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ABSTRACT BOOK

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Efficiency of Dithiothreitol and Sucrose on Bull Semen Cryopreservation

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The aim of this study was to assess the effects of supplementation of dithiothreitol (D) or sucrose (S) as antioxidants on the sperm parameters, plasma membrane integrity, antioxidant activities, sperm molility characteristics and DNA integrity in Tris extender for cryopreservation of bull semen.Totally 24 ejaculates were collected from the three Holstein bulls with the aid of an artificial vagina twice a week. A Tris-based extender (T) and 5% Glycerol (G) was used as the base for the experimental extenders. Each ejaculate was split into three equal aliquots and diluted using both of 5 mM D or 25 mM S, and without additives (control; C). The extended samples were equilibrated slowly to 4°C for 4 h and then froze using a digital freezing machine. Frozen straws were thawed individually in water bath at 37°C for 30 s to analyse motilities, acrosome and total abnormality, plasma membrane integrity, antioxidant activities and sperm motility characteristics. The volume of ejaculates was measured in a conical tube and sperm concentration was determined by Accucell photometer. Sperm motility was indicated using phase-contrast microscope (200x). Sperm motility characteristics were determined by sperm analysis system. DNA integrity was evaluated by comet assay using image analysis system. When compared to the control, D had greatest motility ($P < 0.05$). However, addition of D and S did not significantly increase the percentages of post-thaw sperm progressive motitility, acrosome and total abnormalities and plasma membrane integrity ($P > 0.05$). Control group gave the lowest MDA but this result was not supported with the GPx activity ($P < 0.01$; Table 1). Sperm motion characteristics such as VAP, VCL and BCF gave significantly different results except for VSL, ALH and LIN ($P < 0.05$). D and S were showed better DNA integrity than C (Table 2). In conclusion, it may be stated that, using D and S may improve the DNA integrity. In addition D gave greater motility result than S and C in T extender with 5% G.

Keywords: Antioxidant activity, bull semen, cryopresrevation

Table 1 - Mean (±SEM) CASA progressive motility, CASA sperm motility, acrosome and total abnormalities, plasma membrane integrity, glutathione peroxidase (GPx) and malondialdehyde (MDA) in frozen–thawed bull semen

Groups	Progressive Motility (%)	Motility (%)	Acrosome (%)	Total Abnormality (%)	HOST (%)	MDA (nmol/ml)	GPx (U/ml)
Control 5% G	25.50±3.70	49.50±3.94b	2.13±0.55	10.50±1.41	41.13±2.79	1.73±0.15a	10.34±0.48bc
5% G+5mM D	25.38±2.97	52.25±2.78a	2.38±0.42	11.38±1.29	39.00±3.39	1.85±0.13b	9.46±0.53ab
5% G+25mM S	18.38±2.57	44.75±4.41b	2.50±0.27	11.38±0.65	36.63±2.17	1.83±0.14b	10.82±0.38c
P	N.S.	<0.05	N.S.	N.S.	N.S.	<0.05	<0.01

Table 2 - Mean (±SEM) CASA sperm motion characteristics and DNA integrity in frozen–thawed bull semen

Groups	VAP (µm/sec)	VSL (µm/sec)	VCL (µm/sec)	ALH (µm)	BCF (Hz)	LIN (%)	Tail moment (µm/s)
Control 5% G	99.53±4.79a	80.34±4.87	173.00±8.96a	7.64±0.36	22.06±0.79b	47.88±1.79	9.58±0.22a
5% G+5mM D	102.16±4.43ab	82.05±4.15	178.80±9.11ab	7.76±0.32	22.24±0.82b	47.38±1.08	7.33±0.13b
5% G+25mM S	107.20±3.39c	83.95±2.70	186.71±7.60c	8.33±0.33	17.45±1.25a	46.13±1.41	7.78±0.27b
P	<0.05	N.S.	<0.05	N.S.	<0.05	N.S.	<0.05