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Effect of Extender and Thawing Methods on Post Thawing Preservation

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Abstract: Twenty four ejaculates obtained from 4 native buffalos were used to study the effect of extender and thawing methods on sperm motility and acrosomal integrity during post thawing preservation for 4 h at 5°C. The extenders used were Tris Egg Yolk Citric Acid Fructose Glycerol (TEYCAFG), Illinois Variable Temperature Egg Yolk Glycerol (IVTEYG), Cornell University Extender Egg Yolk Glycerol (CUEEYG) and Minnesota Egg Yolk Glycerol (MEYG). The semen was frozen in 0.5 mL French straws using liquid nitrogen vapor. The thawing of frozen semen was made by immersing the straws in water at 37°C for 12-15 sec (rapid) and in water at 5°C for 2 min (slow). During the post thawing preservation at 5°C. The percentages of motile sperm and damaged acrosomes differed significantly ($p<0.01$) between extenders and between preservation periods. It was observed that the semen frozen in TEYCAFG extender maintained the highest sperm motility and lowest acrosomal damage. Rapid thawing resulted significantly in higher ($p<0.01$). Sperm motility during post thawing preservation at 5°C than did slow thawing there was no significantly difference in percentage of damaged acrosomes between thawing methods.

Key words: Buffalo, extender, sperm, preservation

INTRODUCTION

Today, there are about 151.5 million water buffalo in the world. Of these about 96.6 % are found in Asia. For a thousand years or more, this important animal species has provided draft power, milk, meat and hide to millions of people, particularly small-scale farmers.

One of the earliest techniques in farm animals is Artificial Insemination (AI), which uses superior males. This technique was further improved with the development of cryopreservation of semen and techniques to regulate ovarian function for synchronizing estrus and ovulation.

Today animal breeding, using new methods, could increase widely the mean of production of low producing native animals by crossing them with exotic improved bulls. One of these used methods for widely application of the sperm of exotic excellent breeds is the using of AI technique and modern semen dilution methods. Using this technique, per ejaculation of an excellent bull can used to inseminate 300-400 cows. Therefore possibility of using per ejaculate of an excellent bull widely in herd becomes possible.

Extenders for treating ejaculated semen are very important, so success in this area depends on cryopreservation ability of extender. Since past, Tris, IVT, CUE and Minnesota extenders have used in USA for animals such as cattle, pig and fowls, but using of these types of extenders for diluting in freezing condition for buffalo semen, have not been used.

The superiority of Tris fructose yolk glycerol extender over citrate fructose yolk glycerol extender for post thawing preservation of bovine semen was reported by Steinbach and Foote (1964). No such study has been reported on buffalo semen. The present study was undertaken to determine the comparative efficacy of 4 extender and 2 thawing methods for post thawing preservation of buffalo semen.

MATERIALS AND METHODS

Twenty four ejaculates, 6 each from 4 native buffalo were collected twice weekly using on artificial vagina. The spermatozoa with little entrapped fluid were diluted using four extenders by split sample technique. The 4 extenders used were Tris Egg Yolk Citric Acid Fructose

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Glycerol (TEYCAFG) (Austin *et al*., 1992; Mohan *et al*., 1991; Jainudeen *et al*., 1982), Cornell University Extender Egg Yolk Glycerol (CUEEYG) (Austin *et al*., 1992; Sahni *et al*., 1972), Illinoise Variable Temperature Egg Yolk Glycerol (CUEEYG) (Johar *et al*., 1993; Azawi 1993; Hassan *et al*., 1994; Pursel *et al*., 1974; Susuki *et al*., 1981) and Minnesota Extender Egg Yolk Glycerol (MEEYG) (Sexton *et al*., 1982; Gisen *et al*., 1983). The pH of TEYCAFG, CUEEYG, IVTEYG and MEEYG was adjusted to 6.8. After 14 h of storage in liquid nitrogen, 2 frozen semen straws from each extender per ejaculate were thawed in water at 37°C for 12-15 sec (rapid) and in water at 5°C for 2 min (slow). The semen from two straws was expressed into 2 mL glass vial and preserved for 4 h at 5°C in a water bath kept inside a cold handling box. The percentage of motile sperm and damaged acrosomes were determined at 0 (Immediately after thawing), 1, 2 and 3 h of preservation. The sperm motility was estimated using a phase contrast microscope at a magnification of 450X. The acrosomal damage was studied using Gremsa staining technique of Bearden (1990). The data were analysised using four-factor analysis of variance as per Snedecor and Cochran (1968) after transformation of the percentages.

RESULTS AND DISCUSSION

The mean percentages of motile sperm and damaged acrosomes at different hours of post thawing preservation are presented in Table 1 and 2, respectively. The percentage of motile sperm was observed to differ significantly ($p<0.01$) between extenders. The semen frozen in TEYCAFG gave the highest percentage of motile sperm followed by that in CUEEYG, IVTEYG and MEEYG (Table 1).

The significantly highest sperm motility maintained in TEYCAFG and CUEEYG during post thawing preservation than in IVTEYG and MEEYG is in conformlty with that of earlier workers on bovine semen (Johar and Norten, 1973). The sperm motility decrease significantly ($p<0.01$) during post thawing preservation. The decrease was rapid in IVTEYG for both thawing method and in CUEEYG for slow thawing method (Table 1). It was observed that rapid thawing resulted in significantly higher ($p<0.01$) sperm motility during post thawing preservation at 5°C than did slow thawing.

In the present study, as the time for rapid thawing was restricted to 12-15 sec, the seminal temperature might be around 5°C (Bearden *et al*., 1990) and hence sperm did not suffer cold shock during preservation at 5°C. The interaction between extender and thawing method was found to be significant ($p<0.01$).

Table 1: Percentage of motile sperm (Mean ±S.E) at different hours of post thawing preservation at 5C° in various extender after thawing by two methods

	Rapid thawing (h)			
Extender	0	1	2	3
IVTEYG	42.30±4.17	36.01±4.50	30.14±4.65	24.14±3.58
TEYCAFG	62.14±2.01	61.00±2.41	59.76±2.89	49.12±4.11
CUEEYG	58.14±2.89	56.23±2.63	52.13±4.14	31.3±2.47
MEEYG	44.28±1.1	42.01±2.1	49.14±3.1	31.13±2.8
	Slow thawing (h)			
Extender	0	1	2	3
IVTEYG	39.15±3.13	31.47±5.11	28.17±3.39	19.14±1.17
TEYCAFG	59.14±3.54	52.14±4.15	46.75±1.14	40.58±4.40
CUEEYG	55.14±2.77	47.67±3.31	41.01±3.41	35.30±3.01
MEEYG	40.12±2.8	32.17±3.03	22.17±3.03	19.8±4.01

The mean percentage of damage acrosomes recorded during post thawing preservation of 5°C differed significantly ($p<0.01$) between extenders but not between the thawing methods. The lowest and the highest percentages of damaged acrosomes were recorded TEYCAFG and IVTEYG and MEEYG extenders respectively (Table 2)

Table 2: Percentage of damage acrosomes (Mean±S.E) at different hours of post thawing preservation at 5° C in various extender after by tow methods

	Rapid thawing (h)			
Extender	0	1	2	3
Rapid thawing				
IVTEYG	21.65±2.54	26.17±4.21	25.15±2.78	27.28±3.01
TEYCAFC	12.35±1.05	14.46±2.14	15.00±3.37	16.01±3.01
CUEEYG	12.90±1.14	15.01±2.01	15.88±3.01	16.75±3.01
MEEYG	18.64±2.44	23.11±2.01	25.17±2.45	27.85±3.01
	Slow thawing (h)			
Extender	0	1	2	3
IVTEYG	20.15±3.11	22.15±2.98	23.17±2.08	25.01±3.11
TEYCAFC	11.85±1.44	12.34±2.40	13.13±2.15	15.17±2.55
CUEEYG	14.46±3.22	15.86±2.66	16.84±3.22	19.07±2.01
MEEYG	19.92±3.55	20.45±1.89	22.01±2.00	24.14±2.11

It is evidence from the present study that the quality of semen during post thawing preservation at 5°C was better when buffalo semen was frozen in TEYCAFG extender than in CUEEYG, IVTEEYG and MEEYG extenders and better thawing at 37°C for 12-15 sec than at 5°C for 2 min

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