Vol. XVII (1-2), 1980

DEEP-FREEZING OF BUFFALO SPERMATOZOA BY STRAW METHOD

Khaliq Ahmad*, Rashid Ahmad Ch., Waheed Ahmed and Ala-ud-Din**

The split-ejaculate technique was used for 21 semen samples from five buffalo bulls. The straw method of deep freezing was adopted, using five different extenders which comprised varying proportions of lactose and fructose along with equal proportions of egg yolk, glycerol and antibiotics. The statistical analysis of the data showed the extender comprising 11% lactose (37.5 ml)+ 6% fructose (37.5 ml)+egg yolk (20 ml)+glycerol (5 ml) as the best among all the extenders tried in this experiment.

INTRODUCTION

Artificial insemination as a means of livestock improvement is being practised almost all over the world. In view of the increasing demand of milk and meat in the country and the potential of artificial insemination for enhancing productivity of these valuable commodities, this technique has been introduced in Pakistan. Its use is expanding to the remote areas of the country through frozen semen. The most important aspect of spermatozoan preservation either in the liquid or frozen state is the type of the extender used. It is necessary to select such an extender as provides the sperm cells nearly the same melieu as is present in the seminal plasma and promises better preservation of buffalo spermatozoa in the frozen state. For this purpose five semen extenders were tried in the present experiment.

Redenz (1933) for the first time discovered that reducing sugars could be utilized by the spermatozoa in order to maintain their motility under anaerobic conditions. O'Dell and Almquist (1957) found that the addition of 1.25% of fructose to skim milk extender resulted in a significant increase in freezibility of bull spermatozoa as compared to untreated controls. They suggested that addition of sugar resulted in higher percentage of motile spermatozoa following storage at –79°C for 10 days than samples with added fructose. Tomar and Desai (1961) showed that the addition of glucose and fructose to egg yolk-

*Department of Animal Reproduction, University of Agriculture, Faisalabad

**Department of Physiology and Pharmacology, University of Agriculture, Faisalabad

glycine medium had a prompting action on motility of spermatozoa in buffalo semen. Martin and Emmens (1961) studied the effect of time of equilibration and addition of fructose on survival and fertility of bull spermatozoa deep frozen to –79°C. They showed that revival rate of spermatozoa was higher after longer equilibration period and that the addition of fructose significantly improved the motility percentage after thawing. Adler *et al* (1968) freezed the bull semen with Laiciphos+egg yolk (10%)+Glycerol (5%) extender and lactose (11%)+egg yolk (20%)+glycerol (5%) in straws and pellets respectively. They compiled the results on 60–90 day nonreturn rate which averaged 68.5% to 1993 inseminations with semen in straws and 59.0% to 1872 inseminations with pelleted semen. Gaffaux (1968) compared lactose-egg yolk-glycerol (5%) extender and Laiciphos-egg yolk-glycerol (14%) for deep freezing bull semen in pellets and straws respectively. The conception rate obtained after 60–90 day non-return rate was 66.7 and 67.8% respectively. Roy (1974) used yolk-citrate-glycerol extender for semen from 10 Murrah buffalo and four Holstein-Friesian bulls, freezed in plastic straws and stored in liquid nitrogen for 1–2 months. Conception rate of 227 buffalo females inseminated was 85.9% and of 200 cows inseminated was 80.6%. El-Kafrawi and Barrada (1974) diluted ejaculates from buffalo and Friesian bulls with either egg yolk-skim milk-glycerol (EYS) or egg yolk-citrate-glycerol (EYC), frozen in straws in liquid nitrogen and stored for eight months. They obtained post-thawing motility percentage of 52.3 and 48.9% respectively for Friesian semen in EYS and EYC and 46.2 and 36.1% buffalo semen.

MATERIALS AND METHODS

A total of 21 semen samples from 5 bulls of Nili-Ravi breed were collected with the help of artificial vagina following the technique of Salisbury and Willet (1940). After evaluation, the samples having a motility percentage of at least 60 per cent and a sperm concentration not less than 0.80 billion per ml were selected for further processing. Each of the samples was split into five portions which were diluted with five extenders, the composition of which is given in Table 1.

Table 1. *Composition of experimental extenders*

Ingredients	*Extenders*				
	A	B	C	D	E
11% Lactose (ml)	75.00	56.25	37.50	18.75	—
6% Fructose (ml)	—	18.75	37.50	56.25	75.00
Egg yolk (ml)	20.00	20.00	20.00	20.00	20.00
Glycerol (ml)	5.00	5.00	5.00	5.00	5.00
Penicillin (IU/ml)	1000	1000	1000	1000	1000
Streptomycin (mg/ml)	1.00	1.00	1.00	1.00	1.00

Dilutions were made in a water bath held constantly at 37°C. Immediately after dilution, filling of the straws with diluted semen was performed with the help of automatic suction machine. The open ends of the straws were sealed with polyvinyl chloride powder. Thereafter the straws were transferred in a refrigerator at a temperature of 5°C for an equilibration period of 6 hours. Freezing operation was performed in a wide-mouth freezing chamber by holding the straws on a wire-net, 6 cm above the surface of liquid nitrogen for 8 minutes. Then onward the straws were stored in liquid nitrogen for about 24 hours before thawing. Thawing of frozen semen was carried out in a water bath at 37°C by immersing the straws for 30 seconds. Post-thawing rate of motility of spermatozoa was recorded in all the extenders. The data thus collected was subjected to the analysis of variance (Snedecor and Cochran, 1967) Since the difference between bulls was non-significant, data for all the bulls used in the experiment were pooled for the analysis. Multiple comparison was made to ascertain differences among individual means for treatments using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Each of 21 semen samples was creamy white in colour with normal appearance. The volume varied from 1.5–6.0 ml with an average of 3.12 ml. Sperm concentration was in the range of 0.85–1.65 billion per ml with an average of 1.23 billion.

Table 2. *Analysis of variance for post-thawing motility of spermatozoa*

Source of variation	Degree of freedom	Mean squares	F ratio
Sample	20		
Treatments	4	1812.19	**50.69
Error	80	35.75	

**Significant at 1.0 per cent level of probability.

Multiple Comparison of Means

Extenders	*C*	*B*	*A*	*D*	*E*
Means	42.14	40.71	32.61	23.81	11.43

The data in Table 2 revealed highly significant differences among treatments i.e. extenders. Multiple comparison of means exhibited non-significant differences between extenders C and B. These two extenders were significantly better than all other extenders. Means of extenders A, D and E also exhibited significant differences among themselves. Since the maximum survival of spermatozoa at thawing was observed in extender C, this extender would be considered the extender of choice among all others tried in the present study for successful deep-freezing of buffalo bull semen by straw method.

Spermatozoa have the ability to utilize energy from extraneous energy yielding materials. Lactose and fructose form the main ingredients of extenders used in the present experiment. Both are reducing sugars and could be utilized by the spermatozoa in order to maintain their motility as shown by Redenz (1933). The addition of fructose to lactose improved the sperm survival at thawing. Such improvement in sperm survival and fertility by fructose additions to different extenders has also been reported by many workers (O'Dell and Almquist, 1957; Tomar and Desai, 1961; Martin and Emmens, 1961). However, the fructose levels suggested by these workers do not agree with that used in the present study. On the other hand, glycerol level as used in the present experiment is in accordance to some workers (Adler *et al;* 1968; Goffaux, 1968; Bandyopadhyay *et al;* 1974). Straw method of deep-freezing was selected in the present study as it had been reported to be a better method (Roy, 1974; El-Kafrawi and Barrada, 1974; Wiggin and Almquist, 1975). The straws were frozen by holding them 6 cm above the surface of liquid nitrogen which is in agreement with Roussel *et al,* (1974) who concluded that semen frozen at 6.3 cm height above the liquid nitrogen resulted in best survival rate.

The highest value of the sperm survival rate in extender C indicates the suitability of this extender for successful deep-freezing of buffalo spermatozoa. This phenomena may also be explained in a way that the combined effect of lactose and fructose in this extender as well as in extender B along with egg yolk and glycerol ensure minimum sperm damage during freezing operation and as a consequence a better rate of sperm survival at thawing is achieved.

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