PRESERVATION OF BUFFALO BULL SPERMATOZOA AT HIGH DILUTION IN DIFFERENT DILUENTS

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This experiment was planned to study the effects of various dilution rates on the preservation of spermatozoa of buffalo bull in cow skim milk (CSM) buffalo skim milk (BSM), cow skim milk + 10% glycerol (GSMG) and buffalo skim milk + 10% glycerol (BSMG). The spermatozoa motility was maximum in CSMG on day 4 and 7 of preservation. Addition of glycerol showed a definite beneficial effect on sperm motility both in CSM and BSM. An increasing adverse effect on the motility was noticed in all the diluents when the concentrations were reduced to 5 and 2.5 millions of spermatozoa per ml. of diluents. However, the optimum number of spermatozoa to be preserved in one ml. of diluent was 10 million.

INTRODUCTION

Milk has been successfully used as a preservative for bovine semen. improvement of livability of spermatozoa in skim milk-glycerol diluent at 5°C has been reported. Beneficial effect of glycerol on sperm livability depends on both the level and method of glycerol addition in the preservative (Almquist and Wickersham, 1962).

This experiment was planned to study the effects of various dilution rates of buffalo bull spermatozoa on their preservation in cow's skim milk (CSM), buffalo's skim milk (BSM), cow skim milk + glycerol (CSMG) (10 : 1) and buffalo's skim milk + glycerol (BSMG) (10 : 1) at 5°C.

MATERIALS AND METHODS

Two Ravi-Nili buffalo bulls used in this study were maintained under the routine conditions of management at Animal Reproduction Department, University of Agriculture, Lyallpur, Semen was collected twice a week by using artificial vagina. Sperm concentration was estimated with haemocytometer technique (Walton, 1927), using 2 per cent eosin solution and dilution medium (Smith & Mayer, 1955).

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Skim milk was heated in water bath at 92°C for 10 minutes and then immediately cooled to 37°C by placing it in ice-water. It was then stored at 5°C for further use. Glycerol was added as recommended by Almquist (1959) and streptopenicillin at the rate of 1,000 ug. per ml. of diluent. The semen dilutions were made so as to obtain four semen dilutions viz., 20, 10, 5 and 2.5 million spermatozoa per ml. in each of the four above mentioned diluent.

Percentage motility of spermatozoa was visually evaluated on 1st, 4th and 7th day of storage using 0-10 scale. To avoid bias in motility rating semen of higher concentration was diluted to 2.5 millions per ml. by respective diluents. Evaluation was made on slides warmed to 37°C.

RESULTS

Effect of Glycerol: The motility data obtained on 4th (Table 2) and 7th day (Table 1) were statistically analysed but that of day 1 were not analysed since the variations were negligible.

The motility data on day 4 and 7 of storage revealed significant differences between collections, diluents, and different concentrations within different diluents ($P < 0.01$). Maximum motility on day 4 (38 per cent) and day 7 (23 per cent) was observed in CSMG. These values for BSMG, CSM and BSM were 36, 31 and 30 per cent respectively on 4th day and 22, 15 and 14 per cent respectively on 7th day of preservation. The analysis of variance revealed significant ($P < 0.01$) beneficial effects of glycerol addition on the sperm motility. The glycerol containing diluents showed 38 per cent motility as compared to 31 per cent in non glycerolated diluents. The results revealed that cow milk was a better semen diluent than buffalo milk.

DILUTION EFFECTS

The motility significantly varied in varying concentrations of spermatozoa in four diluents ($P < 0.01$). The difference between 20 and 10 million spermatozoa per ml. of diluent with CSM, CSMG and BSM were not significant. However, in BSMG the motility varied significantly ($P < 0.01$) in fractions containing 20 and 10 million spermatozoa. There was a progressively adverse dilution effect in all the diluents when the concentration of spermatozoa was reduced to 5 & 2.5 million ($P < 0.01$).

DISCUSSION

In the present study a highly significant variation in the preservation of spermatozoa motility had been observed in different ejaculates on day 4 and 7

of semen storage. The motility variation in different ejaculates of semen obtained from bulls had been reported to be because of nutrition and frequency of semen collection (Bratton, *et. al.*, 1948). In the present study the above mentioned factors may not be a cause of variation under the same environment and throughout the period of study. However, certain unknown psychological factors might have caused the existing variation within the collections of this study.

TABLE 1: *Analysis of variance of spermatozoa motility stored at 5°C in four diluents and four concentration on 7th day.*

Source of variation	Degree of freedom	Sum of squares	Mean square	F.R.
COLLECTIONS:	15	1201.02	80.07	10.86**
Between bulls	1	28.12	28.12	3.81
DILUENTS:	3	7505.86	2501.953	339.39**
Skim milk Vs. glycerolated skim milk.	1	7350.78	7350.78	997.12**
Cow's skim milk Vs. Buffalo's skim milk.	1	122.07	122.07	16.56**
Diluents X source of milk.	1	33.01	33.01	4.48*
CONCENTRATION:	3	6103.12	2034.373	275.96**
CONCENTRATION WITHIN DILUENT:	9	31.65	3.517	0.48
Concentration X source of milk.	3	16.21	5.403	0.73
Concentration X glycerolated and non-glycerolated skim milk.	3	13.28	4.427	0.60
Concentration X diluents X source of milk.	3	2.16	0.720	0.10
Within samples.	15	1453.11	96.81	13.13**
Error.	465	3428.12	7.372	
Total:	511	19750.00		

**Significant at 1 per cent level.
*Significant at 5 per cent level

TABLE: *Analysis of variance of spermatozoa motility stored at 5°C in four diluents and four concentrations on 4th day.*

Source of variation	Degree of freedom	Sum of squares	Mean square	F.R.
COLLECTION:	15	2123.38	142.23	21.25**
between bulls	1	23.64	23.63	3.5
DILUENTS:	3	6098.00	2372.877	345.20**
Skim milk Vs. glycerolated skim milk.	1	6757.03	6757.03	999.86**
Cow's skim milk Vs. buffalo's skim milk.	1	225.78	225.78	33.41**
Diluents X source of milk.	1	15.82	15.82	2.34**
CONCENTRATION:	3	6799.02	2266.340	335.36**
CONCENTRATION WITHIN DILUENT:	9	244.34	27.149	2.02**
Concentration X source of milk.	3	4.38	1.460	0.22
Concentration X glycerolated and non-glycerolated skim milk.	3	145.31	48.437	7.17**
Concentration X diluents X source of milk.	3	94.65	31.550	4.67**
Within samples.	15	2511.54	167.44	24.78**
Error.	465	3142.38	6.758	
Total:	511	21652.93		

**Significant at 1 per cent level.

Useful work regarding the efficiency of milk in various forms as diluents had been reported (Saack, Almquist and Patton, 1955 and Saack, Almquist and Flipse, 1956). Cow skim milk (Almquist and Wickershem, 1962 and Vlachos, Nikolaidon and Tasakolof, 1963) and buffalo skim milk (Athar, 1970 and Chaudhry, 1968) had been successfully used for the preservattion of the bovine and buffalo spermatozoa at 5°C.

The addition of glycerol markedly improved the spermatozoa preservation ability of cow skim milk and buffalo skim milk ($P < 0.01$). These results are in agreement with that of Sike and Merilan (1958), and Stewart (1964). The beneficial effect of glycerol had been atttributed to its penetration into the sperm cells where it is oxidatively catabolized for energy processes. Apart from its "fructose sparing activity" it also helps in decreased production of lactic acid (White, 1957).

No comparative study had been reported to evaluate the efficiency of CSM and BSM for preserving the motility of buffalo spermatozoa. In the present study the motility of buffalo spermatozoa was significantly better in cow skim milk than in buffalo skim milk on day 4 and 7 of preservation at 5°C ($P < 0.01$). This variation could be attributed to the compositional differences in cow and buffalo skim milk. It needs to be investigated as to which factor in buffalo milk is responsible for lowering the preserving ability of spermatozoa. The interaction which existed between diluents and source of milk is also perhaps due to the variation in the composition of milk from two species. It is hoped that further studies on this problem will elucidate these findings and the usage of buffalo milk as a semen diluent will depend on favuorable findings of future work. Series of fertility trials showed that if the spermatozoa concentration is maintained, content semen from high fertility bulls could be diluted from 1 : 100 to 1 : 300 without any dilution effect (Willet and Larson, 1952). Adverse effect of dilution has, however, been reported by other workers (Albright *et. al.*, 1958 and Ahmad, 1961). In the present study buffalo spermatozoa also showed adverse effects of high dilutions. In CSM, CSMG and BSM no differences in motility of spermatozoa were observed when each ml. diluted semen contained 20 and 10 million cells. However, in glycerolated buffalo skim milk the motility percentage decreased when spermatozoa concentration was lowered from 20 to 10 million ($P < 0.01$). The percentage motility had a definite decline in all the diluents when the concentration was further reduced to 5 and 2.5 million. The effect of rate of dilution on buffalo spermatozoa motality has not been reported previously. The present results indicate that buffalo and bovine spermatozoa behave in the same manner as far as dilution effects are concerned. On the basis of these observations, it is recommended that for routine artificial insemination work the buffalo semen should be diluted in a manner that each ml. of the semen have more than 10 million spermatozoa.

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