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PRESERVATION OF BUFFALO BULL SEMEN USING LOW AND HIGH DILUTION RATES AT 5°C AND 37°C.

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Skim milk as a diluent preserved the spermatozoa better and for a longer time as compared to egg-yolk citrate, egg-yolk glycine and egg-yolk glucose bicarbonate diluents at 37°C. All these diluents maintained best livability of the spermatozoa at low dilution rates. Egg-yolk glycine diluent maintained greater viability of the spermatozoa and was least harmed by the high dilution effect and prolonged storage at 5°C. Egg-yolk glucose bicarbonate was found to be the poorest diluent under storage at 37°C and 5°C. Yolk containing diluents showed more pronounced effects of high dilution than the milk diluent.

In different concentrations of 20, 10, 5 2.5 10^6 spermatozoa per ml of the diluent, motility percentage of the sperms progressively declined in all the diluents when the concentrations were reduced from 2.0 to 2.5 millions.

INTRODUCTION

The use of artificial insemination is confronted with some important problems to the preservation of semen in different diluents and the effect of dilution rates upon motility of spermotozoa. Sharma and Mahajan (1961) reported that the motility of buffalo spermatozoa was maximum when diluted at 1 : 10 level in yolk-glucose bicarbonate diluent, but dilution beyond 1 : 25 in the same diluent had deterimental effect on motility. Salisbury *et al.* (1943) observed a lesserability of bull spermatozoa to maintian their motility at 5°C by gradually increasing the rate of dilution of semen to 16 part of yolk citrates, whereas studies of Albright *et al* (1958) on spermatozoa molitity at 5°C in various diluents and their combinations revealed that glycine had a property which augmented motility retention in semen extended in yolk citrate, whole milk or skim milk at 5°C storage. However, Ahmad and Ehlers (1967) observed that spermatozoa motility in heated skim milk was better than yolk citrate on day 4 at 1 : 250 dilution rate. It was, therefore, decided to further investigate as to what extent the buffalo bull semen could be diluted in various diluents without having any adverse effect on the rate

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of motility of spermatozoa. The present study was, therefore, conducted to investigate the effect of diluents, viz., egg-yolk-citrate, egg-yolk glucose bicarbonate, egg-yolk glycine and skim-milk in varying concentrations (20, 10, 5 and 2.5×10^6 spermatozoa per ml of the diluent) on the preservation of buffalo spermatozoa. The treatments were stored at 37°C for 24 hours and at 5°C for seven days.

MATERIALS AND METHODS

Two Nili-Ravi buffalo bulls of nearly the same age, were used. The bulls were maintained under routine managemental conditions. Semen was collected by artificial vagina, and after collection, it was immediately evaluated for its volume, consistency, sperm concentration and motility rate. A total of 10 ejaculates having sperm motility not less than 60 per cent and concentration not less than 0.6 billion sperms per ml, of the semen, were selected for study.

Sperms from each ejaculate were diluted in four diluents viz, egg-yolk citrate (d_1), egg-yolk glucose bicarbonate (d_2), egg–yolk glycine (d_3) and skim milk (d_4) so as to maintain four concentration levels of 20, 10, 5, and 2.5 million spermatozoa per ml., in each of the diluents, which were further sub-divided into two equal parts to be stored at 37°C and 5°C, respectively. The spermatozoa motility of the samples stored at 37°C was recorded at 0, 3, 6, 12 and 24 hours, while the same for those stored at 5°C was noted on 0, 4th and 7th day of the storage.

For the purpose of comparison among various treatments analysis of variance method (5) was used. Individual differences were tested by Duncan's Multiple Range Test at 5 and 1 per cent level of significance to observe the effect of various dilution rates on the viability of spermatozoa stored at 37°C and 5°C.

RESULTS AND DISCUSSION

The result regarding the effect of dilution rates in all the four diluents on the motility of spermatozoa are given in table 1. The results showed that lower the dilution rates, the higher was the motility of spermatozoa on storage. In low dilution rates, i. e. 20 million spermatozoa per ml. motility was considerably higher (18.5 to 24 per cent) than in higher dilution rates. i.e., 2.5 million spermatozoa per ml. (3.0 to 32.5 per cent) irrespective of the diluents used.

The differences in dilution rates were found non significant upto 3 hours at 37°C but at 6 hours, dilution rate of 20 million spermatozoa per ml. was significantly ($P<0.01$) higher than that of 5 and 2.5 million spermatozoa per ml. The dilution rate of 20 to 10 million spermatozoa per ml. and 5 to 2.5 million per ml. were similar in their affect to maintain spermatozoan motility. However, at 12 hours storage of semen at 37°C. highly significant ($P<0.01$) differences were observed among all the four dilution rates.

The influence of dilution rates upon the rate of motility (Table 2) was found to be noticeable on 4th day of semen storage, and continued decline in the rate of motility was observed from low to high dilution rates (26.5 to 46 per cent) when compared to other three diluents, viz., skim milk, egg-yolk citrate and egg-yolk glucose bicarbonate, amongst which the rate of motility ranged from 15.5 to 40.5 per cent at the dilution rates of 20 to 2.5 million spermatozoa per ml. of the diluent. On the other hand, after seventh day of storage, the rate of motility was higher in low dilution rates irrespective of the diluent used. However, in higher dilution rates, the motility was considerably low.

The differences in the influence of dilution rates upon the motility percentage at 5°C storage were statistically highly significant ($P<0.01$) for any two of the individual diluents studied. However, a progressive adverse effect of dilution was noted in all the diluents when the dilution rates were reduced from 20 million to 2.2 million sperms per ml.

Great strides have been made in determining the extent to which semen can be diluted without reducing its viability. Series of trials show that if a concent concentration of spermatozoan is maintained, semen from high fertility bulls could be diluted from 1 : 100 to 1 : 300 without any dilution effect. Adverse effect of dilution has, however, been reported by some other workers. (3,4)

In the present study, the buffalo spermatozoa also showed adverse effects of high dilution both at 37°C and 5°C. Percentage motility has shown a definite decline in all the diluents when the dilution rates were reduced from 20 million to 2.5 million spermatozoa per ml. of the diluent. Adverse effects of dilution in egg-yolk citrate had been observed by Ahmad and Ehlers (1) who suggested that high dilution was deterimental for spermatozoa on prolonged storage at 5°C. The relatively low dilution rate indicated yolk citrate to be mediocre diluent during storage at 5°C.

TABLE 1. *Motility percentage of buffalo bull spermatozoa stored at 37°C.*

Hours.	Egg Yolk-citrate				Egg yolk-glucose-bicarbonate				Egg yolk-glycine				Skim milk			
	Spermatozoan concentration. (million)				Spermatozoan concentration (million)				Spermatozoan concentration (million)				Spermatozoan concentration (million)			
	20	10	5	2.5	20	10	5	2.5	20	10	5	2.5	20	10	5	2.5
0	66.0	66.0	66.0	66.0	65.0	65.0	65.0	65.0	66.0	66.0	66.0	66.0	67.0	67.0	67.0	67.0
3	52.5	52.5	51.0	51.0	50.5	49.0	48.0	46.0	51.0	50.0	49.0	46.0	59.0	59.0	59.0	59.0
6	40.5	38.0	34.5	30.5	31.0	25.5	19.0	14.1	36.5	31.5	26.0	22.0	51.0	51.0	51.0	51.0
12	33.5	28.0	22.5	17.5	18.5	11.5	4.5	3.0	27.0	20.5	14.0	7.5	42.5	40.5	36.0	32.5
24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0 0	0.0	0.0	0.0	0.0	15.5	10.5	5.0	2.0

TABLE 2. *Motility percentage of buffalo bull spermatozoa stored at 5°C.*

Days	Egg yolk-citrate				Egg yolk-glucose bicarbonate				Egg yolk-glycine				Skim milk			
	Spermatozoan concentration (million)				Spermatozoan concentration (million)				Spermatozoan concentration (million)				Spermatozoan concentration (million)			
	20	10	5	2.5	20	10	5	2.5	20	10	5	2.5	20	10	5	2.5
0	65.0	65.0	65.0	65.0	64.0	64.0	64.0	64.0	65.0	65.0	65.0	65.0	66.5	66.5	66.5	66.5
4	36.5	28.5	23.0	17.5	33.0	27.5	22.0	15.5	46.0	40.5	35.5	26.5	40.5	35.0	29.0	25.0
7	20.5	13.0	6.4	3.2	14.0	8.2	5.0	3.0	25.0	17.5	11 8	8.5	20.0	13.0	7.0	3.7

In the light of the results of the present study, it is recommended that for a routine artificial insemination, the buffalo semen should be diluted in a manner that each ml. of the diluted semen has not less than 20 million spermatozoa.

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