HYPO-OSMOTIC SWELLING TEST ON BUFFALO (*Bubalus bubalis*) SEMEN

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ABSTRACT

The experiment was conducted in semen of Murrah buffalo bulls. The semen was exposed to three different concentrations of hypo-osmotic solution (150, 100 and 75 milliosmole) at different time periods at 37°C. At 100 mOsm there was highly significant ($p < 0.01$) difference at 45 and 60 minutes between different bulls. It was concluded that 100 mOsm concentration of hypo-osmotic solution was suitable for semen evaluation in buffalo species.

Keywords: Murrah buffalo, hypo-osmotic swelling test, different gradient

INTRODUCTION

Buffaloes are outstanding domestic animals because their milk is superior in quality, having higher fat and solid not fat content. Breeding and reproductive efficiency in buffaloes is poor and no major breakthrough in augmenting reproduction has yet taken place. As the bull is half of the herd, sire indexing is being utilized to mark out the elite male germplasm. The application of artificial insemination (A.I.) and frozen semen are recommended for improvement of buffalo production. The technology of deep freezing of semen has remarkably increased the preservability and storage of semen for artificial insemination and is contributing enormously to exploitation of proven sires in improving animal productivity and conservation of the species. The knowledge gained through the present investigation may provide a guide line for suggesting alteration/ modifications with a view to improve overall freezing of buffalo spermatozoa. Thus, it will be a contribution for augmenting the productivity and conservation of this precious animal wealth. So the present investigation is aimed to study the effect of different hypo-osmotic gradient solution (150, 100 and 75 milliosmole) on buffalo spermatozoa in the neat semen, pre-freezing after equilibration and post-thaw semen of different Murrah buffalo bulls.

MATERIALS AND METHODS

The experiment was conducted in three Murrah buffalo bulls of 4 to 6 years of age belonging to the university dairy farm, Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan,

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Mathura, Uttar Pradesh, India. Experimental bulls of 4 to 6 years of age with good body condition (score 5-6) were selected and maintained under optimum managemental practices as per the standard criteria fixed for maintenance of breeding bulls in bull stations. The pre-freezing seminal parameters of the three buffaloes' semen had >60 percentage progressive forward motility and less than 20 percentage total abnormality.

Semen was collected with an artificial vagina (n = 25), immediately split into three portions, marked, and placed in the water bath at 32°C for further evaluation. Plasma membrane integrity of spermatozoa was determined by the hypo-osmotic swelling test (HOST) as per Jeyendran *et al*. (1984). Hypo-osmotic solution of different osmolality (150, 100 and 75 milliosmole) was prepared as follows.

Solution A (150 mOsm/L) was prepared by dissolving following reagents in the given concentrations as

Sodium Citrate : 7.35 gm
Fructose : 13.51 gm
Doubled Distilled Water : 1000 ml

Solution B (100 mOsm): 1 ml of solution A + 0.5 ml double distilled water.

Solution C (75 mOsm): 1 ml of solution A + 1 ml double distilled water.

The percentage of HOST positive spermatozoa were calculated as

$$\text{HOS positive Spermatozoa} = \frac{\text{Number of spermatozoa exhibiting tail coiling}}{\text{Total number of spermatozoa observed}} \times 100$$

The semen was assessed at three different stages: neat semen immediately after collection, after equilibration (5°C in cold handling cabinet for 3 h) and post thawing (37°C for 30 seconds) of semen at different concentration of hypo osmotic solution. The post thaw semen was evaluated after incubation at 37°C for 1 h and at intervals of 15 minutes (0, 15, 30, 45 and 60 minute of incubation) upto 60 minutes.

The results were analyzed statistically after arcsine transformation of percentage data by using SPSS 15 (SPSS, Chicago, IL, USA).

RESULT AND DISCUSSION

In neat semen, the experimental result did not reveal any significant difference for this parameter between different bulls at different concentrations of HOST solution. It has been found that a 100 mOsm osmotic solution was a better choice for buffaloes (Pratap *et al*.1999, Revell and Mrode, 1994, Karmur *et al.,* 2002), but slightly higher than those reported by Shukla (2002) and Pandey and Gupta (2004) (Table 1).

In equilibrated semen, the HOS positive spermatozoa varied significantly ($P<0.05$) between different bulls at 150 and 100 mOsm and at 75 mOsm, the bull 902 being non-significantly higher than the other bulls (Table 2). In the present study, maximum HOS positive spermatozoa were found in 100 mOsm and minimum was at 75 mOsm. The plasma membrane integrity of spermatozoa was reduced at equilibration as compared to its value in neat semen (Correa and Zavos, 1994; Pramanik *et al.*, 1998; Rasul *et al*., 2001; Karmur *et al*., 2002; Pant *et al*., 2002; Rana and Dhami, 2003; Singh *et al*., 2004).

Semen post thawed at 37°C for 30 seconds at 15 minutes intervals of incubation showed that the HOS positive spermatozoa at 150 mOsm

between different bulls at 0, 15, 30, 45 and 60 minutes did not reveal any significant difference (Table 3) and at 100 mOsm there was no significant difference at 0, 15 and 30 minutes, but at 45 and 60 minutes there was a highly significant ($p<0.01$) difference between different bulls (Table 3). At 75 mOsm, there was a significantly difference at 0, 15, 30, 60 minutes ($p<0.01$) and a highly significant ($P<0.01$) difference at 45 minutes. However, it dropped significantly ($P<0.01$) within a bull at the three concentrations of osmotic solution from 60 to 0 minutes (Table 4). Maximum HOS positive spermatozoa (overall value irrespective of bull) immediately following thawing in 100 mOsm osmotic solution (Correa and Zavos, 1994; Revell and Mrode, 1994, Singh *et al.,* 2004), while corresponding minimum HOS positive spermatozoa were found in 75 mOsm solution were observed. This is due to more than 50% of spermatozoa usually being injured by the process of cryopreservation (Watson, 1995) and formation of ice crystals in the extra and intracellular environment, and increasing solute concentration (Mazur, 1998).

Further, it has been reported that there is no information regarding the different concentrations of hypo-osmotic solution on the buffalo semen.

It was concluded that 100 mOsm concentration of hypo-osmotic solution was suitable for semen evaluation in buffalo species.

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