INFLUENCE OF GLUCOSE AND SODIUM BICARBONATE LEVELS IN CARBON DIOXIDE SATURATED DILUENTS ON BOVINE AND BUBALINE SEMEN

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An experiment is reported on the sperm motility and livability for semen diluted in carbon dioxide saturated diluents. A weekly collection of semen was made from each of a Sahiwal cow bull and a Niliravi buffalo bull, for five consecutive weeks. Each ejaculate was diluted in six carbonated diluents, containing sodium bicarbonate in two levels of 0.2 and 0.3% and glucose in three levels of 0.5, 1.0 and 1.5%. The diluted semen was stored at room temperature (21-31°C). Percentage motility of spermatozoa was examined daily for as long as the sperm survived.

Overall differences in mean percentage sperm motility among diluents were statistically nonsignificant, for both Sahiwal and Niliravi semen. Differences in mean percentage sperm motility between various levels of sodium bicarbonate and between those of glucose, as well as bicarbonate x glucose interaction, were also nonsignificant. However, the diluent containing sodium bicarbonate and glucose at 0.2 and 1.0% levels, respectively, maintained better sperm motility (above 50% for four days) than other diluents. Spermatozoa survived for 10 days in any of the experimental diluents. Room temperatures of 30°C or above seemed to affect the sperm survival in carbonated diluents.

INTRODUCTION

The use of artificial insemination in an animal breeding programme provides an opportunity to accelerate genetic improvement through widespread use of desired sires. One of the main problems in an extensive use of artificial insemination is the preservation and transportation of semen. Current artificial insemination practices utilize dilution and storage of bovine semen at low temperatures to preserve spermatozoa *in vitro*. In Pakistan, the

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usefulness of diluents which preserve semen at low temperatures has its limitations because adequate facilities of refrigeration are neither available in villages nor during transportation. Under these circumstances, development of efficient diluents for preservation and storage of semen at room temperature is of considerable importance.

Certain diluents, like the Illini variable temperature (IVT) diluent, have been used in which carbon dioxide is employed as a means of inactivating spermatozoa, and, thus, prolonging the sperm survival at room temperature. The present experiment was designed to ascertain the optimum combination of glucose and sodium bicarbonate in carbonated diluents. The aim of this study was, therefore, to develop a diluent that suits local conditions for storage of bovine and buffalo semen at room temperature.

MATERIALS AND METHODS

A cow bull of Sahiwal breed and a buffalo bull of Niliravi breed were used in the experiment. These bulls were regular herd sires maintained by the Artificial Insemination Cell of the University of Agriculture at Lyallpur. The bulls were approximately of the same age and similar body condition. They were kept under similar optimal management and hygienic conditions.

The semen was collected once a week from each bull, Niliravi on Monday and Shahiwal on Thursday, for five consecutive weeks. The collection was made by the use of artificial vagina. The collection vial was immediately placed in a water bath at 33-37°C. Each fresh semen sample was tested for its appearance, colour, consistency, sperm motility, sperm concentration, and proportion of abnormal and dead spermatozoa, in order to ascertain that physically and biologically uniform samples were used for the study.

Each ejaculate was diluted in six carbonated diluents, containing sodium bicarbonate in two levels of 0.2 and 0.3 per cent and glucose in three levels of 0.5, 1.0 and 1.5 per cent. The complete compositon of the experimental diluents is given in Table 1. Each diluent was saturated with carbon dioxide gas to bring the initial pH to 6.4. The semen dilution was made at the rate so as to obtain a concentration of 20 million motile spermatozoa per ml. The diluted semen was stored at room temperature, which ranged 21-31°C during the course of the experiment, and percentage sperm motility was observed daily till it fell to zero.

TABLE 1 : *Composition of Six Carbon Dioxide Saturated Diluents Used in the Experiment*

Ingredient	Diluent					
	1	2	3	4	5	6
Sodium citrate (g)	2.0	2.0	2.0	2.0	2.0	2.0
Glucose (g)	0.5	1.0	1.5	0.5	1.0	1.5
Sodium bicarbonate (g)	0.2	0.2	0.2	0.3	0.3	0.3
Potassium chloride (g)	0.04	0.04	0.04	0.04	0.04	0.04
Sulphanilamide (g)	0.3	0.3	0.3	0.3	0.3	0.3
Distilled water	added to make 100 ml of each diluent.					
Penicillin G (thousand I. U.)	100	100	100	100	100	100
Dihydrostreptomycin sulphate (mg)	100	100	100	100	100	100
Buffer pH after carbonation	6.4	6.4	6.4	6.4	6.4	6.4

RESULTS AND DISCUSSION

The average percentage sperm motility of both Sahiwal and Niliravi bulls semen, diluted in different carbonated experimental diluents, over the successive days of storage at room temperature, is given in Table 2. The initial sperm motility was 80 per cent for each ejaculate, and it declined over successive days of storage. It was upto the tenth day that spermatozoa remained alive in any of the diluents, by which time the sperm motility fell to zero. The decay in motility was more gradual, for both Sahiwal and Niliravi semen, in the diluent containing 0.2 per cent sodium bicarbonate and 1.0 per cent glucose levels as compared to the other diluents. Among all other diluents there was little difference in sperm motility for any of the two levels. The carbonated diluent with 0.2 per cent sodium bicarbonate and 1.0 per cent glucose levels maintained over 50 per cent sperm motility for four days for both Sahiwal and Niliravi semen. In other diluents semen retained above 50 per cent motility for only two to three days when stored at room temperature. There was a gradual increase in the pH for all the diluents. It seems that, for both Sahiwal and Niliravi semen, spermatozoa died by the time the pH increased to 7.0.

A number of earlier studies on IVT and other carbonated diluents demonstrated similar results as obtained in the present study on diluent having

sodium bicarbonate and glucose at levels of 0.2 and 1.0 per cent, respectively (Momongan *et al.*, 1960; Kalev *et al.*, 1961; Bartlett and VanDemark, 1962; Vera Cruz *et al.*, 1964; Sharma and Mahajan, 1966). However, there are other studies which indicated that 50 per cent or higher sperm motility was retained for six to seven days, or even more, when bovine or bubaline semen was diluted in carbonated diluents and stored at room temperature (Van Demark and Bartlett, 1958; Lunca and Feredean, 1965; Khan, 1969).

Carbon dioxide reversibly inhibits the spermatozoal metabolism, thus prolonging the sperm survival. This is accomplished by the occurrence of a

TABLE 2 : *Average Percentage Sperm Motility on Different Days of Storage at Room Temperature (21-31°C) for Semen Diluted in Six Carbonated Diluents.*

Day	Diluent with 0.2% B			Diluent with 0.3% B		
	0.5%G	1.0%G	1.5%G	0.5%G	1.0%G	1.5%G
	1	2	3	4	5	6
Sahiwal semen						
1	80	80	80	80	80	80
2	69	71	64	60	59	62
3	54	62	56	48	48	51
4	43	52	43	36	38	39
5	34	49	30	25	26	24
6	21	33	17	17	15	12
7	11	22	8	8	6	5
8	4	14	3	3	0	2
9	0	5	1	1	0	0
10	0	1	0	0	0	0
Nilravi semen						
1	80	80	80	80	80	80
2	69	72	65	63	63	64
3	54	63	57	51	51	55
4	38	52	42	41	33	40
5	26	41	31	28	23	29
6	16	33	21	17	13	19
7	10	20	11	6	8	8
8	5	11	3	2	2	2
9	1	4	0	0	1	0
10	0	1	0	0	0	0

B = Sodium bicarbonate
G = Glucose

reversible reaction that forms carbonic acid, which, in turn, lowers the pH of the diluent:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

Variations in the time of survival of spermatozoa, as indicated by motility observations in reported studies on carbonated diluents, would seem to be attributable to the differences in the composition of diluents used and in the temperature of storage of diluted semen. For room temperature of 30°C or above, the sperm survival in carbonated diluents was adversely affected, as observed in the present study, because of difficulties in the initial adjustment and subsequent maintenance of pH. The carbonic acid formed as above is a weak acid, and is also very unstable. It appears that at a temperature of 30°C and above, the reverse reaction is catalysed at an accellerated rate. As a consequence, the survival of spermatozoa would obviously be affected and sperm motility declined. This indicates the importance of room temperature for semen diluted in carbonated diluents. In most of the studies where sperm motility was maintained at 50 per cent or above for six or more days, the room temperature was in the vacinity of 25°C, or even lower.

Analysis of variance for percentage sperm motility data is presented in Table 3. Sperm motility differences between diluents, between glucose levels, between sodium bicarbonate levels, as well as glucose x sodium bicarbonate

TABLE 3 :- *Analysis of Variance of Percentage Sperm Motility for Semen Diluted in Six Carbonated Diluents and Stored at Room Temperature (21-31°C)*

Source of variation	Degrees of freedom	Mean square	
		Sahiwal semen	Niliravi semen
Between diluents	5	197.77NS	135.13NS
Glucose levels	2	108.97NS	50.70NS
Sodium bicarbonate levels	1	458.34NS	288.67NS
Glucose x sodium bicarbonate	2	156.29NS	141.39NS
Within diluents	54	830.72	853.92

NS = nonsignificant

interaction, were all statistically nonsignificant for both Sahiwal and Niliravi semen. Similarly percentage sperm motility differences on each successive day of storage, for days two to seven, were also nonsignificant for all these effects

and interaction. These results suggest that various levels of glucose and sodium bicarbonate used in the experimental diluents were not markedly different to indicate superiority of a particular composition. However, the diluent with 0.2 per cent sodium bicarbonate and 1.0 per cent glucose exhibited a trend for maintaining better sperm motility for both Sahiwal and Nilkrayi semen.

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