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Article:

Effect of different concentrations of sodium dodecyl sulfate (SDS) in cryodiluent on freezability and DNA integrity of bull spermatozoa

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Abstract

The current study aims to investigate the effect of different concentrations of sodium dodecyl sulfate (SDS) in tris-egg yolk-based extenders on post-thaw semen characteristics in order to improve freezability and DNA integrity of bull spermatozoa. Semen samples were collected from four adult fertile healthy bulls; Samples were collected weekly from each bull for six successive weeks using an artificial vagina. After initial microscopic evaluation, ejaculates from each bull were diluted in a Tris-based buffer egg yolk extender and cooled to 5°C. The Semen extender was supplemented by different concentrations of SDS; 0 (as a control group), 0.25, 0.5, 1 and 1.5 %. Samples were processed for cryopreservation. Frozen straws were thawed in water bath at 37°C for 30 sec and then evaluated for post- thaw motility, viability index, mitochondrial activity, plasma membrane and acrosome integrities. Comet assay was applied to determine the DNA integrity of spermatozoa. SDS at concentrations of 0.25% resulted in the best motility, mitochondrial activity and acrosome, membrane, and DNA integrities of post- thaw bull spermatozoa. Increasing concentration of SDS above 0.25% appeared to have deleterious effect of sperm acrosome, membrane and DNA integrities, Defects in sperm DNA manifested as increased in the percentage of fragmented DNA, DNA content in tail of comet, tail length and Olive tail moment. In conclusion, 0.25% SDS is the optimum concentration for cryopreservation of bull spermatozoa in order to improve freezability and DNA integrity.

Keywords: Bull spermatozoa, DNA integrity, Freezability, SDS.

Introduction

Artificial insemination (AI) has made important contributions to the progress of controlled reproduction in cattle over the last few decades. Short-term or long-term sperm preservation and AI achieve several benefits, such as genetic improvement, genetic resource protection, decreased incidence of sexually transmitted diseases, and enhanced transportation of genetic material across countries [1].

Many problems limit the use of frozen sperm from extremely superior bulls in AI; one significant difficulty that arises during cryopreservation is the loss in the quality and fertility of bull sperm due to different physiological and mechanical stresses [2]. Even when controlled semen freezing strategies are used, cryopreservation affects the functional and structural integrity of bovine spermatozoa. This is a consequence of reactive oxygen species (ROS) production during cryopreservation process due to excessive lipid peroxidation of polyunsaturated fatty acids (PUFAs) present in the membrane of mammalian

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spermatozoa [3]. These alterations in cryopreserved sperm are responsible for lowered fertility rates and poor sperm viability in the female reproductive tract [4]. Increasing concentrations of (ROS) may lead to further sperm cell deterioration during semen storage at low temperatures [5], resulting in significant deleterious effects on the structural integrity, functional activity [6] and compromising the fertilizing capacity of bull sperm [7].

Despite the fact that male reproductive fluids have a variety of antioxidant molecules which act as a natural defense system against the detrimental effects of ROS [8]. Nevertheless, this system is deemed insufficient under cryopreservation-mediated stress and fails to protect spermatozoa against oxidative insults during cryopreservation process [9]. Excess ROS generation in semen during the freeze-thawing process has been associated with alterations in the endogenous defense system and damage to sperm membranes [10]. Consequently, to minimize oxidative damage during the freezing-thawing procedures of bull spermatozoa, optimization of cryopreservation protocols for bull sperm by reinforcement of a semen extender with a suitable cryoprotectant (CPA) became an active field of research [11]. Cryoprotectants (CPAs) are molecules with strong metal chelation and ROS scavenging activity that could contribute to alleviating the undesirable effects of sperm chilling or freezing, thereby extending their viability and activity [8]. SDS is a water-soluble alkyl ionic detergent capable of denaturing native membrane proteins [12]**.** SDS has been added as a cryo-diluent additive to improve the protective effect of egg yolk [13]**.** SDS prevents early sperm capacitation in frozen and thawed spermatozoa [14]. However, SDS in the diluent has proved beneficial for the cryopreservation of sperm from a variety of domestic and wildlife species [15]**.**

There are few studies that have addressed the effect of SDS on quality of post-thaw bovine semen. Therefore, this current work was conducted to study the effects of different concentrations of SDS in tris-egg yolk based extender on post-thaw semen characteristics in order to improve freezability and DNA integrity of bull spermatozoa.

Materials and Methods

Ethical approval

The protocol of the current study has been approved by the Veterinary Medical Research Ethics Committee, Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt, according to the OIE standards for use of animals in research with number **Soh.Un.Vet/00050R**.

Chemicals and Semen Collection:

SDS and other chemicals used in this study were obtained from Sigma-Aldrich, USA. This study was performed on 4 adult healthy bulls that were maintained on the experimental farm of the Animal Reproduction Research Institute (ARRI). Their age ranged between 3 and 4 years and their body weight was 550-700 Kg. Throughout the experimental period, the animals were kept under similar conditions of nutrition and management, each animal received a daily ration composed of about 15 kg green berseem, 4-5 kg concentrates mixture in pellet form, and 4-5 kg rice hay. Water was allowed ad libitum.

In the present study, ejaculates were collected early in the morning, using the pre-warmed artificial vagina (42˚c), and semen samples were collected weekly from each bull for six successive weeks (October – December 2022). Two successive ejaculates with about 10 min intervals were collected from each animal on one occasion. Immediately after collection, semen samples were evaluated for ejaculate volume, and individual motility (%) using a pre-warmed stage of phase-contrast microscope.

Processing of Semen for Cryopreservation

After microscopic evaluation, the two ejaculates from each bull were pooled together. Each pooled semen sample was diluted with a Tris-based egg yolk extender. According to Shiva Shankar Reddy et al. [16], Tris-based buffer comprised of Tris 33.2 g/l, citric acid 18.3 g/l, dextrose 7.8 g/l, 7% (V/V) glycerol, 20% (V/V) egg yolk and antibiotics (gentamycin sulphate 500µg/mL, tylosin tartrate 100µg/mL, lincomycin HCl 300µg/mL, and spectinomycin HCl 600µg/mL [17] and double distilled water to make a volume of 200 mL at 37 ˚C in an incubator in an appropriate dilution rate to obtain a concentration of 40×106 sperm/ml for all the ejaculates. Samples were cooled slowly to 5˚C over 1.5 hours.

SDS was added in the above-prepared extender to obtain five different final concentrations: 0, 0.25, 0.5, 1 and 1.5 % (Control, T1, T2, T3, and T4, respectively). Each pooled ejaculate was split into five equal aliquots and diluted with SDS supplemented extender; then packaged in 0.25 mL straws (IMV, France). The straws were sealed by using an automatic filling and sealing machine and placed 4 cm above liquid nitrogen in the vapor phase in a foam box for 15 minutes before being plunged into the liquid phase [18]. Straws were transferred to a liquid nitrogen tank and stored.

Evaluation of post-thaw sperm parameters:

Frozen straws were thawed in a water bath at 37°C for 30 sec, immediately after thawing; straw contents were evaluated for the following parameters:

1. Viability and morphological related parameters:

1.1. Motility of Frozen-Thawed Semen

Motility estimations were assessed by using a phase contrast microscope with a warm stage maintained at 37 °C,

at hourly intervals for a period of 3 hours. A wet mount was made using a 15µL drop of semen placed directly on a pre-warmed microscope slide and covered by a coverslip. Sperm motility estimations were performed at 200 x magnification in three different microscopic fields for each semen sample by the same researcher. The mean of the three successive estimations was recorded as the final motility score [19].

1.2. Viability index:

For assessment of sperm viability, thawed samples (5straws) representing each treatment were incubated at 37ºC in narrow glass tubes. Estimation of motility was done at hourly intervals for a period of 3 hours. The viability index was calculated to be equal to half of the post-thaw motility in addition to the summation of recorded motility at 1st, 2nd and 3rd hours post-thawing.

1.3. Sperm Mitochondrial Activity

The MTT test, used to determine the spermatozoal mitochondrial activity, MTT reduction assay depends on the ability of metabolically active cells to reduce tetrazolium salt (3[4, 5-dimethylthiazol-2-y1]-2, 5-diphenyltetrazolium bromide) to formazan. MTT assay was performed according to the method of Mosmann**,** [20] for each frozen semen sample six wells of the 96-well micro-plate were used. The 100 µl of semen sample plus 10 µl of MTT stock solution (5 mg MTT/ml of PBS) was placed in each well.

The rates of MTT reduction were determined using an ELISA reader (Thermo Reader) at a wavelength of 550 nm. The optical density of semen samples was measured three times (immediately after thawing, after half an hour, and after one hour of incubation at 37 ◦C). MTT reduction rates (optical density) for each semen sample was calculated by concurring the difference between the first and each of the second (MTT1) and third (MTT2) readings of the ELISA reader.

1.4. Sperm morphological abnormalities

Sperm morphological abnormalities were evaluated by a dual staining procedure, according to Didion et al. [21]. After thawing, spermatozoa were incubated with an equal volume of 0.2% trypan blue for 10 min and washed twice (centrifugation at 700g for 6 min) with PBS (phosphate buffer saline). Smears were made on glass slides and dried quickly on a warm stage. Slides were stained with 10% Giemsa stain for 40 min. They were rinsed under a stream of distilled water, air-dried, and covered with coverslips. At least 200 sperm cells were counted. The abnormal spermatozoa were classified as head, mid-piece, and tail abnormalities [22].

2. Functional integrity related parameters:

2.1. Plasma Membrane Integrity

The hypo-osmotic swelling test (HOST) was used to evaluate the functional integrity of sperm membrane; the procedure described by Jeyendran et al. [23] was used to determine the percentage of HOST-positive cells in each sample based on curled and swollen tails. This was performed by incubating 30 µL of semen with 300 µL of hypo-osmotic solution (0.735g of sodium citrate dihydrate and 1.351g of fructose in 100 mL of sterile, de-ionized water). The sample was incubated at 37°C for 30-45min in a 2 ml micro-centrifuge tube. After incubation, 0.2 mL of the mixture was spread with a cover slip on a warm slide for examination by using phase-contrast microscopy (400×). Then 200 sperm were examined. Sperm with swollen or coiled tails were recorded [24].

2.2. Acrosome Integrity

Acrosome integrity was evaluated by a dual staining procedure, according to Didion et al. [21]. As mentioned above, Spermatozoa were classified as acrosome intact (light purple - dark pink acrosome) and damaged/lost acrosome (unstained or blue acrosome) [22].

2.3. Sperm cell DNA integrity: Comet assay (Single cell gel electrophoresis assay)

The alkaline comet assay (single-cell electrophoresis) for spermatozoa was carried out according to Hughes et al. [25]. A total of 200 sperm cells were examined under a fluorescent microscope (Leica, Germany, 400X, at 254 nm wavelength). The percentage of non-fragmented (compact sperm head) and fragmented (elongated tail of a comet) sperm nuclear DNA was calculated. The intensity of the fluorescent stain in the comet tail region is presumed to be related to the DNA content, and DNA damage is estimated from measurements of the percent DNA in the tail, tail length, and olive tail moment, using an image analysis system (Comet-Score program). Spermatozoa with fragmented DNA showed increased migration of the DNA from the sperm nucleus towards the tail forming a "comet" as shown in **Figure 1 [A]**, while spermatozoa with intact DNA do not form a “comet” as shown in **Figure 1 [B]**.

Statistical analysis

Data were presented as mean ± SEM (standard error of the mean). One-way ANOVA and Duncan's multiple range tests were done for the obtained data using SPSS program version 16.0 and $p \leq 0.05$ was considered statistically significant.

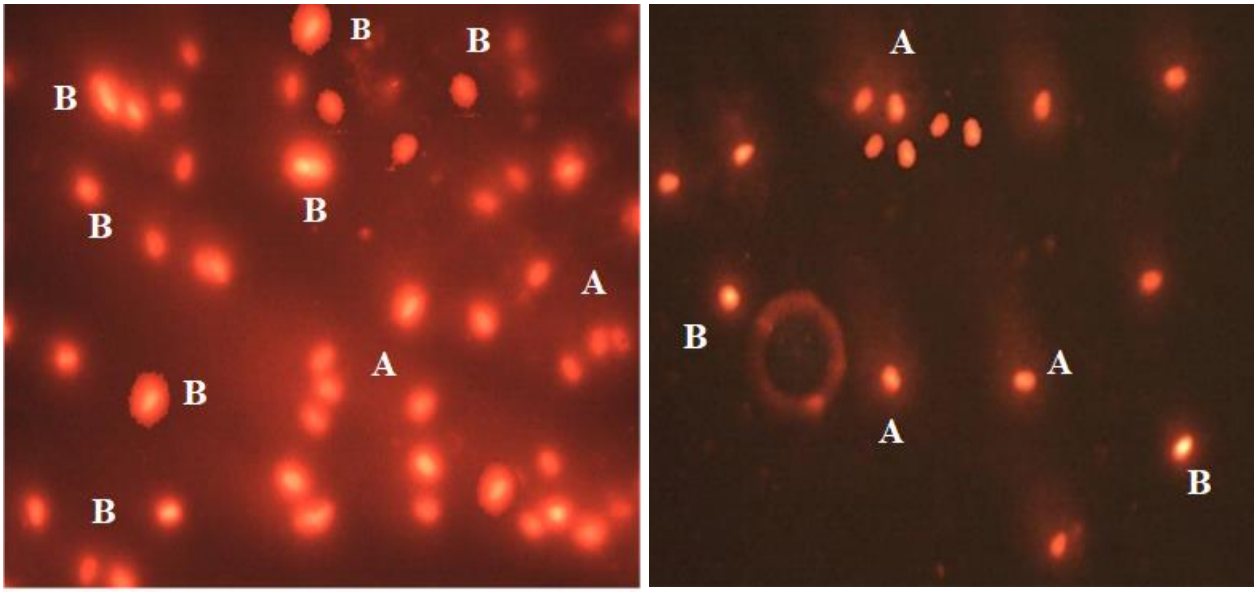

Figure 1 shows post-thaw bull spermatozoa after comet assay. Where [A] represents spermatozoa with DNA fragmentation, while [B] represents spermatozoa without DNA fragmentation.

Results

The results of the present study showed that the addition of appropriate concentration of SDS is fundamental for bull semen cryopreservation. SDS at concentrations of 0.25% resulted in the optimum motility, membrane, acrosome, and DNA integrities of bull spermatozoa.

Effects of supplementation of different concentrations of (SDS) in the Tris egg yolk extender on sperm motility, viability index, integrity of plasma membrane and acrosome, mitochondrial activity, total abnormalities of bull sperm at post-thawing are shown in **Table 1**. Post-thaw sperm motility, plasma membrane and acrosome integrity were significantly higher ($P<0.05$) in semen samples containing 0.25% SDS than in others containing (0.5% - 1%- 1.5% SDS) and control samples ($P<0.05$). Results revealed that some of the tested parameters did not differ significantly between the control group and other SDS treatments 0.5– 1- 1.5%, also SDS supplementation was not equally beneficial at all different concentrations.

Effects for SDS supplementation at different concentrations in the Tris egg yolk extender on DNA integrity (different comet parameters) of bull spermatozoa at post-thawing are presented in **Table 2**. The addition of 0.25% SDS resulted in the highest ($P \leq 0.01$) DNA integrity (96.25±0.25 %), While the addition of 0.5 - 1 - 1.5% SDS to the semen extender was found to exert no significant difference from the control group on DNA in the tail of the comet, percentage of non-fragmented DNA, tail length and Olive tail moment. On the other hand, the addition of 1 % and 1.5 % SDS resulted in the lowest DNA integrity (94.50±0.5% and 95.0±0.0% respectively).

Discussion

The freezing methodology, cryoprotective diluent composition, dilution and cooling rates, and thawing procedure all affect sperm viability during the freezing-thawing process. The goal of this experiment was to find the ideal SDS concentration in the tris-egg yolk extender for the cryopreservation of bull spermatozoa, it was found that 0.25% SDS in a tris-egg yolk based extenders was fundamental; this greatly enhanced the frozen-thawed spermatozoa's motility, plasma membrane, and DNA integrity. Moreover, using the same SDS concentration helped to maintain acrosome integrity the best. Addition of 0.125% SDS in freezing extender had a significant effect ($p < 0.05$) on post- thaw kinetic and functional parameters of buffalo bull semen [26]. Similarly, 0.035% SDS preserved the motility and fertilization capacity of mouse spermatozoa [27]. According to El-Badry et al. [28] addition of 0.03% SDS to extenders for semen freezing is fundamental for cryopreservation of equine spermatozoa, enhancing motility and acrosome integrity. Furthermore, after freezing and thawing, the boar sperm's motility and intact-acrosome percentage were significantly enhanced by the addition of 0.5% orvus ES paste (OEP) to a diluent containing 20% egg yolk [29]. Tsutsui et al. [30] reported that addition of 0.5–1.0% OEP to the extender for freezing canine semen was effective in prolonging post-thaw survival of spermatozoa and improving canine sperm membrane integrity [31] and acrosomal integrity [32].

It has been demonstrated that SDS is only beneficial in the presence of egg yolk, suggesting that it functions by altering the tertiary structure of the egg yolk lipoproteins [33], improves the post-thaw survival of spermatozoa by

acting as a surfactant, solubilizing and increasing the dispersion of egg yolk globules within the diluent,

enhancing the contact between protective egg yolk materials and the sperm cell membrane [30], thereby increasing the cryoprotective effects of egg yolk, which stabilize plasma and acrosomal membranes, and protecting spermatozoa from the toxic effects of glycerol during the freeze-thaw process [32]

The current study revealed that, using of high concentrations of SDS (greater than 0.25%) had a negative impact on plasma membrane, acrosome integrity, and post-thaw motility, this supported by findings of Aboagla & Terada [34], the motility and acrosome integrity of frozen-thawed goat spermatozoa were significantly decreased when the extender contained >0.05% SDS. Aboagla & Terada [34] hypothesized that when SDS is used at a high concentration in the extender, the amount of free SDS molecules increases and may bind to the sperm membrane directly, with devastating results. The addition of 0.5,1 and 1.5% SDS to tris egg yolk-based extender was found to have no discernible effects on the various sperm traits examined in the current study. Likewise, SDS had no effect on sperm acrosomal morphology in Sika deer spermatozoa [35].

Table 1 Effect of different concentrations of (SDS) in the Tris egg yolk extender on post-thaw viability and morphological related parameters, plasma membrane and acrosome integrities of bull sperm

Semen traits	Sodium dodecyl sulfate (SDS) concentrations					
	Control (0%)	T1 (0.25%)	T2 (0.5%)	T3 (1%)	T4 (1.5%)	*P*-value
Motility (%)	45.00±0.04^{b}	52.50±2.50^{a}	42.50±2.50^{b}	40.00±0.01^{b}	40.00±0.03^{b}	0.011
Viability index	127.50±0.05^{ab}	153.75±8.75^{a}	121.25±6.25^{ab}	115.00±0.05^{ab}	110.00±0.03^{ab}	0.008
Plasma membrane integrity (%)	46.50±1.5^{b}	55.00±3.00^{a}	44.50±5.00^{b}	44.00±1.00^{b}	40.50±5.00^{b}	0.010
Acrosome Integrity (%)	52.50±1.50^{b}	60.50±1.50^{a}	47.50±2.50^{bc}	45.50±1.50^{bc}	42.50±2.50^{c}	0.008
MTT reduction rate	0.50±0.01^{b}	0.56±0.01^{a}	0.43±0.02^{c}	0.44±0.02^{c}	0.46±0.01^{bc}	0.001
Total Abnormality (%)	8.50±0.50^{ab}	6.00±1.00^{b}	8.00±1.00^{ab}	8.00±0.01^{ab}	9.00±0.01^{a}	0.13

Values are mean ±SE. a, b,c Values with different superscripts within rows differ significantly, p < 0.05.

Table 2 Effects of different concentrations of (SDS) in the Tris egg yolk extender on DNA integrity (different comet parameters) of bull spermatozoa at post-thawing

Comet parameters	Sodium dodecyl sulfate (SDS) concentrations					
	Control (0%)	T1 (0.25%)	T2 (0.5%)	T3 (1%)	T4 (1.5%)	P-value
Sperm with intact DNA (%)	95.25±0.25^{ab}	96.25±0.25^{a}	95.50±0.00^{ab}	94.50±0.50^{b}	95.00±0.00^{b}	0.044
DNA in head of Comet (%)	94.51±0.02^{b}	95.76±0.76^{a}	94.37±0.47^{b}	93.91±0.09^{b}	93.48±0.07^{b}	0.016
DNA in tail of Comet (%)	5.49±0.02^{a}	4.24±0.76^{b}	5.53±0.47^{a}	6.08±0.08^{a}	6.50±0.07^{a}	0.015
Comet tail length (pixels)	6.54±0.38^{a}	6.08±0.25^{a}	6.26±0.27^{a}	6.75±0.25^{a}	6.93±0.12^{a}	0.28
Olive tail moment	0.55±0.005^{a}	0.42±0.025^{b}	0.49±0.005^{a}	0.51±0.035^{a}	0.52±0.030^{a}	0.011

Values are mean ±SE. a, b,c Values with different superscripts within rows differ significantly, p < 0.05.

Conclusion

According to our results, (0.25% SDS) is the optimal concentration for improving the freezability and functional integrity of sperm cells during the cryopreservation of bull sperm in tris-egg yolk diluent.

Authors' contribution

Research idea and proposal: Mostafa Omar Rayan, Alaa El-Din Zain, Diya El-Badry and Abeer M. Anwer. Methodology: Diya El-Badry, Mostafa Omar Rayan and Abeer M. Anwer. Data collection: Mostafa Omar Rayan. Draft writing, data analysis and statistics: Mostafa Omar Rayan. Revision and editing: Alaa El-Din Zain and Diya El-Badry. Paper submission: Mostafa Omar Rayan

Conflict of interest

There is no conflict of interest.

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