Journal of Animal and Veterinary Advances 6 (11): 1340-1342, 2007
ISSN: 1680-5593

Effect of Extender on Sperm Motility and Acrosomal Integrity of Frozen Buffalo Sperm

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Abstract: The comparative efficacy of the 4 extender (TEYCAFG, CUEEYG, IVTEEYG and MEEYG) was studied by split sample technique using 24 ejaculate (6 each from 4 buffalo bulls) after separation of seminal plasma. The percentage of motile sperm in frozen semen was significantly higher ($p<0.01$) in TEYCAFG (71.56±1.21) and CUEEYG (67.56±1.01) than in MEEYG (39.15±2.27) and IVTEEYG (34.41±3.01). The difference between the former 2 and between the latter 2 extenders was not significant. The percentage of damage acrosomes was significantly higher ($p\leq0.05$) in IVTEEYG than in TEYCAFC and CUEEYG, but the difference between MEEYG and IVTEEYG, was not significant.

Key words: Buffalo, extender, sperm, acrosomal integrity, frozen

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. Perusal of literature revealed diversity of opinion about the best extender for freezing buffalo semen. In all the earlier studies, only post thawing sperm motility was used as the criterion for evaluation. A comprehensive study using different extenders was lacking. Hence, an attempt has been made to study the relative efficacy of four extender for freezing of buffalo semen based on sperm motility and acrosomal integrity.

MATERIALS AND METHODS

A total of 24 ejaculates, 6 each from 4 buffalo bulls aged 4-6 years, were collected twice weekly using an artificial vagina. If a buffalo donated less than 2 mL of semen, a second ejaculate was collected and pooled tighter and considered as a simple ejaculate. The spermatozoa with little entrapped fluid was split into 4 parts and diluted at 1:5 fraction A Tris Egg Yolk Citric Acid Fructose Glycerol Extender (TEYCAFG) (Austin *et al*., 1992; Mohan and Sahi, 1991; Jainudeen and Santhana, 1982), Cornell University Extender Egg Yolk Glycerol (CUEEYG) (Austin *et al*., 1992; Sahni and Roy, 1972), Illinoise Variable Temperature Egg Yolk Glycerol (IVTEYG) (Johar *et al*., 1973; Azawi *et al*., 1993; Hassan and Groham, 1994; Pursel *et al*., 1974; Suzuki and Niwa, 1981) and Minnesota Extender Egg Yolk Glycerol (MEEYG) (Sexton and Geisen, 1982; Gisen and Sexton, 1983). The diluted s emen was then cooled to 5°C within 11 h and the fraction B of the extender (amount equal to that of fraction A) was added in 3t at an interval of 15 min. After 5 h of equilibration the semen was frozen in medium size French straws by rapid horizontal vapor freezing

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technique and then stored in liquid nitrogen. After 14 hours of storage the semen was thawed in water at 37°C for 12-15 sec.

The percentages of motile sperm and damaged acrosomes were recorded at different stage. Fresh semen after primary dilution and cooling to 5°C, after equilibration and after freezing. The acrozomal damage was studied using Giemsa staining technique. The analysis of variance and critical difference test were done as per Snedecor and Cochran (1968) after transforming the percentages into angles.

RESULTS AND DISCUSSION

The mean percentages of motile sperm and damaged acrosomes are presented in Table 1 and 2. The mean percentage of motile sperm in frozen semen in TEYCAFG and CUEEYG was with those of Austin *et al.* (1992) and Mohan and Sahi (1991). But it was much lower than that reported by Sahni and Roy (1972) in semen frozen into pellets.

The percentage of motile sperm in frozen semen did not vary significantly between TEYCAFG and CUEEYG (Table 1). But Austin *et al.* (1992) observed higher post-thawing motility of buffalo semen frozen in CUE than in Tris based extender. The percentage of motile sperm in frozen semen was found to be significantly higher ($p<0.01$) in TEYCAFG and CUEEYG than in MEEYG and IVTEEYG (Table 1). Similar present observations were recorded by workers on other animals' semen (Mohan and Sahi, 1991; Usmanov, 1973). The present observation differed from of Johar *et al.* (1973) who recorded higher post-thawing motility of Cattle spermatozoa in IVTEEYG than in MEEYG.

The higher percentage of motile sperm observed in this study in TEYCAFG than in CUEEYG could be due to removal of seminal plasma (Deka and Rao, 1984). The effect of interaction between extender and stage was significant in percentage of motile sperm. This might be due to more drop in sperm motility in frozen semen in CUEEYG and IVTEEYG compared in TEYCAFG and MEEYG.

The different forms of damaged acrosomes are shown in Table 2. The percentage of motile sperm and damaged acrosomes differed significantly ($p<0.01$) between stages of processing of semen. The percentage of damaged acrosomes was significantly higher ($p<0.05$) in IVTEEYG than in CUEEYG and CUEEYG, but the difference between IVTEEYG and TEYCAFG was not significant.

Table 1: Percentage of motile sperm (Mean±S.E*) in various extenders at different stages of processing semen

	Extenders			
Stages	TEYCAFG	CUEEYG	MEEYG	IVTEEYG
Fresh man	89.86±0.54			
After cooling to 5°C	85.5±0.64^{bc}	81.7±0.62^{ab}	64.23±0.54^{de}	60.25±1.21^{k}
After equilibration	80.49±1.02^{cd}	78.33±0.33^{bcd}	59.88±0.75^{k}	56.33±.99^{k}
After freezing	71.56±1.01^{g}	67.56±1.01^{f}	39.15±1.16	34.41±1.01^{e}

Means not bearing at least one superscript in common differ significantly ($p<0.01$), Mean of 24 observation

Table 2: Percentage of damaged acrosomes (Mean±SE*) in various extenders at different stages of processing of semen

	Extenders			
Stages	TEYCAFG	CUEEYG	MEEYG	IVTEEYG
Fresh man	2.18±0.20			
After cooling to 5°C	5.25±0.55	5.99±0.66	6.25±0.74	6.23±0.65
After equilibration	8.55±0.88	9.01±0.88	14.66±1.21	18.25±0.55
After freezing	14.66±1.10	15.02±1.05	20.55±1.54	25.41±1.55

Mean of 24 observation

ACKNOWLEDGEMENT

The authors wish to thank the staff of buffalo farm for allowing us to use facilities and buffalo the present study.

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