Effect of Fructose, Trehalose or Sucrose Supplementation of the Tris-Based Extender Including Cholesterol-Loaded Cyclodextrin on the Freezability and Post-Thaw Parameters of Ram Spermatozoa

SUMMARY

The ram sperm undergo different type of damages during cryopreservation process which consequently results in low pregnancy rate in sheep. Among them damages the sperm membrane is the major factor that affects the sperm survival post-cryopreservation. Many studies have been conducted to minimize the adverse effect of cryopreservation on sperm plasma membrane. Therefore, the objective of present study was to protect the sperm membrane during cryopreservation by using different sugars (fructose, sucrose and trehalose) in combination with cholesterol in semen extenders. The information based upon post-thaw parameters would help to choose the best combination for ram sperm cryopreservation.

Five Kıvırcık rams were used and semen was collected using electro-ejaculator. Initial motility, concentration and abnormal sperm percentage were evaluated. After evaluation, semen was split into two parts: one was treated with CLC (3mg/120x10^6 sperm) and second remained untreated. After treatment, CLC treated and untreated parts was further divided into three aliquots and diluted with either fructose, sucrose or trehalose supplemented extenders. Then all the diluted aliquots were packed into straws and cooled to 4°C for two hours. After equilibration at 4°C, straws were frozen in liquid nitrogen vapor and stored in liquid nitrogen. Straws were thawed after freezing and sperm characteristics (motility, live/dead, membrane integrity, abnormal sperm and acrosome integrity) were estimated.

The post-thaw results showed that there is no statistical difference in motility amongst the groups, however, CLC-trehalose (54.5±3.28%) and CLC-fructose (54±2.86%) has highest motility compared to other groups. Live sperm percentage was significantly ($P<0.05$) higher in CLC-fructose and sucrose as compared to untreated fructose and sucrose. There is no significant difference in abnormal sperm percentage between CLC treated and untreated group. CLC-trehalose has highest percentage of total intact membrane and live-intact membrane sperm compared to other groups.

The CLC-treated groups has significantly ($P<0.05$) low percentage of sperm with reacted acrosome as compared to CLC-untreated sperm.

In conclusion; although the sugars utilized in this study has influenced some of the post-thaw paramaters the protective influence of CLC on freezing ram spermatozoa was more prominent than sugars.

Key words: CLC, cryopreservation, fructose, ram sperm, sucrose, trehalose