

Short Communication

Effect of Buffers on Freezing of Buffalo Bull Semen

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ABSTRACT

Sixteen semen samples (ejaculates) from two Nili-Ravi buffalo bulls (8 from each) were preserved in liquid nitrogen at -196°C for 24 hours to study the effect of five extenders on post-thaw motility, liveability (hours) and absolute index of liveability of spermatozoa. Immediately after collection and evaluation, the semen samples were diluted with respective extenders viz. A, B, C, D and E having different concentrations of Tris and Sodium citrate fructose, glucose, lactose, egg yolk, glycerol and antibiotic penicillin and dihydrostreptomycin. After an equilibration period of 6 hours, samples were frozen by fast method and stored in liquid nitrogen for 24 hours. Thawing was carried out at 37° for 30 seconds in water bottle to observe post-thaw motility percentage, liveability (hours) of spermatozoa and absolute index of liveability for comparing the relative efficiency of the five extenders. The average values of post-thaw motility percentage were 45.9 ± 5.2 , 38.1 ± 5.2 , 50.0 ± 6.4 , 41.9 ± 7.9 , and 40.9 ± 6.3 for extenders A, B, C, D and, respectively. Corresponding values for liveability at 37°C were 8.6 ± 0.9 , 7.6 ± 0.9 , 9.2 ± 1.1 , 8.3 ± 0.9 and 7.6 ± 1.0 hours. The absolute index of liveability at 37°C averaged 178.0 ± 38.4 , 130.3 ± 24.2 , 207.5 ± 41.4 , 153.5 ± 55.3 and 141.0 ± 42.5 for extenders A, B, C, D and E, respectively. Post-thaw motility for two bulls (B_8 and B_9) averaged 44.7 ± 6.0 and $42.0 \pm 5.5\%$ while the post-thaw liveability (hours) at 37°C averaged 8.4 ± 1.2 and 8.1 ± 1.0 , respectively. The absolute index of liveability at 37°C for two bulls averaged 168.3 ± 42.3 and 155.7 ± 38.5 . The effect of five extenders on post-thaw motility, liveability (hours) at 37°C and absolute index of liveability was highly significant ($P < 0.01$). The effect of two bulls on these parameters was non-significant. It was found that the extender C containing Tris and Sodium citrate in 1:1 ratio was the best for successful preservation of buffalo bull semen at -196°C . The order of merit for five extenders used was C, A, D, E and B.

Key words: Freezing; Buffers; Buffalo bull; Semen

INTRODUCTION

The buffalo, a great national asset and with its population 26.3 million heads (Anonymous, 2004-05), is the major source of milk in the country. Low productivity of our dairy animals is an obstacle to meet the nutritional requirements of our ever increasing human population. The existing gap of milk and meat supply in the country can be well abridged by exploiting the productive and reproductive potential of the buffalo. Artificial insemination is one of the most important techniques ever devised for the genetic uplift of livestock.

Among various factors affecting fertility of frozen semen, composition of extending medium is the most important one. Different extenders have been tried for deep freezing of buffalo bull semen. Among these Tris (hydroxymethyl) aminomethane has successfully been used as an organic buffer for deep freezing of bull semen (Foote, 1970). Another organic buffer, sodium citrate, has also proved very useful for this purpose (Glen *et al.* 1956).

The present study was designed to study the effect of two buffers Tris and sodium citrate dihydrate, combined in different proportions on the freezability of buffalo bull semen.

MATERIALS AND METHODS

Experimental material. The semen of two buffalo bulls of Nili-Ravi breed, maintained at the Semen Production Unit, Department of Animal Reproduction, University of Agriculture, Faisalabad was used in this study.

Semen collection and evaluation. In order to get a complete ejaculate of good quality, each bull was given enough time for sexual stimulation and one false mount was allowed for sexual preparation before the collection of first ejaculate. The second ejaculate was collected 10-15 minutes following the first collection. Ejaculates showing motility estimate of at least 60 % were pooled. A total of 16 pooled samples were available for further processing.

Experimental methods. Five experimental extenders were used, the composition of which is given in Table I. Egg yolk was separated by complete removal of albumen. Egg yolk was poured into a cylinder by puncturing the yolk membrane. The required amount of Tris, sodium citrate dihydrate, citric acid, fructose, glucose, lactose, penicillin and streptomycin for each extender were weighed and placed in separate beakers.

Distilled water was added to dissolve these ingredients and make the final volume of 73 ml. Then the

required volume of egg yolk (20 mL) and glycerol (7 mL) were added to each solution and each extender was shook with a separate glass rod for uniform mixing of the ingredients. The extenders were stored in 5 pre-labeled, clean and sterilized beakers which were kept in a water bath at 37°C until used for dilution of semen samples. In extension of semen each pooled sample was split into five portions each of which was diluted 1: 10 with one of the five experimental extenders.

Filling and sealing of straws. In filling and sealing of French straw of five different colors corresponding to five extenders and made of polyvinyl chloride, were used in this experiment. In equilibration and freezing the semen filled straws were stored for an equilibration period of 6 hours at 4°C in the refrigerator. Rate of motility of spermatozoa was checked at the end of equilibration period. Freezing was performed in a wide mouth freezing chamber containing liquid nitrogen. Small plastic goblets filled with liquid nitrogen were used to collect the straws from freezing chamber by holding the goblet in one hand with the help of long forceps and gathering the straws with the other hand. Immediately after plunging into plastic goblets, the straws were transferred to bigger steel goblets, having a sieve at the lower end. These steel goblets with straws were kept vertically in the storage container filled with liquid nitrogen for at least 24 hours before thawing. Thawing of straws was carried out in a water bath at 37°C. The thawed semen was separately procured into dry sterilized vials for each extender and was transferred to an incubator to determine liveability of spermatozoa at 37°C. The liveability (in hours) was recorded for all extenders by recording the percentage of motile spermatozoa at hourly intervals till the death of all spermatozoa. The initial rate of motility after thawing and liveability of spermatozoa at 37°C were used to compute absolute index of liveability by using the following formula (Melovenof, 1962).

$$Ia = (T \times R)$$

Where

Ia = is the absolute index of liveability

T = is the time interval between observations

R = is the average percentage motility for every two consecutive observations, starting from the time of incubation.

Data for post thawing rate of motility, liveability and absolute index of liveability were subjected to analysis of variance. Duncan's multiple range test was applied for multiple mean comparison (SAS, 1998).

RESULTS

Physical characteristics and evaluation of unfrozen semen samples was performed. The motility percentage of semen samples, immediately after extension and equilibration averaged 62.5 ± 2.6 and 63.7 ± 2.3 in the experimental bulls.

Post-thaw motility. The post-thaw motility for extenders A, B, C, D and E averaged 45.9 ± 5.2 , 38.1 ± 5.2 , 50.0 ± 6.4 , 41.9 ± 7.9 and $40.9 \pm 6.3\%$, respectively. Analysis of variance revealed that the effect of extenders on post-thaw motility of spermatozoa was highly significant ($P < 0.01$). The comparison of means revealed that the order of merit for five extenders was C, A, D, E and B (Table II).

On the basis of these results, it can be concluded that extender C (containing Tris, citric acid, sodium citrate dihydrate, fructose, glucose and lactose) proved to be better than all other extenders on the basis of post-thaw motility percentage after freezing operation.

Post-thaw liveability At 37 °C. The post-thaw liveability for extenders A, B, C, D and E averaged 8.6 ± 0.9 , 7.6 ± 0.9 , 9.2 ± 1.1 , 8.3 ± 0.9 and 7.6 ± 1.0 hours, respectively. Analysis of variance revealed that the effect of extenders on postthaw liveability of spermatozoa was highly significant ($P < 0.01$). The post-thaw liveability for the bulls B₈ and B₉ averaged 8.4 ± 1.2 and 8.1 ± 1.0 hours, respectively.

The absolute index of liveability for extenders A, B, C, D and E averaged 178.0 ± 38.4 , 130.3 ± 24.2 , 207.5 ± 41.4 , 153.5 ± 55.3 and 141.0 ± 42.5 , respectively. Analysis of variance revealed that the effect of extenders on absolute index of liveability of spermatozoa was highly significant ($P < 0.01$).

Table I. Composition of experimental extenders

Ingredients	Experimental				
	A	B	C	D	E
Tris (gm)	2.42	1.82	1.21	0.60	-
Citric acid (gm)	1.34	1.00	0.67	0.34	-
Sodium Citrate (gm)	-	0.52	1.04	1.56	2.08
Fructose (gm)	0.25	0.25	0.25	0.25	0.25
Glucose (gm)	0.25	0.25	0.25	0.25	0.25
Lactose (gm)	1.00	1.00	1.00	1.00	1.00
Penicillin (units/ml)	100000	100000	100000	100000	100000
Streptomycin (mg/ml)	100	100	100	100	100
Glycerol (ml)	7	7	7	7	7
Egg Yolk (ml)	20	20	20	20	20
Distilled Water upto (ml)	100	100	100	100	100

Table II. Average values \pm S.D. of absolute index of liveability after thawing for different extenders and bulls

Extenders	Absolute index of liveability		
	B ₈	B ₉	Average
A	182.8 ± 42.0	173.1 ± 34.7	178.0 ± 38.4
B	135.6 ± 27.7	125.0 ± 20.7	130.3 ± 24.2
C	215.2 ± 42.2	199.6 ± 40.5	207.5 ± 41.4
D	165.3 ± 52.1	141.6 ± 58.5	153.5 ± 55.3
E	142.5 ± 46.4	139.3 ± 38.5	141.0 ± 42.5
Average	168.3 ± 42.1	155.7 ± 38.5	162.0 ± 40.3

The absolute index of liveability for bulls B₈ and B₉ averaged 168.3 ± 42.3 and 155.7 ± 38.5 respectively, being higher for the bull B₈. Analysis of variance showed that the effect of bulls on absolute index of liveability of spermatozoa was significant ($P < 0.05$). Bull B₈ was better than B₉ in this respect. The interaction between extenders and bulls was, however, non significant.

DISCUSSION

These results are similar to other workers. Bomstein and Steberi (1959) reported that Tris (hydroxymethyl aminomethane) was useful in prolonging survival of washed bovine spermatozoa stored at room temperature. Bartlett and Van Demark (1961) also found that the addition of 0.0125 M Tris to an Illinois Variable Temperature (IVT) extender prolonged sperm survival at 26°C. Davis *et al.* (1963) found that the survival of bovine spermatozoa in 0.2 and 0.25 M Tris buffered-yolk glycerol (TYG) extender was generally superior to that in a standard citrate-yolk-glycerol (CYG) extender at 5.25 and -85°C. The 0.1 and 0.15 M Tris buffered extenders were inferior ($P < 0.01$) and the bending of the sperm tails in these two extenders suggested that these levels of Tris were hypotonic. At -85°C, nearly 90 % of the sperms showing motility before freezing were still motile immediately after freezing in 0.2 and 0.25 M Tris-yolk-glycerol (TYG).

Steinbach and Foote (1964) extended bovine semen in citrate-yolk-glycerol (CYG), Tris-yolk-glycerol (TYG) and skim-milk-glycerol (SMG), frozen in a dry ice alcohol bath and transferred to liquid nitrogen for storage at -196°C. Foote (1970) reported that the bovine sperm motility following freezing and thawing in Tris extender was slightly superior to that in citrate extender but there was no difference in their fertility. Flukinger *et al.* (1976)

reported that Tris based extenders were better than skim milk based extenders for the preservation of bovine spermatozoa. Similarly, Singh and Matharoo (1975) and Tuli *et al.* (1982) found that the Tris proved to be better than sodium citrate dihydrate for the preservation of buffalo bull spermatozoa.

The results of the present study revealed that the combination of Tris (hydroxymethyl aminomethane) and sodium citrate dihydrate in equal proportions should be used as buffer for deep freezing of buffalo bull spermatozoa. However, if only one buffer is to be used, Tris should be preferred over sodium citrate dihydrate.

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