COMPARATIVE STUDY OF PLASMA MEMBRANE INTEGRITY OF SPERMATOZOA BY USING HOS MEDIUM AND DISTILLED WATER AND THEIR RELATION WITH POST THAW MOTILITY

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ABSTRACT

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This study was carried out on 148 frozen thawed semen samples from cattle (58 from 11 HF bulls, 36 from 7 crossbred bulls, 33 from 6 Sahiwal bulls and 21 from 4 Jersey bulls) and 57 frozen thawed semen samples from 11 buffalo bulls maintained at Animal Breeding Centre (a Unit of ABRO India), Salon, Rae Bareli, UP. Plasma membrane integrity of cryopreserved sperms was studied with the help of HOS solution (Osmolarity 0.080 Osmole/kg, pH 7.31) and double distilled water (Osmolarity 0.001 Osmole/kg, pH 5.93). The mean percentages of HOS reactive sperms in HOS solution and double distilled water for cattle and buffalo were 57.61±0.57, 48.98±0.34 and 57.06±0.75, 47.14±0.34, respectively. Except, Crossbred, the percentage of HOS reactive sperms in HOS solution was significantly ($P<0.05$) higher than the percentage of reactive sperms in distilled water in HF, Jersey and Sahiwal breed of cattle. The results revealed that the HOS solution was more suitable hypo-osmotic medium for assessing the plasma membrane integrity of frozen thawed spermatozoa as compared to Double distilled water. Highly significant positive correlations were observed between HOS reactive sperms in HOS solution and in distilled water as well as with post thaw motility (PTM) in both the species ($r = 0.42$ to 0.75).

Key words: HOS medium, Plasma membrane integrity, HOS reactive sperms, Cattle, Buffalo, Post Thaw Motility.

INTRODUCTION

The Artificial Insemination (AI) technology is considered to be the most powerful tool in the hands of Animal Breeders for the genetic improvement of the livestock as well as to control the sexually transmitted diseases. The genetic gains through AI are achieved by maximum utilization of an elite sire to produce thousands of superior progenies, which otherwise is not possible through natural mating. However, the major problem in AI appears to be the non-availability of the efficient methods for predicting fertility potential of frozen semen. In India, the most widely used quality assessment criterion i.e. post thaw motility (PTM) of sperms alone was not found to be sufficient enough for this purpose. Alternatively, the plasma membrane integrity of spermatozoa, which is very much crucial for the metabolism, capacitation, acrosome reaction and penetration of sperm into the oocyte (Burks and Sailing, 1992), is gaining importance as an indicator in determining the fertility potential of the spermatozoa. Several researchers have studied the plasma membrane integrity by exposing the sperms to Hypo-osmotic

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solution (Jeyendran, *et al.,* 1984; Zekariya *et al.,* 2005), while others have used double distilled water for the same (Lomeo and Giambersio, 1991; Pratap *et al.,* 2000; Mokashi *et al.,* 2008) with contradictory results. In view of this, the present study was designed to compare the efficiency of HOS medium (Osmolarity 0.080 Osmole/kg, pH 7.31) and double distilled water (Osmolarity 0.001 Osmole/kg, pH 5.93) for assessment of plasma membrane integrity of sperms and to find out the correlation between post-thaw motility with plasma membrane integrity.

MATERIALS AND METHODS

This study was conducted on 148 frozen thawed semen samples collected from cattle (58 from 11 HF bulls, 36 from 7 crossbred bulls, 33 from 6 Sahiwal bulls and 21 from 4 Jersey bulls) and 57 frozen thawed samples from 11 buffalo bulls maintained at Animal Breeding Centre, Salon, Raebareli, UP, during the year 2009-10.

The semen samples with minimum 70 % initial motility were processed for freezing. The post thaw motility and plasma membrane integrity of the frozen semen samples were assessed 24 hours after freezing. Plasma membrane integrity was assessed on the basis of percentage of HOS reacted sperms using HOS medium with Osmolarity of 0.080 Osmole/kg and pH 7.31 (prepared by adding 0.3675 gm of tri-sodium citrate and 0.6750 gm of fructose in 50 ml of double distilled water as suggested by Jeyendran *et al.,* 1984) and double distilled water with Osmolarity of 0.001 Osmole/kg and pH 5.93.

The effect of both media on HOS reactive sperms percentage was analyzed by Least squares analysis technique (Harvey, 1975) including types of media as an effect in the model.

RESULTS AND DISCUSSION

The mean percentages of HOS reactive sperms found in HOS medium were significantly ($P<0.01$) higher than in double distilled water for both cattle and buffalo bulls. The mean values of HOS reacted sperms in HOS medium were 57.61±0.51 and 57.06±0.75 per cent respectively, whereas in distilled water, the mean values were 48.98±0.34 and 47.14±0.62 per cent, respectively, for cattle and buffalo bulls. Similar observation was reported by Fazano *et al.* (1993); Lin *et al.* (1998) and Zekariya *et al.* (2005). In contrast, Pratap *et al.* (2000) and Mokashi *et al.* (2008) observed higher percentages of HOS reactive sperms in double distilled water than in hypo-osmotic medium. Among the different breeds of cattle, the percentage of HOS reactive sperms in HOS medium were found significantly ($P<0.05$) higher than double distilled water in HF, Jersey and Sahiwal cattle, whereas, in CB the variation was found to be statistically non significant. The average percentage of reacted sperms in HOS media was observed to be 58.86 % in HF, 58.02% in Jersey, 57.71% in Sahiwal and 53.21 % for CB, respectively. While the figures for double distilled water were 47.73%, 48.51%, 46.13% and 53.21% for HF, Jersey, Sahiwal and Crossbreds respectively. The difference in mean percentages of HOS reactive sperms in two different media may be due to difference in osmolarity and pH of the media. However, Bahamondes *et al.* (2001) did not find significant difference in mean percentages of HOS reactive sperms in hypo-osmotic medium and double distilled water. The mean post-thaw motility of frozen semen samples was observed as 57.33±0.44 and 57.63±0.66 percent, respectively, for cattle and buffalo bulls under study.

The results revealed positive and highly significant ($P<0.01$) correlations between the aforesaid parameters. This indicated that frozen semen samples with high post-thaw motility had the better plasma membrane integrity, which indirectly reflected better fertilizing capacity of sperms. The results of present study confirm with the findings of Serikoonjaenak *et al.,* (2007), who also found a positive and significant correlation between plasma membrane integrity and post-thaw motility.

From the results it is inferred that HOS medium with the Osmolarity of 0.080 Osmole/kg is more suitable for assessing plasma membrane integrity of the frozen thawed semen samples derived from cattle and buffalo

bulls than double distilled water and that there exists a better correlation between HOS reactive sperms and post-thaw motility.

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