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Protection of cell membrane of buffalo spermatozoa during cryopreservation

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ABSTRACT

Cell membrane of buffalo spermatozoa was substantially damaged in the egg yolk tris dilutor during cryopreservation as evidenced by greater release of transaminases and dehydrogenases enzymes. Insulin and foetal calf serum were found to protect the sperm cell membrane as evinced by reduced leakage of these enzymes and imporved level of motile, live and normal spermatozoa during cryopreservation.

Key words : Buffalo, spermatozoa, cryopreservation, additives

Sperm cell membrane is subjected to damage during freezing and thawing resulting into leakage of certain vital intracellular enzymes which affect sperm cell functions both, in vivo and in vitro (Dhami and Sahni, 1994). Therefore, an experiment was conducted to study the leakage of various marker enzymes and thereby assess the cell membrane damage during cryopreservation of buffalo spermatozoa in tris dilutor, as well as protecting the membrane by addition of foetal calf serum, insulin and cholesterol.

MATERIALS AND METHODS

Experimental material comprised of 18 ejaculates from three Murrah buffalo bulls. Immediately after collection, neat semen was evaluated by standard laboratory tests. A portion of all semen samples was diluted in egg yolk tris (EYT) dilutor (Vasanth, 1979) and EYT supplemented with foetal calf serum @ 10% (EYT-S), insulin @10 mg/ml (EYT-I) and cholesterol @ 1 mg/ml (EYT-C). All diluted semen samples were processed for deep freezing (Dhami and Sahni, 1994). Remaining portion of undiluted semen and a portion of diluted semen samples in all four dilutors at various stages of freezing were centrifuged at 3000 rpm for 15 min. at 4-50C to get seminal plasma in which enzymes were estimated. Semen samples were evaluated at all stages, i.e. 0 Hr dilution, pre-freeze (PF) and post thaw (PT) by sperm motility, livability and abnormality, transaminases (Sigma Technical Bulletin, 1959), i.e. glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) and dehydrogenases (Blackshaw, 1963), i.e. lactic dehydrogenase (LDH) and succinic dehydrogenase (SDH). Data were analysed statistically (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Quality of neat semen was satisfactory, as evidenced by average values for spermiogram and enzyme content in the neat semen (Table 1). There was no adverse effect of dilution on structural and functional integrity of sperm cell membrane in all dilutors, as the values of fresh semen and after dilution (0 Hr) did not vary significantly in respect of physico-morphological characters and enzymes studied. It indicated that satisfactory level of spermatozoan viability, livability and membrane integrity were maintained upon dilution due to optimum osmolarity of all dilutors (Kishor, 1997).

Sperm cell membrane damage was less during 0 Hr to PF stage of semen freezing, as evidenced by low leakage of various enzymes studied in EYT medium (Table 1). The observations were further corroborated by optimum level of motile, live and normal spermatozoa in the EYT during 0 Hr to PF stage. However, foetal calf serum, insulin and cholesterol seem to have protected the sperm cell membrane, as evidenced by lower leakage of enzymes studied and higher values of viable and normal spermatozoa in EYT-S, EYT-I and EYT-C during this stage of semen freezing. The results observed above may be conceived because of protection of spermatozoa from severe cold shock by foetal calf serum (Adam, 1990; Pandey 2001). Insulin also might have helped spermatozoa to face adverse conditions of cooling and cold shock better due to high energy state (Booth and McDonald, 1982).

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Table 1: Meat ± SE of physico-morphological and biochemical characters of semen of Murrah bulls during freezing stages.

		DILUTORS			
Sl. No.	Seminal characters/Freezing stages	EYT	EYT-S	EYT-I	EYT-C
1.	**Motile sperm, %** (66.66 ± 1.71)				
	0 Hr	61.11 ±2.16	63.05±1.72	63.05±1.85	63.61±1.97
	PF	51.66±1.85	56.11±1.74	55.27±1.78	57.05±1.78
	PT	42.22±2.36^{a}	48.61±2.13^{b}	47.77±2.14^{b}	48.33±2.28^{b}
2.	Live sperm % (71.44±1.54)				
	0 Hr	67.66±1.63	69.55±1.71	69.55±1.71	69.77±1.55
	PF	63.33±1.32	65.72±1.70	66.61±1.88	66.17±1.43
	PT	50.16±0.99^{a}	56.72±1.36^{b}	57.22±1.65^{b}	56.27±1.53^{b}
3.	Abnormal sperm % (11.33±2.00)				
	O Hr.	11.55±0.85	8.27±0.54	10.33±1.09	9.38± 0.94
	PF	12.50±0.98	9.94±0.76	10.88±1.11	10.50±1.05
	PT	15.11±0.93^{a}	12.61±0.82^{b}	13.11±1.17^{ab}	12.16±1.18^{b}
4.	GOT Units/ml (104.66±7.06)				
	O Hr.	391.27±97.37	273.77±65.61	206.77±40.93	183.16±27.79
	PF	449.50±102.59	339.55±70.33	253.94±43.27	291.38±62.32
	PT	538.88±102.89^{a}	417.16±69.19^{bc}	342.44±42.96^{b}	447.11±75.49^{bc}
5.	GPT Units/ml (31.00±1.57)				
	O Hr.	44.66±1.10	41.94±1.77	38.11±2.28	42.44±1.82
	PF	60.94±2.07	58.16±1.16	55.55±2.58	57.38±2.18
	PT	107.44±1.04^{a}	93.66±1.95^{b}	90.33±1.40^{b}	100.22±1.33^{ab}
6.	SDH mg/ml (315.16±2.72)				
	O Hr.	416.94±2.62	390.50±2.81	369.77±3.13	405.22±3.56
	PF	599.33±2.82	506.55±3.14	446.83±2.68	588.94±2.49
	PT	1064.64±13.3^{a}	983.11±5.40^{b}	982.88±2.59^{b}	1081.72±3.61^{a}
7.	LDH, mg/ml (237.05±4.10)				
	O Hr.	352.11±3.73	325.77±3.49	306.44±3.56	334.22±3.67
	PF	544.00±3.68	438.50±3.60	386.77±4.70	540.11±2.66
	PT	977.50±3.54^{a}	921.55±4.03^{b}	917.22±5.06^{b}	1011.94±3.28^{a}

Figures in parentheses indicate values in the neat semen. Means bearing common superscript do not differ significantly on overall basis.
PF = Pre-freeze, PT = Post-thaw

The level of GOT enzyme increased 5.15 times from neat semen to frozen thawed semen in EYT reflecting substantial leakage of the enzyme through damaged sperm cell membrane. The insulin protected sperm cell membrane and function maximally as evinced by minimum leakage of this enzyme (3.27 times) in the EYT-I medium as compared to foetal calf serum (3.99 times) and cholesterol (4.27 times). The results may be attributed to the fact that carrier substances present in the cell membrane can transport glucose only under the influence of insulin. By this mechanism in the presence of added insulin, probably more glucose reached inside the cell where intracellular metabolic machinery took it over for energy generation and thus made the sperm more capable to withstand the rigors of freezing and thawing better (Booth and McDonald, 1982). Similarly, level of GPT increased 3.47 times in the EYT, where as in the EYT-I medium, it increased by 2.91 times only as compared to 3.02 times in EYT-S and 3.23 times in EYT-C. As such, the results are well conceivable.

Regarding the leakage of dehydrogenases, SDH increased 3.38 times in the EYT from neat to frozen semen which was reduced to minimum of 3.12 times in the presence of insulin and foetal calf serum (Table 1) which were thus beneficial for cryopreservation of buffalo semen. Highest increase of 3.43 times was recorded in the EYT-C medium. It seems that foetal calf serum, rich in macro- and microbiomolecules, protected the structural and functional integrity of sperm cell membrane by providing nutrients, cryoprotectants and chelating agents (Gupta and Saxena, 1999). Similar effect of the serum and insulin was observed regarding leakage of LDH (4.12 times in EYT, 3.87 times in EYT-I, 3.89 times in EYT-S and 4.27 times in EYT-C) and as such results are conceivable. Protective effect of foetal calf serum and insulin is also corroborated by higher level of motile, live and normal spermatozoa in the frozen semen in EYT-I and EYT-S as compared to EYT.

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