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Cysteamine supplementation revealed detrimental effect on cryosurvival of buffalo sperm based on computer-assisted semen analysis and oxidative parameters

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ABSTRACT

The aim of this study was to investigate the effect of addition of cysteamine to the semen extender on post-thaw semen quality. A total of 30 ejaculates were collected from six bulls. Each ejaculate was divided into five equal parts and diluted to final concentration of 80 million sperms/mL using Optixcell® (IMV, France) semen extender supplemented with different concentrations of cysteamine (0, 0.75, 1.25, 2.5 and 5 mM) and cryopreserved. In the frozen-thawed samples, the VAP, VSL, VCL ALH and sperm motility of control samples was greater ($P < 0.05$) than cysteamine treated samples. The sperm abnormality and malondialdehyde (MDA) concentration were found highest in 5 mM cysteamine treated samples. The cysteamine treated samples travelled significantly less distance in cervical mucus as compared to control. Further, cysteamine decreased acrosomal integrity of sperm. In incubation test, control samples showed better sperm motility as compared to treatment groups. Further, cysteamine supplementation decreased the total antioxidants and increased the MDA concentration of sperm. From the study, we hypothesized that cysteamine cannot stimulate synthesis of glutathione (GSH) intracellularly in sperm to combat free radicals because during the maturation, sperm lost its cytoplasm which is necessary for biochemical reaction in which cysteamine reacts with cystine to form a mixed disulfide which taken up by cells and split into cysteine in the cytoplasm. Synthesis of GSH depends on the availability of cysteine. In conclusion, the results of our study strongly emphasize that cysteamine would not be a suitable additive in extender for freezing buffalo bull semen.

1. Introduction

The exposure of spermatozoa to the process of cryopreservation is responsible for the production of reactive oxygen substances (ROS) that decrease post-thaw motility, viability, intracellular enzymatic activity, sperm functions and fertility (Aitken et al., 1989; Sariozkan et al., 2015). Moreover, the endogenous antioxidant system is inherently insufficient especially in the buffalo semen to protect the sperm from the oxidative stress resulting in higher lipid peroxidation of sperm membrane (Nair et al., 2006). This fact is the foundation of strategies consisting of the supplementation of different antioxidants in extenders which overcome the detrimental effect of cryopreservation.

Glutathione (GSH), a potent antioxidant enzyme in mammalian cells, protects the cell from oxidative damage

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(Meister and Tate, 1976). In vivo, GSH synthesis increases during development and maturation of oocyte in the ovary (Perreault et al., 1988) and protects oocyte in later stages of fertilization (Telford et al., 1990). After fertilization, GSH participates in sperm decondensation and transformation of the fertilizing sperm head into the male pronucleus (Perreault et al., 1988; Yoshida et al., 1993). Synthesis of glutathione depends on the availability of cysteine inside the cell (Furnus and de Matos, 1999). Cysteamine, a low molecular weight thiol compound, reacts with cystine and form a mixed disulfide which is taken up by a mammalian cell via the leucine transport carrier and split into cysteine in the cytoplasm (Ishii et al., 1981). Thus, cysteamine reduce cystine to cysteine and enhance glutathione synthesis (Issels et al., 1988). A number of studies have been taken this fact into account and demonstrated that the addition of cysteamine to the in vitro maturation media improves the rate of embryo development (de Matos et al., 1996, 2002; Takahashi et al., 1993; Anand et al., 2008; Olson and Seidel, 2000). However, addition of cysteamine in freezing extender of semen of various species generated contradictory results (Bucak et al., 2007, 2009; Tuncer et al., 2014; Najafi et al., 2014; Sariozkan et al., 2015; Akalin et al., 2016; Buyukleblebici et al., 2016; Gungor et al., 2016). For the first time, Bucak et al. (2007) used cysteamine as additive in sperm cryopreservation and found a dramatic increase in motility (73.0% compared with 47.5%) of ram semen, compared to base extender. To our knowledge, the supplementation of cysteamine in a semen extender for cryopreservation of buffalo semen has not been reported. Therefore, this study was to determine concentration of cysteamine in freezing extender, in an attempt to improve post-thawing semen quality of buffalo semen.

2. Materials and methods

2.1. Cysteamine

Cysteamine was purchased from Sigma–Aldrich Chemicals Pvt Limited (Cat No.: M 6500).

2.2. Semen collection

Six Murrah buffalo bulls (age 3–5 years) maintained at bull shed of Semen Freezing Laboratory, Central Institute for Research on Buffaloes, Hisar, Haryana, India were selected for the study. Semen of these bulls was collected twice a week using artificial vagina technique and freezing was performed for five times for the study. Pre-freezing sperm motility was assessed subjectively under phase contrast microscope equipped with a warm stage (37 °C) at 400× magnification and only ejaculates having ≥70% sperm motility were used for this experiment.

2.3. Semen processing

The ejaculates of each bull were divided into five equal fractions and diluted to final concentration 80 million sperms/mL using Optixcell®(IMV, France) extender supplemented with different concentrations of cysteamine (0, 0.75, 1.25, 2.5 and 5 mM). Thereafter, the extended

Table 1
Effect of cysteamine on sperm kinetics and motile parameters of fresh extended sperm.

Cysteamine (mM)	VAP (μm/s)	VSL (μm/s)	VCL (μm/s)	ALH(μm)	BCF (Hz)	STR (%)	LIN (%)	TM (%)	PM (%)	RM (%)
0	110.28 ± 22.36	81.97 ± 14.64	194.17 ± 49.62^{b}	8.20 ± 1.58^{c}	25.22 ± 3.89^{a}	74.38 ± 3.48^{a}	45.12 ± 4.62^{a}	71.11 ± 9.46^{b}	51.03 ± 7.34^{b}	64.05 ± 10.25^{b}
0.75	99.29 ± 20.87	76.88 ± 15.37	170.02 ± 44.24^{ab}	7.44 ± 1.52^{bc}	25.15 ± 3.89^{a}	77.00 ± 2.85^{a}	47.14 ± 4.40^{ab}	71.61 ± 12.90^{b}	52.98 ± 10.18^{b}	63.46 ± 13.91^{b}
1.25	104.53 ± 20.68	81.36 ± 14.64	180.23 ± 43.04^{ab}	7.59 ± 1.45^{bc}	26.61 ± 2.72^{a}	77.37 ± 2.97^{a}	47.28 ± 4.27^{ab}	72.61 ± 8.19^{b}	54.24 ± 6.07^{b}	64.58 ± 8.79^{b}
2.50	93.24 ± 17.23	76.12 ± 13.47	153.05 ± 33.71^{ab}	6.50 ± 1.14^{ab}	28.55 ± 3.54^{a}	80.89 ± 3.04^{b}	51.82 ± 5.12^{b}	66.80 ± 12.62^{b}	50.97 ± 10.50^{b}	57.90 ± 13.57^{b}
5.00	88.98 ± 26.47	78.49 ± 23.91	141.58 ± 30.86^{a}	5.51 ± 0.82^{a}	35.90 ± 3.13^{b}	87.27 ± 1.39^{c}	57.46 ± 8.22^{c}	44.31 ± 4.90^{a}	35.63 ± 9.68^{a}	37.44 ± 10.44^{a}

VAP: average path velocity; VSL: straight linear velocity; VCL: curvilinear velocity; ALH: average lateral head displacement; BCF: beat cross frequency; STR: straightness; LIN: linearity; TM: total motility; PM: progressive motility; RM: rapid motility.
^{abc}Different superscripts in columns indicate significant difference ($P < 0.05$).