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Effect of Glucose Supplementation in Skim Milk Diluter on Motility, Plasma Membrane and Acrosomal Integrity of Nili-Ravi Buffalo Bull Spermatozoa Stored at 5°C

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Abstract.- This experiment was designed to evaluate the effect of glucose supplementation in skim milk diluter on motility, plasma membrane and acrosomal integrity of Nili-Ravi buffalo bull spermatozoa stored at 5°C. Semen from three buffalo bulls was collected and diluted at 37°C with a final concentration of 10 x 10^6 motile spermatozoa per ml in skim milk diluter containing 5, 10 and 30mM glucose or without glucose (control). The diluted semen was cooled to 5°C in 2 hours and stored at 5°C for five days. Motility and plasma membrane integrity of buffalo bull spermatozoa was similar ($P > 0.05$) in all four experimental diluters at 1st day of storage. However, sperm motility and plasma membrane integrity on 3rd and 5th day of storage was significantly ($P < 0.05$) higher in diluter containing glucose (5, 10 or 30mM) as compared to control. Sperm acrosomal integrity remained similar ($P > 0.05$) in all experimental diluters for five days of storage at 5°C. No differences were found among experimental diluters supplemented with 5, 10 and 30mM glucose ($P<0.05$), therefore, 5mM glucose supplementation is recommended. In conclusion, glucose supplementation improved the preservability of Nili-Ravi buffalo bull spermatozoa in skim milk diluter at 5°C.

Keywords: Glucose, semen quality, liquid semen, buffalo bull spermatozoa.

INTRODUCTION

Artificial insemination is a technique of animal breeding which has been intensively used to improve the genetic potential of farm animals in developed countries. This technique is based on rapid dissemination of germplasm to large number of females from small number of superior sires in terms of economically important traits (Vishwanath and Shannon, 1997).

Artificial insemination with frozen semen is used in buffalo with limited success due to poor freezability and fertility (Anzar *et al*., 2003). Liquid semen in artificial insemination programme could be a choice because of higher fertility rates with almost 3-5 times less number of spermatozoa per dose as compared to frozen semen (Sharma and Sahni, 1988; Shannon and Vishwanath, 1995). Use of tris, citrate and milk based diluters for liquid storage of buffalo semen at 4-5°C temperatures is common practice (Sansone *et al.*, 2000). However, ability to conserve semen quality, availability and economics make skim milk superior for liquid storage of buffalo bull semen (Akhter, 2006). For proper functioning of the metabolic activities of spermatozoa energy sources are naturally present, but during dilution and storage period continuous energy depletion occurs (Sansone *et al*., 2000).

Glucose is a major naturally occurring energy source for animal cells that may be used as energy supplement in diluter for the continuation of metabolic activities and functioning in liquid state (Akhter, 2006). Therefore, it was hypothesized that addition of glucose as energy supplement in skim milk diluter may improve the preservability of Nili-Ravi buffalo bull semen stored at 5°C. This experiment was designed to evaluate the effect of glucose supplementation in skim milk diluter on motility, plasma membrane and acrosomal integrity of Nili-Ravi buffalo bull spermatozoa stored at 5°C.

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MATERIALS AND METHODS

Powdered skimmed milk (SKIMZ®; CANDIA) 10% (w/v) without glucose supplementation was used for preparation of diluter, as control. Experimental diluters were prepared by adding 5, 10 and 30mM of glucose to control diluter. Streptomycin sulphate @ 1 mg/ml and benzyl penicillin @ 1000 i.u /ml were added in each diluter at room temperature. Semen from three Nili-Ravi buffalo bulls was collected for 3 weeks (replicates) with artificial vagina maintained at 42°C. After collection, semen was transferred to the laboratory for initial evaluation. Sperm motility was assessed microscopically at 400X using phase contrast microscope. Sperm concentration was assessed by Neubauer haemocytometer. Semen ejaculates having motility > 60% were split into four aliquots and diluted at 37°C with skim milk diluter without or with 5, 10 and 30mM glucose. The diluted semen was cooled from 37°C to 5°C in 2 hours and stored at 5°C for five days.

Sperm plasma membrane integrity was determined by hypo-osmotic swelling assay (HOS). The HOS solution (osmotic pressure ~ 190mOsm/kg) contained sodium citrate 0.735g and fructose 1.351g, dissolved in 100ml distilled water. To perform assay, 50µl semen sample was mixed with 500µl pre-warmed (37°C) HOS solution and incubated at 37°C for 30 to 40 minutes. After incubation, a drop of semen sample was examined using phase contrast microscope (400X). One hundred spermatozoa were counted and the percentage of cells with curled tails (swollen/intact plasma membrane) was recorded as HOS positive (Akhter *et al*., 2008).

Sperm acrosomal integrity was determined by mixing 500 µl of semen samples with 50µl of 1% formal citrate solution and a drop of semen sample was examined under phase-contrast microscope (1000X). One hundred spermatozoa were counted to determine the percentage of acrosomal intact spermatozoa (Akhter *et al*., 2010).

The data on sperm motility, plasma membrane and acrosomal integrity were analyzed using ANOVA and presented as mean (± SE). A 5% ($P < 0.05$) level was used to determine statistical significance.

RESULTS

Motility of buffalo bull semen

The data on the effect of glucose supplementation in diluter on motility of buffalo bull spermatozoa are presented in Figure 1. The motility of buffalo bull spermatozoa did not differ ($P>0.05$) in all experimental diluters on 1st day of storage. However, sperm motility was higher ($P<0.05$) in diluters containing 5, 10 and 30mM glucose on 3rd and 5th day of storage at 5°C as compared to control.

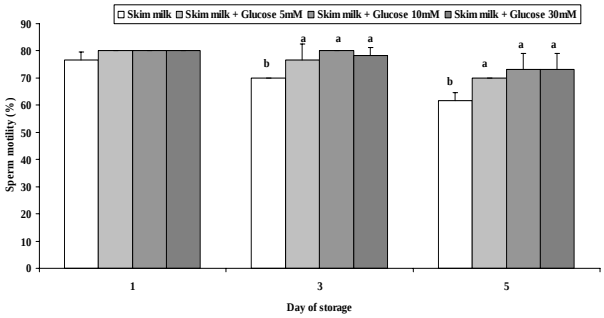

Fig. 1. Effect of glucose supplementation in diluter on motility of buffalo bull spermatozoa on different days of storage at 5°C. Bars with different letters differ significantly ($P<0.05$) at a given day.

Plasma membrane integrity of buffalo bull semen

The data on the effect of glucose supplementation in diluter on plasma membrane integrity of buffalo bull spermatozoa are presented in Figure 2. There was no difference ($P>0.05$) in plasma membrane integrity of buffalo bull spermatozoa in all experimental diluters on 1st day of storage. However, sperm plasma membrane integrity was recorded higher ($P<0.05$) in diluters containing 5, 10 and 30mM glucose on 3rd and 5th day of storage at 5°C compared to control.

Acrosomal integrity of buffalo bull semen

The data on the effect of glucose supplementation in diluter on acrosomal integrity of buffalo bull spermatozoa is presented in Figure 3.

Sperm acrosomal integrity of buffalo bull spermatozoa remained similar (P>0.05) in experimental diluters on 1st, 3rd and 5th day of storage at 5°C.

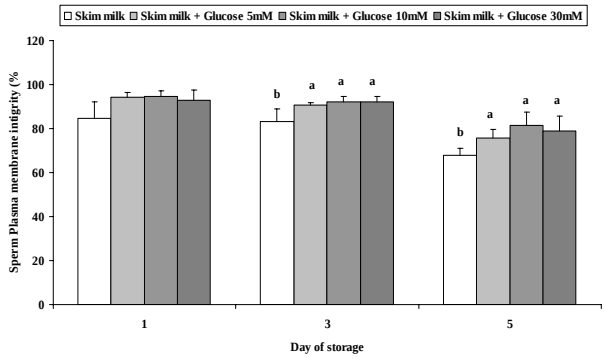

Fig. 2. Effect of glucose supplementation in diluter on plasma membrane integrity of buffalo bull spermatozoa on different days of storage at 5°C. Bars with different letters differ significantly (P < 0.05) at a given day.

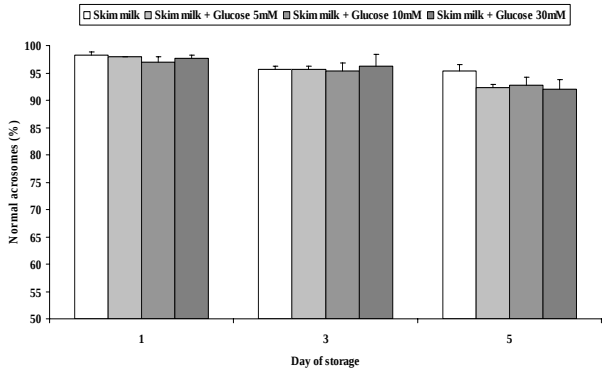

Fig. 3. Effect of glucose supplementation in diluter on acrosomal integrity of buffalo bull spermatozoa on different days of storage at 5°C.

DISCUSSION

The energy sources required for normal metabolic activities of spermatozoa are depleted continuously during dilution and storage of semen. Therefore, we tested glucose supplementation in diluter as energy source to improve the quality of Nili-Ravi buffalo bull semen (motility, plasma membrane and acrosomal integrity) stored at 5°C.

Sperm motility is one of the common methods routinely used for the assessment of buffalo bull spermatozoa in artificial insemination programme. In the present study, sperm motility remained similar (P>0.05) in all experimental diluters on 1st day of storage. However, higher (P<0.05) motility was observed in diluters supplemented with 5, 10 and 30mM glucose as compared to control on 3rd and 5th day of storage at 5°C. Our findings are inline with the study on Egyptian buffalo semen, where supplementation of glucose in tris based diluter improved the motility of buffalo bull spermatozoa (El-Azab *et al.*, 1984). Similarly, supplementation of 2% glucose in Tris based diluter at 5°C, improved motility of Murrah buffalo bull spermatozoa (Kumar *et al.*, 1992).

Sperm plasma membrane integrity is a semen quality parameter of extreme importance because it is highly related with fertility of bull semen (Brito *et al.*, 2003). In our study, percentage sperm plasma membrane integrity did not differ in all experimental diluters on 1st day of storage. However, percentage sperm plasma membrane integrity was higher in diluter supplemented with 5, 10 and 30mM glucose as compared to control on 3rd and 5th day of storage at 5°C. It is noteworthy to mention that data on plasma membrane integrity is very much similar to that of sperm motility *i.e.*, similar pattern was observed in sperm motility and plasma membrane integrity. In this study, percentage of sperm with normal acrosomes remained similar in all experimental diluters on 1st, 3rd and 5th day of storage. Similar observations were recorded on acrosomal integrity of buffalo spermatozoa preserved at 5°C in skim milk diluter (Akhter *et al.*, 2008, 2010).

In conclusion, glucose supplementation in skim milk diluter improved the quality (motility and plasma membrane integrity) of buffalo bull semen stored at 5°C upto 5 days. The values were similar in skim milk diluter supplemented with 5, 10 and 30mM glucose. Therefore, 5mM glucose is recommended to improve the preservability of liquid Nili-Ravi buffalo semen used for artificial insemination programme.

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