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PRESERVATION OF BOVINE SPERMATOZOA IN MODIFIED SKIM MILK AT 5 °C.

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Nine collections of semen from two Sahiwal bulls, made over a period of six weeks, were used to study the efficiency of four modified skim milk extenders in maintaining motility of spermatozoa stored at 5 °C for seven days. The semen extenders were skim milk, skim milk with 1.2% glucose, skim milk with 10% glycerol, and skim milk with 20% egg yolk.

The extender containing skim milk with egg yolk maintained sperm motility at the highest level during storage period (53.4% on day 4), while that containing skim milk with glycerol showed the lowest motility rate (8.2% on day 4). Skim milk alone and skim milk with glucose had, respectively, 43 and 44% of spermatozoa motile on day 4. It appeared that a high level of glycerol, as used in one of the experimental extenders, had detrimental effect on the survival of spermatozoa because of its hypotonic action.

INTRODUCTION

One of the main problems is extensive use of artificial insemination is the preservation of semen. Among the widely used extenders, skim milk has been found to be satisfactory for preserving bovine semen. However, there appears to be a considerable scope for the improvement of skim milk as a semen extender.

The present research was designed to study the effect of skim milk in combination with egg yolk, glucose or glycerol on the motility and livability of bull spermatozoa.

MATERIALS AND METHODS

Semen of three bovine bulls, two of Sahiwal breed and one of Thari breed, belonging to the Department of Animal Reproduction, University of Agriculture, Lyallpur, was used in this study. However, only one ejaculate could be obtained from Thari bull. The data on Thari semen were thus too

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meagre, and have, therefore, not been included in this investigation. Data on nine collections from two Sahiwal bulls, made over a period of six weeks, were used in the experiment.

Semen was collected once or twice a week, by the use of an artificial vagina. Immediately after collection each ejaculate was evaluated for colour, density, sperm concentration and rate of sperm motility. Only ejaculates having sperm concentration of over 0.6 billion per millilitre and percentage sperm motility of 50 and above were used.

After evaluation each ejaculate was divided into four parts, and each part was diluted with one of the four experimental extenders. The composition of the four extenders is given in Table 1. Skim milk used in the preparation of extenders was given standard heat treatment to eliminate toxicity. The semen was diluted so as to give a concentration of at least 20 million spermatozoa per millilitre of diluted semen.

TABLE 1. *Composition of experimental extenders*

Ingredient	*Extender*			
	SM	SMG	SMGly	SMY
Skim milk (ml)	100	80	90	80
Glucose 6% solution (ml)	—	20	—	—
Glycerol (ml)	—	—	10	—
Egg yolk (ml)	—	—	—	20
Combiotic* (mg)	125	125	125	125

*A product, containing 300,000 IU of penicillin G Procaine, 100,000 IU of penicillin G sodium, and 1.0 g of streptomycin sulphate, manufactured by Pfizer Laboratories Ltd.

The diluted semen was stored in a refrigerator at 5 °C. Daily observations were made for the percentage of spermatozoa that were motile in each diluted semen sample during seven days of storage. The data thus collected for the day 1, day 4 and day 7 of storage were subjected to analysis of variance, for differences between extenders, using the one-way classification technique (Snedecor and Cochran, 1967). The data were also analysed for multiple comparison of mean percentage sperm motility for individual extenders, using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The mean percentages of spermatozoa that were motile on the day 1, day 4 and day 7 of storage, for Sahiwal semen diluted in different experimental extenders, are given in Table 2. The motility declined over the storage period, and the decay was greatest for the extender containing skim milk with glycerol (SMGly). In this extender, only 8.2 per cent of spermatozoa were found motile on the fourth day, and none on the seventh day of storage. The rate of decay in sperm motility was least for the extender containing skim milk with egg yolk (SMY). In this extender, 53.4 per cent spermatozoa were motile on the fourth day, and 36.1 per cent on the seventh day of storage.

TABLE 2. *Mean percentage sperm motility on different days of storage of Sahiwal semen diluted in four experimental extenders*

Extender	Number of semen samples	*Mean sperm motility* (%)		
		Day 1	Day 4	Day 7
SM	9	65.8	43.0	18.8
SMG	9	64.3	44.0	19.4
SMGly	9	64.1	8.2	0
SMY	9	67.3	53.4	36.1

The analyses of variance for sperm motility differences between extenders on day one, day 4 and day 7 of storage are presented in Table 3, Table 4 and Table 5, respectively. The difference between extenders on the first day of storage was statistically non-significant, as would be expected. However, the differences between extenders both on the fourth day and the seventh day of storage were highly significant ($P<0.01$). Table 4 and Table 5 also show the multiple comparison of mean percentage sperm motility for individual extenders on the day 4 and day 7 of storage, respectively. The differences SMY–SMGly, SMG–SMGly, and SM–SMGly, on both the days, were highly significant ($P<0.01$). However, on the seventh day the differnces SMY–SMG and SMG–SM were also found to be highly significant ($P<0.01$).

TABLE 3. *Analysis of variance of percentage sperm motility in various extenders on day one of storage of Sahiwal semen*

Source of variation		Degrees of freedom	Mean square
Between extenders	..	3	20.03NS
Within extenders	..	32	110.14

NS Non-significant

TABLE 4. *Percentage sperm motility in various extenders on day 4 of storage of Sahiwal semen*

(a) Analysis of variance

Source of variation		Degrees of freedom	Mean square
Between extenders	..	3	3560.06**
Within extenders	..	32	143.10

(b) Multiple comparison of extenders

Extender	Mean (Z)	Z—SMG	Z—SM	Z—SMGly
SMY	53.44	9.44	10.44	45.28**
SMG	44.00	—	1.00	35.84**
SM	43.00	—	—	34.83**
SMGly	8.16	—	—	—

**Significant at 1 per cent level of probability.

TABLE 5. *Percentage sperm motility in various extenders on day 7 of storage of Sahiwal semen*

(a) Analysis of variance

Source of variation		Degrees of freedom	Mean square
Between extenders	..	3	1960.02**
Within extenders	..	32	119.20

(b) Multiple comparison of extenders

Extender	Mean (Z)	Z—SMG	Z—SM	Z—SMGly
SMY	36.11	16.67**	17.34**	36.11**
SMG	19.44	—	0.67	19.44**
SM	18.77	—	—	18.77**
SMGly	0	—	—	—

**Significant at 1 per cent level of probability.

Results obtained in this study from the use of skim milk as a semen extender (SM) are in agreement with the results obtained by various other workers (Almquist *et al.*, 1954; Cheema and Ahmed, 1970). The same was true of the extender containing skim milk with glucose (SMG) (Valerani, 1948; Cheema and Ahmed, 1970). Glucose is utilized by spermatozoa to maintain their motility under the existing anaerobic conditions. It diffuses into the cell and undergoes glycolysis. Glucose was used at 1.2 per cent level so as to make the solution isotonic for spermatozoa. With regard to the percentage of spermatozoa that were motile, the present results have shown little difference between extenders containing skim milk alone or skim milk with glucose.

The decay in sperm motility was rather sharp for the semen diluted in the extender containing skim milk with glycerol. In a number of other studies, however, the addition of glycerol resulted in the maintenance of better sperm motility compared to the extenders containing no glycerol (Flipse and Almquist, 1956; Almquist, 1962; Cheema and Ahmed, 1970). These researchers have indicated that glycerol has a protective action on spermatozoa under low temperature conditions. It acts by modifying the process of ice crystal formation and dissolution in the medium, so that damage due to pressure and other mechanical effects is reduced. Glycerol is also utilized by the spermatozoan cell in its oxidative metabolism, since the cell wall is readily permeable to this ingredient.

Some Russian workers (Platov, 1960; Torbin, 1966) have reported results from their studies that do not agree with the results reported by the above authors. They observed that the dilution of semen with liquid solution of glycerol brought about death of spermatozoa comparable with the dilution of semen with water. It was indicated that glycerol had a great ability for hypotonicity, and the hypotonic action of glycerol can be avoided by adding more of the monosaccharide sugars in the extender. The present results are in agreement with the findings of the Russian workers. The poor maintenance of

sperm motility during the storage period in the extender containing skim milk with glycerol seems to be attributable to the high level of glycerol used that would have caused hypotonic action. Such an action would appear to have dissolved the nutrients inside the spermatozoan cell, and, thus, affected the motility and survival of spermatozoa.

In the present investigation, the extender containing skim milk with egg yolk gave best results in maintaining the motility of spermatozoa. Egg yolk contains glucose, various proteins and amino acids, which make it a rich nutritive medium for spermatozoa. Moreover, two of the egg yolk constituents, viz. lipoprotein and lecithin, protect the integrity of lipoprotein sheath of the spermatozoan cell (Blackshaw and Salisbury, 1957). Similar results as in the present study have been reported by other workers (Sikes and Merilan, 1958; Kale, 1963; Joshi and Singh, 1968). It appears that skim milk can be improved as a semen extender by the addition of egg yolk.

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