

ABSTRACT

The objective of this study was to find out the optimal equilibration time and better extender for the cryo-preservation of Buffalo bull semen. Four different equilibration times 2, 4, 6 and 8 hours and two extenders i-e Bioxcell[®] and Egg Yolk Tris Glycerol (EYTG) were used to observe their effects on post-thaw motility of buffalo bull spermatozoa. For this purpose five Nili-Ravi buffalo bulls maintained at Buffalo Research Institute, Pattoki were selected. Semen was collected from buffalo bulls using an artificial vagina. Total twenty semen samples (four from each bull) were collected. Pooled ejaculates, possessing more than 60% visual sperm motility, were divided into two aliquots, diluted with either of the two extenders and filled in 0.5 ml French straws. These semen straws were cooled to 4 °C in 2 hours, equilibrated for different times and frozen in a programmable freezer before plunging them into liquid nitrogen (-196 °C). Thawing of frozen semen was done after 24 hours at 37 °C for 15 seconds.

It was observed that in fresh buffalo bull semen, values of mass motility, individual sperm motility and sperm concentration averaged 2.40 ± 0.11 %, 73.88 ± 0.87 % and 935.50 ± 53.60 millions per ml respectively; where as percentage of sperm motility after dilution in Bioxcell[®] and EYTG extenders was 67.00 ± 0.65 % and 70.00 ± 0.57 % respectively.

It was recorded that average pre-freeze sperm motility percentage at 2, 4, 6 and 8 hours equilibration was 51.90 ± 0.33 , 52.85 ± 0.35 , 52.60 ± 0.35 and 52.80 ± 0.31 in Bioxcell[®] and 52.40 ± 0.33 , 53.05 ± 0.34 , 53.20 ± 0.39 and 53.35 ± 0.38 in EYTG extenders, respectively; where as average values of post-thaw sperm motility percentage after 24 hours storage for 2, 4, 6 and 8 hours equilibration were 37.40 ± 0.41 , 38.60 ± 0.54 , 38.30 ± 0.42 and 38.75 ± 0.53 in Bioxcell and 38.00 ± 0.45 , 39.80 ± 0.48 , 39.70 ± 0.59 and 40.45 ± 0.57 in EYTG extenders, respectively.

Analysis of variance for post-thaw motility revealed a significant difference between two extenders and four equilibration times. Multiple comparisons by the application of L.S.D technique for post-thaw motility in four equilibration times showed that the difference of means among 4, 6, and 8 hour equilibration was non-significant but it was found significant when compared to 2 hours equilibration time ($P < 0.05$). Mean values of post thaw motility for two extenders when subjected to T-test showed a significant difference ($P < 0.05$). In conclusion, equilibration times of 4, 6 and 8 hours were found significantly superior to that of 2 hours and semen samples extended in EYTG showed better post thaw sperm survivability than Bioxcell[®]. As, in this study, non-significant difference regarding post thaw motility for equilibration times of 4, 6 and 8 hours has been found so the use of 4 hour equilibration time can safely be recommended along with Egg Yolk Tris Glycerol (EYTG) extender for cryo-preservation of Buffalo bull semen.