J. Natn. Sci. Coun. Sri Lanka 1979 7(2) : 111—114

SHORT COMMUNICATION

Preservation of Murrah Buffalo Semen at 4°C

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(*Paper accepted* : 31 *July* 1979)

The present consumption levels of milk in Sri Lanka is estimated at 1.4 ounces per caput per day which is 70% of the consumption levels estimated in 1963.[4] This would mean that our present milk production levels need to be increased by nearly 800%, if we are to achieve nutritional standards of 5.49 ounces per caput per day, recommended by the Medical Research Institute of Sri Lanka. This large gap between production levels and consumption needs, calls for an immediate strategy for the genetic improvement of our indigenous cattle resources from a national viewpoint. Owing to this present need, the hitherto neglected Sri Lankan indigenous buffalo is now being recognized as a potential milk producer on account of its ruggedness to thrive under adverse environmental conditions and poor nutrition. In 1967, largescale importation of Murrah and Surti Buffalo breeds for cross breeding purposes with local buffaloes to increase milk, meat and draught power were made from India. One of the quickest ways of achieving this objective is by artificial insemination. Artificial insemination service for buffaloes was available in Sri Lanka during 1950/55 period, but did not gain popularity because breeding of buffaloes for milk production was not appreciated then. At present, artificial insemination with buffaloes is not practised in Sri Lanka. Further, no work has been done on the preservation of buffalo semen in Sri Lanka. Hence the present study was undertaken to compare the effectiveness of three diluents in preserving Murrah Buffalo semen at 4°C.

Six ejaculates, one per week, obtained from a six year old Murrah Buffalo bull, were used for preservation. The ejaculates were obtained by the artificial vagina method. The diluents used were, modified tri-hydroxymethyl-methylamine (TRIS), TRIS with egg yolk and Illini-variable-temperature (IVT) medium. The components of each of the above diluents is shown in Table 1. Immediately after collection, the semen samples were assessed for volume, density, motility and dead sperm percentage. The percentage motile spermatozoa was assessed as follows: the semen sample was diluted with 2.9% sodium citrate solution and a drop of diluted semen was placed on a clean, warm slide. Individual progressive motility of spermatozoa was observed under the microscope and an estimate was made of the percentage of motile spermatozoa. The semen samples after assessment were diluted with experimental

diluents to give a dilution rate of 1 : 10 in 15 × 125 mm glass tubes. The tubes were then capped and stored in a refrigerator. The temperature inside the refrigerator during the experimental period varied between 4°C and 6°C. The stored semen was examined daily on a warm stage (38°C) microscope for the assessment of the percentage of motile spermatozoa. Dead spermatozoa percentage was counted after staining the semen for 2 minutes with Eosin Nigrosin stain.

TABLE 1. The components of three diluents

TRIS diluent		
TRIS (tri-hydroxymethyl-methylamine)	2.6497	g
Citric acid monohydrate	1.4652	,,
Cysteine	0.10	,,
Potassium chloride	0.04	,,
Glucose	0.625	,,
Distilled water	100	ml
TRIS with egg yolk diluent		
TRIS	2.5192	g
Citric acid monohydrate	1.3928	,,
Glucose	0.40	,,
Egg yolk	20	ml
Distilled water	80	,,
Modified IVT (Illini-variable-temperature) diluent		
Sodium citrate	20	g
Sodium bicarbonate	2.1	g
Potassium chloride	0.4	,,
Sulfanilamide	3.0	,,
Distilled water	100	ml

1000 iu/ml of Penicillin G and 1000 μg/ml of streptomycin were added to all the diluents.

Semen volume ranged from 4 to 7.5 ml per ejaculate and the average sperm density was 0.66 × 10^9 per ml of semen. The percent motile spermatozoa during storage in the three diluents is shown in Table 2. There was a gradual decrease in the percentage of motile sperm during storage in both TRIS and TRIS with egg yolk diluent and 50% motility was reached at 168 hours. Analysis of variance indicate that there is no significant ($p > 0.01$) difference in the percent motile spermatozoa during storage of semen in TRIS and TRIS with egg yolk diluent. The results observed with TRIS diluent in the present study is in agreement with the observation made by Chaube and Sengupta.[1] However, Sengupta and Chaube,[5] reported that TRIS with egg yolk diluent could maintain 50% motility of buffalo semen for 216 hours. There was a sharp decline in the percentage motile sperm in IVT diluent and 50% motility was reached within 48 hours. Dagweker and Mittal,[3] reported that buffalo semen can be preserved for 4 days with 50% motility in IVT diluent at room temperature. The

reason for the poor response with IVT diluent observed in this study is not clear but could be attributed to the difference in storage temperature, as the semen samples after dilution was stored at 4°C in the present study. The percent dead spermatozoa during storage of buffalo semen in three diluents at 4°C to 6°C is shown in Table 3. Results indicate that percent dead spermatozoa gradully increased during storage in TRIS and TRIS with egg yolk diluent and reached 32% and 12% respectively, at 168 hours. This observation is in agreement with Chaube and Sengupta,[2] who reported that egg yolk percentage in the diluents helps to reduce dead spermatozoa.

TABLE 2. Percent motile spermatozoa during storage of buffalo bull semen in different diluents at 4°C. (mean ± S.D)

Time (hours)	TRIS	TRIS with egg york	IVT
0	80.3 ± 3.0	80.8 ± 3.0	80.0 ± 2.9
24	78.8 ± 1.8	78.0 ± 2.4	71.0 ± 11.1
48	77.0 ± 2.4	75.0 ± 2.9	50.0 ± 2.5
72	73.0 ± 4.0	74.2 ± 1.2	
96	71.3 ± 1.6	72.0 ± 2.4	
120	62.5 ± 2.5	66.0 ± 4.9	
144	65.0 ± 2.9	52.5 ± 5.8	
168	50.0 ± 2.9	50.0 ± 4.3	

TABLE 3. Percent dead spermatozoa during storage of buffalo bull semen in different diluents at 4°C. (mean ± S.D.)

Time (hours)	TRIS	TRIS with egg yolk	IVT
0	10.6 ± 2.9	7.2 ± 1.2	10.4 ± 4.8
24	12.8 ± 0.6	9.2 ± 1.2	15.2 ± 6.5
48	18.0 ± 7.4	10.0 ± 2.7	26.3 ± 8.2
72	18.6 ± 8.8	11.8 ± 9.6	
96	22.3 ± 7.8	11.0 ± 2.7	
120	24.7 ± 5.7	11.8 ± 3.9	
144	28.5 ± 2.5	12.5 ± 2.5	
166	32.0 ± 2.9	12.9 ± 3.1	

In conclusion, the results of the present trial indicate that both TRIS and TRIS with egg yolk diluent could maintain motility of 50% or above of Murrah Buffalo semen upto 7 days. However, IVT diluent maintained similar motilities upto 48 hours, only. The fertility rate following insemination with preserved semen was not tested in this study. However, studies in cattle indicate that there is a good correlation between percent motile spermatozoa and fertility rate. Further research is in progress to evaluate suitable diluents to preserve Murrah, Surti and Local Buffalo semen at room temperature, 4°C and – 196°C.

Acknowledgements

The authors gratefully acknowledge the assistance given in the form of research grant by the Department of Agriculture.

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