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Plasma Membrane Integrity of Sperm in Mithun (Bos Frontalis) Ejaculate

KEYWORDS: Hypo osmotic swelling test, plasma membrane integrity, sperm, mithun

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ABSTRACT *A study was undertaken to standardise the hypoosmotic solution to estimate the functional membrane integrity of sperm in mithun. Sodium citrate and fructose based various hypoosmotic solutions (mOsm/l) were prepared and grouped into G1 (0), G2 (50), G3 (100), G4 (150), G5 (200), G6 (250) and G7 (300). Twenty five ejaculates were collected from five matured mithuns bulls and allowed for macroscopic evaluation followed by 10µl of semen was immediately added to 2ml of each different hypoosmotic solution and incubated for one hour at 37°C in water bath. Sub sequentially, 20µl of diluted semen in hypoosmotic solution were evaluated using phase-contrast microscope. A total of 200 spermatozoa were counted in at least five different fields and sperm tails were classified as non-responded (non-coiled) and responded (coiled and strongly coiled). Result revealed that in total and strong coiling, G4 was superior to G1, G2, G6 and G7 (P<0.05). Further, result from G3, G4, and G5 had a similar increased percentage of coiling were grouped to assess differences among mithuns. The percentage of total coiling was differed significantly (P<0.05) among mithuns. It was concluded that 150 mOsm/l solution was most suitable for mithun spermatozoa.*

INTRODUCTION

As the plasma membrane of sperm is important in metabolic exchanges with the environment and its structural integrity is essential for sperm capacitation and fertilization. In mithun species, semen analyses are based on methods developed for other domestic animal species. Thus, it becomes necessary to test a suitable concentration of hypoosmotic swelling test (HOST) solution to verify the functional membrane integrity and physical structure of spermatozoon of mithun without causing lysis of sperm membrane. Jeyendran et al. (1984) initiated to use the HOST solution to evaluate plasma membrane functional integrity in humans. Later on the same procedure was used in other domestic species *viz*. dogs (Dobranić et al. 2005), bulls (Correa and Zavos 1994, Revell and Mrode 1994), rams (Watson and Duncan 1988), boars (Zou and Yang 2000) and stallions (Neild et al. 1999). Further, perusal of literatures revealed that there was no information on the effect of different osmolarity solution on the sperm functional membrane integrity in mithun. Hence, this present study was designed to establish the most suitable HOST solution (osmolarity) for testing membrane integrity of mithun sperm cell or spermatozoa.

MATERIAL AND METHODS

Five apparently healthy mithun bulls of ~ 3 to 5 yr of age (493 to 507 kg body weight) with good body condition (score 5-6) were selected for the study from the mithun herd, ICAR-NRC on Mithun, Jharnapani, Nagaland, India and were maintained under uniform feeding and management conditions. Trisodium citrate and fructose based seven different osmolarity solutions such as G1 (0), G2 (50), G3 (100), G4 (150), G5 (200), G6 (250) and G7 (300) were prepared by serial dilutions (Correa and Zavos 1994, Revell and Mrode 1994). These solutions were aliquated into 3 ml polyethylene tubes (2ml/tube) and then stored at -20°C until use. Twenty five ejaculates were collected from the five bulls (five ejaculates from each bull) through rectal massage method and were evaluated and accepted for evaluation if the following criteria were met: concentration: >500 million / ml; mass activity >3+, individual motility: >70% and total abnormality: <10%.

A volume of 10µl was gently mixed in each of the 2ml HOST solutions previously described and incubated for one hour in a water bath at 37°C. After incubation, 20µl of the solutions containing semen were placed on a microscope slide, covered with a cover glass and evaluated using a phase-contrast microscope (Nikon, Eclipse 80i; 400× magnification). Total of 200 spermatozoa were counted in at least five different fields and spermatozoa were classified as swelled (coiled) and strongly coiled (Revell and Mrode 1994). The results were analysed statistically and means were analyzed by one way analysis of variance followed by the Tukey's post hoc test to determine significant differences between the different osmolarity solutions using the SPSS/PC computer program (version 10.0; SPSS, Chicago, IL).

RESULTS AND DISCUSSION

Result of different percentage of coiled, strongly coiled and total coiled spermatozoa after exposure to different hypoosmotic solutions were revealed that the coiling percentage was increased from 0 mOsm/l solution and reached a maximum value with the 150 mOsm/l solution after that coiling percentage was began to decrease reaching the minimum score with 300 mOsm/l solution (Figure 1). This pattern was observed both in the strong as well as total coiling of spermatozoa in mithun. As per result in total coiling, G4 was superior to G1, G2, G6 and G7 (P<0.05) and similar pattern was also observed in strong coiling of spermatozoa. There was no interaction among solutions and mithuns (P>0.05) and G3, G4, and G5 had a similar increased percentage of coiling. The percentage of total and strong coiled spermatozoa were differed significantly (P<0.05) among mithuns (Figure 2).

The most widely used sperm parameters to screen the semen at initial stage are sperm count, morphology and motility. But the development of new simple tests to screen the semen quality in terms of functional membrane integrity of sperm could be useful in artificial insemination centre. Moreover HOS test gives an indirect indication of fertilizing potential of sperm.

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In the present study, the results revealed that 150 mOsm/l has significantly ($p< 0.05$) higher total as well as strongly coiled sperm as similar in other domestic animal species (Dobranić et al. 2005, Correa and Zavos 1994, Revell and Mrode 1994, Watson and Duncan 1988, Zou and Yang 2000, Neild et al. 1999) has been recorded. The biochemically active sperm cell begins to coil at the distal end of the tail and proceeds towards the midpiece and head as the osmotic pressure of the suspending media is lowered (Jeyendran et al. 1984). In the present study, the term total coiling is referred to as the percentage of sperm cells evaluated that coiled and the term strong coiling is referred to as the percentage of sperm cells that coiled with more intensity. Differences in the coiling intensity could be indicated that the membrane integrity can present some degree of variation and was being more evident under some hypoosmotic solutions. It is also important that coiling is due to a hypotonic solution and it should be differentiated from pathological conditions (Barth and Oko 1989).

In the present study, the result showed that hypoosmotic solution was effective between 100 and 200 mOsm/l in mithun bulls; however 150 mOsm/l solution has given best result on both strong and total coiling. Similar to reports observed in other species such as cattle (150 mOsm/l) (Revel and Mrode 1994), humans (150 mOsm/l) (Jeyendran et al. 1984) and it is also varied with different species such as in swine, it is 50 to 150 mOsm/l (Zou and Yang 2000) and in stallion, it is 25 to 100 mOsm/l (Neild et al. 1999). These results indicate that there is need to find out the best and suitable hypoosmotic concentration to be used in HOST according to the species being evaluated.

It was concluded that sperm response to hypoosmotic challenge depends on osmolarity. Hypoosmotic solution at 150 mOsm/l produced a higher percentage of sperm with tail swelling than other osmotic solutions and this suggests that HOST at 150 mOsm could be the most suitable for mithun. Moreover, further research is needed to establish the correlation between the results of HOST at 150 mOsm/l and other seminal attributes of sperm quality as well as with the reproductive potential of bulls and finally it could aid the routine analyses of mithun semen.

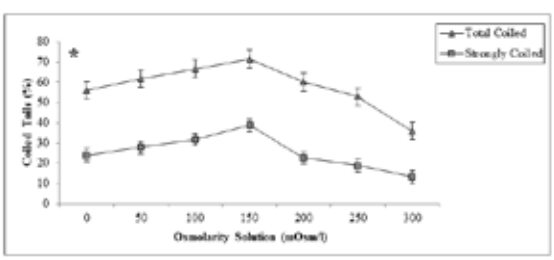

Fig 1: Effect of different concentrations of hypoosmotic solutions (mOsm/l) on the coiling of mithun spermatozoa (mean ± SD) (* indicates P<0.05)

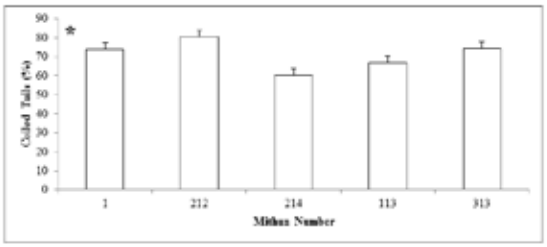

Fig 2: Effect of hypoosmotic solution (150 mOsm/l) on the total coiling of mithun spermatozoa (mean ± SD) (* indicates P<0.05)

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