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Research article

Improvement of buffalo semen freezability by using TRIS extender enriched with different concentrations of trehalose/sucrose

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Abstract

The present study was designed to display the role of Trehalose / Sucrose addition to Tris-Fructose-Egg yolk-Glycerol extender on the rate of freezability and post-thawed characters of buffalo frozen semen. For extension and freezing, buffalo semen samples were extended in Tris-Fructose-Egg yolk-Glycerol without the addition of Trehalose / Sucrose as a control (TFEG-C) and with the addition of different concentrations of Trehalose or Sucrose. The best sperm motility, sperm livability, sperm abnormality, sperm cell membrane and DNA integrities appeared with TFEG-T100 mM/l (33.50±1.50%, 69.40±2.11%, 11.60±0.67%, 64.00±2.76%, 96.50±0.92 and 11.60±0.67%, respectively) and TFEG-S50 mM/l (33.00±2.81%, 68.80±2.25%, 10.40±0.54%, 66.10±2.68% and 95.60±1.44%, respectively). From the present study, it can be concluded that addition of Trehalose (100 mM/l) / sucrose (50 mM/l) to Tris-Fructose-Egg yolk-Glycerol extender might help in improvement of the post-thawed characteristics of buffalo frozen semen.

Key words: Buffalo, Semen, Freezability, Extender, Trehalose, Sucrose.

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1. Introduction

It is well known that the composition of the extender, suitable cryoprotectants and optimal freezing and thawing rates are important factors for successful semen cryopreservation [1]. The quality of frozen semen is the most influencing factor for conception rate [2]. It has been reported that cryopreservation process leads to the generation of reactive oxygen species (ROS) that impair sperm motility, membrane integrity and fertilizing ability [3-5]. These changes are due to oxidative and osmotic stresses [6,7]. Accordingly, the beneficial effects of sugars in the extender, on the post-thaw sperm viability of mammalian species, have been suggested earlier [8, 9, 10]. Trehalose as a membrane-protecting disaccharide confers a greater cryoprotective capacity to the basic extender when added up to 100 mM. Such addition results in an improvement in the sperm motile activity and in vivo fertility [11]. Sperm plasma and acrosomal membranes are especially important with regard to survival following thawing and it is considered that there are changes on the

primary organelles caused by cryopreservation process. Naing et al. [12] stated that combination of monosaccharide (glucose) and disaccharide (trehalose) improved semen quality following cryopreservation and better improvement was observed when trehalose was supplemented with concentration 198.24 mM to the glucose extender in goat semen. A combination of 100 mM trehalose and 5% glycerol was an adequate combination for achieving post-thawing semen quality [13]. The addition of trehalose to the freezing extender leads to the reduction of cryo-damage of buffalo spermatozoa [14].

The present study was designed to display the role of Trehalose / Sucrose addition to Tris-Fructose-Egg yolk-Glycerol extender on the rate of freezability and post-thawed characters of buffalo frozen semen.

2. Materials and Methods

Semen was collected from five mature buffalo-bulls kept in Abbasia frozen semen Center, General Organization for Veterinary Services, Ministry of agriculture, Egypt, by using an artificial vagina. Immediately after collection, semen samples were held in a water bath adjusted at 37° C. After evaluation, only semen samples with at least initial sperm motility of 70%, and normal sperm of 80% were used for further processing. Visual motility, sperm livability, sperm abnormalities and sperm membrane integrity were assessed [15]. For extension, Tris-Fructose-Egg yolk-Glycerol (TFEG) was utilized as described by Foote [16]. Semen samples were pooled and divided into 7 fractions; one diluted with the basic control extender (TFEG-C) and other aliquots of pooled semen samples were diluted with TRIS-Fructose-Egg yolk-Glycerol (TFEG) extender containing the different concentrations of Trehalose or Sucrose according to Woelders et al. [17] and liu et al. [18] with some modifications, in order to provide a concentration of 60 million sperm/ml, as shown in table 1.

Table 1.Tris-Fructose-Egg yolk-Glycerol (TFEG) with the addition of different concentrations of trehalose (T) or sucrose (S).

Ingredients (g/100ml)	Experimental extenders					
	TFEG-T 50mM/l	TFEG-T 100mM/l	TFEG-T 200mM/l	TFEG-S 50mM/l	TFEG-S 100mM/l	TFEG-S 200mM/l
Tris	2.295	2.295	2.295	2.295	2.295	2.295
Citric acid	1.295	1.295	1.295	1.295	1.295	1.295
Fructose	0.999	0.999	0.999	0.999	0.999	0.999
Glycerol %	7.300	7.300	7.300	7.300	7.300	7.300
Trehalose	1.700	3.400	6.800	-	-	-
Sucrose	-	-	-	1.700	3.400	6.800
Egg yolk %	20	20	20	20	20	20

All media contained 0.475 g/L sodium penicillin, 0.8 g/L streptomycin sulfate

After freezing and thawing in water bath adjusted at 37 ˚C for 30 sec., estimation of the post-thawed sperm motility, livability and abnormalities [19] as well as the sperm membrane integrity by using the HOST [20] and DNA integrity by using Acridine orange staining technique [21] were adopted as described by Shahba [22].

The obtained data were tabulated and computed for statistical analysis, where

appropriate, according to the SPSS® program version 10 (1999). Mean ± SEM, ANOVA and LSD were calculated to deduce the effect and the most efficient concentrations of Trehalose / Sucrose on the post-thawed characteristics of buffalo spermatozoa.

3. Results and Discussion

Results

The present results (Table, 2) revealed significant differences in the post-thawed characteristics of buffalo semen with the addition of Trehalose/Sucrose to the extender. The best sperm motility, sperm livability, sperm abnormality, sperm cell membrane and DNA integrities appeared with TFEG-T 100mM/l (33.50±1.50%, 69.40±2.11%, 11.60±0.67%, 64.00±2.76%, 96.50±0.92 and 11.60±0.67%, respectively) and TFEG-S50 mM/l (38.00±2.00%, 72.10±2.42%, 10.10±0.52%, 66.60±2.13% and 98.10±0.66%, respectively).

Discussion

The current study indicated the presence of some improvement in characteristics of buffalo frozen semen, a finding which came in agreement with that observed in some earlier reports [9, 23, 24].

Table 2. Effects of different trehalose and sucrose concentrations on sperm assessment parameters of frozen buffalo semen (Means ± SEM)

Extender	Sperm parameters (%)				
	Motility	Livability	Abnormality	Membrane integrity	DNA integrity
TFEG-C	34.00±1.94^{b}	70.80±1.86^{a}	09.70±0.49^{c}	57.30±2.27^{b}	97.00±1.06^{a}
TFEG-T50mM/l	32.50±1.53^{b}	68.00±1.71^{a}	14.40±0.82^{a}	62.10±3.31^{a}	87.20±8.60^{c}
TFEG-T100mM/l	33.50±1.50^{b}	69.40±2.11^{a}	11.60±0.67^{b}	64.00±2.76^{a}	96.50±0.92^{a}
TFEG-T200mM/l	30.50±1.57^{b}	66.60±1.90^{b}	12.60±0.67^{a}	59.20±3.00^{b}	95.00±0.93^{b}
TFEG-S50mM/l	38.00±2.00^{a}	72.10±2.42^{a}	10.10±0.52^{b}	66.60±2.13^{a}	98.10±0.66^{a}
TFEG-S100mM/l	33.00±2.81^{b}	68.80±2.25^{a}	10.40±0.54^{b}	66.10±2.68^{a}	95.60±1.44^{b}
TFEG-S200mM/l	28.50±1.50^{a}	68.10±2.21^{a}	13.30±1.23^{a}	62.00±3.70^{a}	96.70±0.97^{a}

Values within the same column with different letters differed significantly at least at $P < 0.05$

It has been found that the improved quality of frozen semen, on addition of trehalose or sucrose to the extender, is due to the osmotic changes reducing all injury caused by ice crystallization as non-permeable substances rendering hypertonic media enough to decrease intracellular freezable water [11, 25]. Trehalose / Sucrose interact with the membrane phospholipids and proteins providing the membrane more flexibility against cryo-injuries [26-29]. Sugars also act as a source of energy for spermatozoa by generating energy from intracellular ATP leading to improved post-thaw sperm motility [23,30,31]. A synergistic effect between glycerol as cryoprotectant and trehalose as a non-permeating cryoprotectant in semen extender is more beneficial than of single cryoprotectant [32]. In the present study, addition of Trehalose / Sucrose to buffalo frozen semen extender appeared to have nearly the same effect on the post-thawed quality of buffalo spermatozoa, a finding which came in accordance with that observed in semen cryopreservation of buffalo [31], ram [33] and boar [1]. However, in some previous studies adopted on semen cryopreservation of ram [11, 34, 35], goat [36] and bull [17, 37], there was a

greater cryoprotectant capacity for trehalose than that for sucrose as indicated by the sperm motility and sperm cell membrane integrity after thawing.

From the present study, it can be concluded that addition of Trehalose (100 mM/l) / Sucrose (50 mM/l) to Tris-Fructose-Egg yolk-Glycerol extender might help in improvement of the post-thawed characteristics of buffalo frozen semen.

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