor freezers.

From the present study, it can be concluded that the higher levels of LDH, GSH and ACP is one of the parameter for good freezable semen which can be further exploited in selection of bulls as additional criteria for commercial application of Artificial Insemination programme in large scale.

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