The Indian Journal of Animal Reproduction, 24(1): 75-76, June 2003

SHORT COMMUNICATION

Studies on Seminal Enzyme (GOT, GPT and LDH) Profile and its Relationship with Acrosomal Integrity in Surti Buffalo Bull Semen

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Received: October 6, 2001
Accepted: September 17, 2002

ABSTRACT

Six ejaculates each from 5 Surti buffalo bulls were cryopreserved and studied before and after cryopreservation and at ½, 1 and 2 hours post thawing to evaluate the extent of damage of spermatozoa. The sperm motility and percent live sperms and percent normal acrosomes showed a decline after cryopreservation. The seminal enzymes GOT, GPT and LDH increased in the seminal plasma to significanly high level ($P<0.01$) post freezing and thawing and at different times of post thaw incubation indicating a leakage from the spermatozoa. It was concluded that freezing and thawing of semen causes cryoinjury to the sperms, decrease in the percentage of intct acrosomes and leakage of semen enzymes.

Key words: Acrosome, GOT, semen, seminal enzymes

During the process of semen freezing, acrosomal damage and leakage of intracellular enzymes for spermatozoa are inevitable, inspite of addition of egg-yolk and cryoproctectants 40-50% of the sperms don't survive, even with optimum cryopreservation protocols (Watson, 2000). Cryoinjuries to the sperm cells can cause membrane disruption of the acrosomes, leakage of intra cellular enzymes and changes in sperm cell permeability. The present study was undertaken to investigate the extent of cryoinjury caused to buffalo bull spermatozoa due to freezing based on intracellular enzyme (GOT, GPT and LDH) leakage and to study the cytomorphological changes in relation to acrosomal integrity.

A total of 6 ejaculates were collected from 5 Surti buffalo bulls managed at LRS Vallabhnagar at 3 days interval. These ejaculates were evaluated, diluted and frozen as per methods described by Purohit (2001). The semen samples were stored for some time and were then evaluated for physical traits, enzyme leakage and acrosomal integrity post thawing at 37°C for 1 minute.

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The cryoinjury to buffalo bull spermatozoa was studied by assessing the extent of damage to the acrosomes and by estimating the concentration of GOT, GPT and LDH enzyme levels in the extra cellular fluid before and after freezing and thawing (post-thawing) at 0, ½ , 1 and 2 hours post-thaw incubation period, respectively. The acrosomes were studied using Giemsa staining technique (Watson, 1975). Enzyme (GOT, GPT and LDH) activities were measured by spectrophotometric technique (Hergt and Langin, 1957) using Semi-Auto-Analyzer (Microlab 100, E Merck, Mumbai, India). The data were analysed statistically (Snedecor and Cochran, 1967) for interpretation.

The sperm motility per cent, live per cent of spermatozoa and per cent normal acrosomes of fresh and frozen thawed semen showed decline in sperm motility declined from an average of 66.04±1.06% in fresh to 42.06±3.66% in frozen thawed semen for the five Surti buffalo bulls. Likewise the liver per cent of spermatozoa declined from an average of 72.69±2.28% to 39.360±6.19% and per cent of normal acrosomes declined from an average of 79.66±0.46% to 36.13±6.14%, after freezing and thawing respectively. The average decline in per cent normal acrosomes in the present

study at different intervals of post-thaw incubation was 5.15% , 6.90% and 10.17% at ½, 1 and 2 hours, respectively. These values closely resembled to those observed by Angasaria, 1997 and Chinnaiya and Balakrishnana, 1998.

The overall mean activity of GOT, GPT and LDH enzymes observed in the fresh extended seminal plasma was 47.0±8.68, 15.73±2.29 and 252.70±21.43 IU/L, respectively. The ratio of GOT : GPT ranged between 2.10:1 to 3.5:1, amongst the five bulls with a mean of 2.6:1. These findings are in agreement with Chouhan and Srivastav, 1973; Nafornita et al., 1977 and Varshney et al., 1978. The 't' test analysis revealed that the differences between the bulls were highly significant ($P<0.01$) for seminal plasma GOT, GPT and LDH activity. The mean values of GOT, GPT and LDH activities differed significantly ($P<0.01$) between pre-freeze and post-thaw incubation periods respectively and also differed significantly ($P<0.01$) between the bulls. Harrison and White, 1972 have concluded that enzymatic activity (GOT, GPT and LDH) in seminal plasma originated from disintgrated cytoplasmic droplet, plasma membrane and acrosomal damage when spermatozoa were subjected to stress conditions like cold and osmotic shock.

Overall mean values of leakage of enzymes (GOT, GPT and LDH) in seminal plasma of buffalo bull as a result of cryopreservation are presented in Table 1.

Table 1: Leakage of enzymes (IU/L) in seminal plasma of Buffalo bull as a result of cryopreservation

Enzyme	Post-Thawing			
	0 Min.	30 Min.	60 Min.	120 Min.
GOT	11.06 ±7.23	28.26 ±2.08	42.96 ±2.03	56.63 ±2.29
GPT	7.30 ±2.46	13.67 ±1.08	22.67 ±2.65	32.83 ±2.69
LDH	41.83 ±13.9	117.33 ±1.29	205.83 ±3.07	248.96 ±4.01
Acrosomal disintegration	42.50 ±5.76	47.67 ±3.60	49.40 ±2.72	52.67 ±2.65

The increase in enzymatic activity or leakage might be due to cryoinjury to spermatozoa during freezing and thawing inspite of cryoprotection offered by the egg-yolk. Although, the leakage of all the three enzymes is around 40% (just after thawing), corresponding reduction in sperm motility is only about 25.46% and reduction in normal acrosome is about 43.43%. Inter correlation between enzyme releases due to cryopreservation showed that all the three enzymes GOT, GPT and LDH were positively inter-correlated. The maximum inter-correlation was seen between GOT and GPT enzyme ½ at 1 hr post-thaw incubation period. The correlation between percent intact acrosome and enzyme leakage was negative and was -0.26, -0.18 and 0.48 for GOT, GPT and LDH, respectively. Whereas, the correlation between GOT and GPT and GOT and LDH was however, positive.

It was clear that freezing and thawing of semen caused cryoinjury to sperm, it lead to leakage of enzymes and also tends to decrease the percentage of intact acrosomes.

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