RELEASE OF INTRACELLULAR ALANINE AND ASPARTATE TRANSAMINASE FROM BUFFALO SEMEN DURING CENTRIFUGATION AND THEIR EFFECT ON SPERM SURVIVAL

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ABSTRACT

Ten pooled buffalo semen samples were separated into five equal aliquots i.e A to E. Aliquot A (whole semen not centrifuged), C (centrifuged but seminal plasma not removed) and E (centrifuged and seminal plasma removed) were extended at the ratio of 1:10 with lactose-fructose-egg-yolk-glycerol extender and incubated to note the liveability (hours) and absolute index of liveability at 37°C. Aliquot E showed significant better liveability than A and C ($P < 0.01$). The poorest results were observed in that of aliquot C.

Aliquot B (whole semen not centrifuged), D (whole semen centrifuged but seminal plasma not removed) and seminal plasma removed from aliquot E after centrifugation were used for the determination of alanine and aspartate transaminase (ALT & AST). The ALT levels of 30.29 ± 1.37, 34.75 ± 2.02 and 10.50 ± 0.48 IU/L where as AST concentration of 214.12 ± 3.51, 257.37 ± 3.90 and 60.28 ± 1.75 IU/L were found in aliquot B, D and E, respectively.

It may be concluded from the results that centrifugation induces leakage of ALT and AST which have adverse effects on sperm survival. Therefore, seminal plasma must be removed after centrifugation to improve the sperm survivability and preservation.

INTRODUCTION

Centrifugation and washing had been used for efficient separation of sperm from seminal plasma which improve the viability of buffalo spermatozoa (Ahmad *et al.*, 1996). Similarly, removal of seminal plasma and washing has shown the beneficial effects on motility and acrosome morphology of frozen thawed spermatozoa (Memon *et al.*, 1985). Double washing of buffalo spermatozoa yields better surviveability than single washing or unwashed spermatozoa (Ahmad *et al.*, 1997). Leakage of alanine transaminase (ALT) and aspartate transaminase (AST) after deep freezing of semen has been reported (Iqbal, 1987; Nath *et al.*, 1991; Dhami and Shani, 1993; Younis, 1996), however, literature regarding the leakage of ALT and AST after centrifugation in buffalo semen is scarce.

The present study was carried out to investigate whether leakage of ALT and AST during centrifugation takes place or not and do these leaked enzymes affect the surviveability of buffalo bull spermatozoa.

MATERIALS AND METHODS

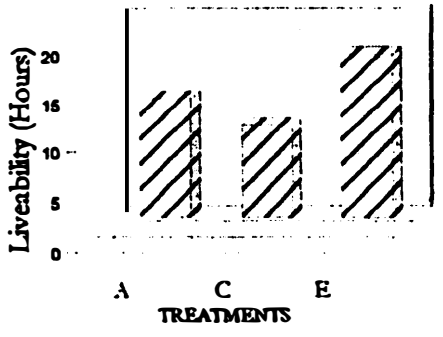
In this study, semen was collected from two Nili Ravi buffalo bulls having same age and kept under similar feeding and management practices. Two semen ejaculates were obtained from each bull on weekly collection day with A.V. First and second ejaculates were pooled. A total of ten pooled (five from each bull) good quality semen samples were used in this study. Immediately after collection and evaluation each pooled semen sample from each bull was divided into five equal aliquots (A to E). Aliquots C, D and E were centrifuged at 3000 rpm for ten minutes (Iqbal, 1987). Seminal plasma was removed from aliquot E while in aliquots C and D it was not removed. The sediment of aliquot E was mixed with lactose-fructose-egg-yolk glycerol extender to make the volume equal to that of semen before centrifugation. Aliquots A (whole semen without

centrifugation), C (semen centrifuged but seminal plasma not removed) and E (seminal plasma removed) were diluted with the extender separately to make the ratio of 1:10. They were incubated at 37°C to note the liveability (hours) and absolute index of liveability following Melovenof (1962).

Aliquots B (whole semen without centrifugation), D (centrifuged but seminal plasma not removed) and seminal plasma removed from aliquot E were used for the estimation of ALT and AST by photometric method (Anonymous, 1980) by using Randox ALT and AST kit and Microlab-100 (Merk) photometer.

RESULTS AND DISCUSSION

The liveability (hours) and absolute index of liveability of spermatozoa in aliquots A, C and E are presented in Fig. 1 and 2. For aliquots A, C and E the liveability (hours) of spermatozoa at 37°C was 12.00 ± 0.71 (10-14), 9.20 ± 0.86 (7-12) and 16.60 ± 1.03 (13-19) whereas the values for absolute index of liveability were 449.03 ± 30.65 (338.52-513.53), 333.44 ± 28.34 (240.02-403.53) and 609.04 ± 38.05 (473.53-691.04) respectively. The data reveals that there is a highly significant difference among the three aliquots ($P<0.01$). Removal of seminal plasma from aliquot E yielded better results than aliquots A and C. The aliquot C from which seminal plasma was not removed after centrifugation showed the poorest results.



A = Whole Semen (WS) not centrifuged (C)
C = WS C with Seminal Plasma (SP)
E = WS C removed SP

Fig.1: Liveability (hours) of buffalo bull spermatozoa at 37°C in whole semen (not centrifuged), whole semen (centrifuged but seminal plasma not removed) and seminal plasma removed after centrifugation.

The results of the present study are in agreement with the findings of Shah (1993), Ahmad *et al.* (1996), Ala-ud-Din *et al.*, (1996) and Ahmad *et al.* (1997). The beneficial effects are due to the removal of a toxic substance, protein in nature present in the seminal plasma, through centrifugation (Shanon, 1965) and this is in high level in buffalo semen as compared to cattle (Ganguli, 1978) and adversely affects the buffalo spermatozoa as compared to cattle (Sahni, 1990).

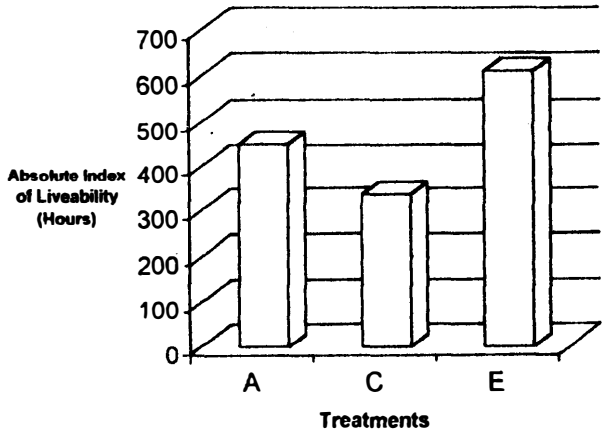


Fig.2: Absolute index of liveability of buffalo bull spermatozoa at 37°C in whole semen (not centrifuged), whole semen (centrifuged but seminal plasma not removed) and seminal plasma removed after centrifugation.

Centrifugation and washing has been used successfully for efficient separation of sperm from the seminal plasma (Ahmad *et al.*, 1997). However, the spermatozoa have shown to be highly vulnerable to mechanical damage which is obvious from the data presented in Fig.3. The ALT levels of 30.29 ± 1.37 (20-42), 34.75 ± 2.02 (20-55) and 10.50 ± 0.48 (6-16) IU/L, respectively, were observed in aliquots B, D and seminal plasma of aliquot E. The AST concentrations were 214.12 ± 3.51 (168-240), 257.37 ± 3.90 (220-307) and 60.28 ± 1.75 (48-75) IU/L respectively for these aliquots. The high levels of both ALT and AST after centrifugation in aliquot D are pointing to the fact that these enzymes were leaked from the spermatozoa. However, the levels of these enzymes are low in aliquot B and also in the seminal plasma of aliquot E. Similar, findings have been reported by Mann (1951), who observed the release of intracellular protein, enzymes and ions into the surrounding medium after centrifugation which suggested mechanical injury to sperm cells. The poorest results observed in aliquot C for liveability (hours) and absolute index of liveability revealed that leakage of ALT and AST resulted into high levels of these enzymes which could have toxic effects on spermatozoa. The activity of ALT and AST in the

seminal plasma is negatively correlated with average fertility of bulls (Pace and Graham, 1970; Singh and Sadhu, 1972; Dhami and Shani, 1993) which supports the findings of the present study. However, if the seminal plasma is separated after centrifugation it has beneficial effects on sperm survivability and preserve ability.

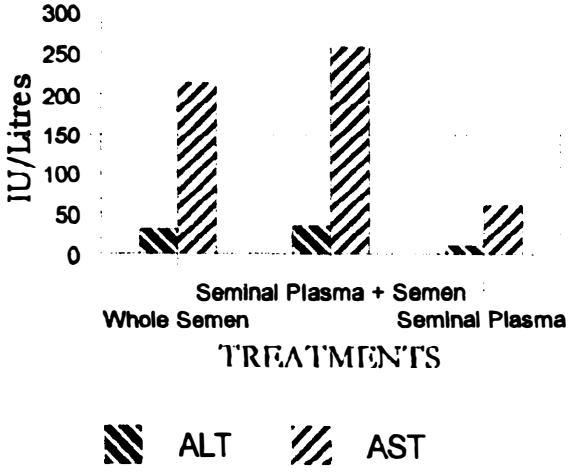


Fig.3: ALT and AST concentration in whole semen (not centrifuged), whole semen (centrifuged but seminal plasma not removed) and seminal plasma of buffalo bull.

It may be concluded from the present study that to improve the preserveability/liveability of semen the seminal plasma being separated by centrifugation should be removed, otherwise leaked ALT and AST will have toxic effect and deteriorate liveability of semen.

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