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DEVELOPMENT OF THE BEST HYPO-OSMOTIC SWELLING SOLUTION FOR EVALUATION OF FUNCTIONAL MEMBRANE INTEGRITY OF SPERMATOZOA OF NILI-RAVI BUFFALO BULL

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ABSTRACT

The functional integrity of spermatozoa in various domestic animals like horse, cattle and swine is evaluated by hypo osmotic swelling test. The hypo osmotic swelling test has not been tested in the fresh semen of Nili-Ravi buffalo bull. The aim of the present study was evaluation of membrane integrity by establishing the best hypo osmotic swelling solution. Ten solutions with different osmolarities were used: 70(HS1), 90(HS2), 100(HS3), 120(HS4), 140(HS5), 150(HS6), 190(HS7), 230(HS8), 280(HS9) and 300 (HS10). Twenty samples were collected (ten from each bull) with forty eight hours interval. The collected semen was placed in water bath at 37°C and was evaluated macroscopically and microscopically for color, volume, mass activity and motility. After evaluation 0.1 ml of semen was mixed with 1ml of hypo osmotic swelling solutions and incubated for one hour at 37°C. A total of two hundred sperms were counted in five fields and sperms were classified as swollen and strongly swollen. The percentages of coiled spermatozoa in ten hypo osmotic swelling solutions mentioned above were 18.3%, 23.5%, 37.3%, 41.4%, 50.2%, 57.5%, 38.6%, 26.0%, 13.9% and 6.2% respectively. The percentages of strongly swelling solutions were 9.2%, 12.0%, 21.6%, 32.8%, 31.0%, 39.1%, 29.3%, 21.7%, 10.0% and 5.7% for ten solutions respectively. HS6 was greater to HS1, HS2, HS7, HS8, HS9 and HS10 in increasing the ability of swelling ($P<0.05$). Similarly HS6 showed greater ability of swelling as compared to HS1, HS2, HS8, HS9 and HS10 ($P<0.05$).These results indicated that the solution having 150 mOsm/L was the best for functional membrane integrity of spermatozoa in fresh semen of Nili-Ravi buffalo bull semen.

Keywords: Hypo osmotic swelling test, Functional membrane integrity, Spermatozoa, Nili-Ravi bull

INTRODUCTION

The major parameters to check the quality of semen are motility, livability and structurally normal spermatozoa for the evaluation of fertility of breeding bull. However evaluation of these parameters does not fulfill criteria for use of breeding and selection of bull for fertility because they are unable to report the functional integrity of sperm plasma membranes and metabolism. These parameters only help to assess the structural integrity of the sperm cell (Neild et al., 1999).To study the functional integrity of spermatozoa; the hypo osmotic swelling test (HOST) has proved the good results. Thus it becomes necessary to adopt the new techniques in HOST for evaluation of functional integrity in semen of Nili-Ravi buffalo bull.

The ability to increase in volume indicates that these cells are active biochemically. This swelling will occur at tail region because the membrane of tail is loosely held as compared to head. HOST has been used by different workers in cattle (Correa and Zavos, 1994; Revell and Mrode, 1994), swine (Vasquez et al., 1997; Zou and Yang, 2000), horses (Neild et al., 1999), porcine (Perez-Llano et al., 2001),dogs (Dobranic et al., 2005), avian spermatozoa (Malecki et al.,2005)and humans (Jeyendran et al., 1984). The best HOST for these species as already established. The objective of this study was to investigate the best concentration of HOST to assess the well-designed integrity of Nili-Ravi buffalo bull semen.

MATERIALS AND METHODS

Experimental animals

The experiment was conducted at the Semen Production Unit, Department of Theriogenology, University of Agriculture Faisalabad, Pakistan. The fresh semen of two Nili-Ravi breeding bulls (B4 and B6) was used in this study. These bulls were in good health condition and were producing the semen of good quality for artificial insemination.

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Preparation of Hypo osmotic solutions

The solution of 300 mOsm/L trisodium and fructose was prepared according to method of Revell and Mrode (1994). The ten solutions with different osmolarities were prepared by diluting in distilled water as stated by Correa and Zavos (1994) and were stored at -20°C temperature till use. The solutions with different osmolarities were (mOsm/L): (HS1) = 70, (HS2) = 90, (HS3) = 100, (HS4) = 120, (HS5) =140, (HS6) = 150, (HS7) = 190, (HS8) = 230, (HS9) = 280,and (HS10) =300.

Collection and macroscopic evaluation of fresh semen

The semen was collected before the sunrise with the help of artificial vagina by mounting on teaser bull. The semen was collected two times in week and ten samples were collected from each experimental bull. The collected semen was placed in a water bath at 37°C and evaluated for volume, color, mass activity and motility. The volume was made by mixing gently 0.1 ml of fresh semen with 1 ml of hypo osmotic solutions and incubated at 37°C for one hour. After completion of incubation the 10 µl of solution containing semen was placed on pre-warmed microscopic slide and covered with a cover slide and studied under the phase contrast microscope at 1000x magnification. A 200 no. of total spermatozoa were counted in five different fields. The spermatozoa were grouped as swollen and strongly swollen according to method used by Revell and Mrode (1994). In addition to entire swelling, a strong coiling reported when the end of tail became much coiled.

Statistical analysis

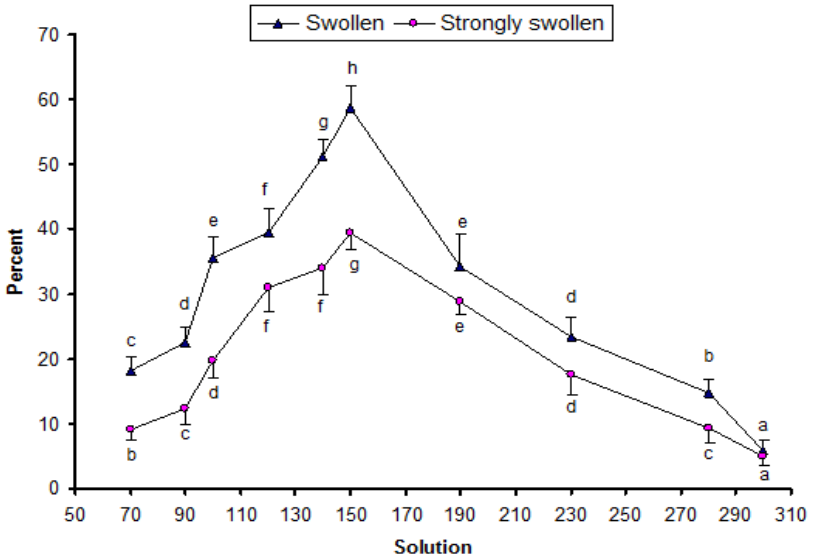
Data presented as the mean ± SD, were analyzed by a two-way analysis of variance (ANOVA) (Neild et al., 1999). Statistical significance was set at $P < 0.05$.

RESULTS

The spermatozoa showed strongly and total coiling patterns of swelling on exposing to solutions of different osmolarities.

According to statistics as in fig 1, bull spermatozoa appeared to undergo increase in swelling from 70 mOsm/L solution and reached a maximum value of 150 mOsm/L. After reaching this peak the swelling began to reduce to attain the minimum value with300 mOsm/L solution. Such a kind of pattern was observed both in strong and total swelling from beginning to end points of solutions. According to overall swelling, HS6 was greater to HS1, HS2, HS7, HS8, HS9 and HS10 in increasing the ability of swelling ($P<0.05$). Similarly HS6 showed greater ability of swelling as compared to HS1, HS2, HS8, HS9 and HS10 ($P<0.05$).

Fig 1: Effect of different concentrations of hypo osmotic solutions (mOsm/L) on the coiling of fresh spermatozoa (mean ± SD). Means with different superscript differed significantly among solutions (Friedman; $P<0.05$



DISCUSSION

The bull spermatozoa exhibited related pattern of swelling as observed in other domestic animals (Correa et al., 1997; Neild et al., 1999), when exposed to hypo osmotic swelling solutions.

It is anticipated that under the hypo osmotic conditions biochemically active spermatozoa with functional membrane integrity will increase in volume to obtain the equilibrium between solution and the internal solute compartments of spermatozoa. Due to increase in volume the swelling starts at tail region of spermatozoa at distal end and moves towards the mid piece and head as the osmotic pressure lowered (Jeyendran et al., 1984). In the present study, percentages of sperm cells swollen studied as total swelling whereas the sperms with more intensity of swelling called strong swelling. Due to differences in swelling intensity it could be stated that the variation in swelling is more pronounced under hypo osmotic mediums. Considering this difference of swelling, it is necessary to differentiate the swelling from cytoplasmic droplet in principal piece. The swelling of sperm indicates the functional integrity whereas cytoplasmic droplets indicate the pathological conditions (Barth and Oko, 1989). According to Jeyendran et al. (1984), the best hypo osmotic solution should cause the visible increase in the volume of sperm without the lysis of sperm membrane. The present study shows this ability at 150 mOsm/L maximum and found similar to in cattle (Revel and Mrode, 1994), 150 mOsm/L in humans (Jeyendran et al., 1984), 100 mOsm/L in boars (Samardzija et al., 2008) but different when compared to 100 mOsm/L found for pig (Phillip Matson et al.,2009), 34mOsm/L for emu (Malecki et al.,2005) and 25 to 100 mOsm/L for equine spermatozoa (Neild et al., 1999). This difference in species justifies need of development of best hypo osmotic solution. Combining the data of swelling and strong swelling, 150 mOsm/L solution showed the best results of swelling. It is well-known that the fraction of swelling varies according to bulls (Prasad et al., 1999), bucks, or season (Kale et al., 2000) and it is strongly correlated to mass movement, progressive motility, live sperm count, total intact acrosome, sperm concentration (Prasad et al., 1999), and fertility (Jeyendran et al., 1984).This indicates that the 150 mOsm/L solution should be used for the functional integrity of buffalo bull spermatozoa and it might aid the routine analyses of bull semen.

CONCLUSION

These results indicated that the solution having 150 mOsm/L was the best for functional membrane integrity of spermatozoa in fresh semen of Nili-Ravi buffalo bull semen.

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