

**Summary of the DVSc thesis** No: 15318, Faculty of Veterinary Medicine, Urmia University.

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**Title of thesis:** Comparison of cholesterol carriers and substitution of fructose and glycerol with trehalose on frozen/thawed ATP content, DNA integrity and kinematics variables of ram spermatozoa

**Summary:**

Current study was aimed to assess the  $\beta$ -cyclodextrin ( $\beta$ CD) and methyl- $\beta$ -cyclodextrin (M $\beta$ CD) on delivery of cholesterol to the ram spermatozoa membrane, and substitution of fructose and glycerol with trehalose on the quality of frozen/thawed ram semen. Samples were collected from the five fertile rams, diluted with tris-citric acid-fresh LDL extender, pooled, and used in the study. In experiment 1, two carriers ( $\beta$ CD vs. M $\beta$ CD) were used and compared to deliver the cholesterol (at 0, 0.5, 1, 1.5, 2, and 4 mg/ml). In the experiment II, trehalose (0, 7, 14, 21, and 28 mM) was substituted with fructose (28, 21, 14, 7, 0 mM, respectively). In the experiment III, the best cholesterol/carrier dose groups of first experiment, was selected to evaluated the fructose/trehalose (14/14 mM) combination compared to fructose (28 mM) alone. The concentration of glycerol in the above experiments was set at 4.5%. In the final experiment (IV), effect of lowering glycerol (4% vs. 4.5%) was assessed on ram semen cryostorage using selected cholesterol/carrier groups. Kinematics (evaluated by CASA), chromatin integrity (evaluated by acridine orange staining), ATP contents, and malondialdehyde amounts were evaluated. Results indicated cholesterol (especially at 1.5 and 2 mg/ml) improved the kinematics, and ATP levels using both carriers. The optimized amounts of trehalose (14mM)/fructose(14mM) reduced peroxidation and DFI levels. Co-administration of optimized levels of cholesterol with proper amounts of trehalose/fructose did not show extra beneficial effects compared to each of them. Trehalose could not protect the spermatozoa at lower amounts (4% vs. 4.5%) of glycerol. In conclusion, either the optimized levels of cholesterol (using  $\beta$ CD or M $\beta$ CD carriers) or substitution of half of fructose with trehalose alone could lead to improvement in frozen/thawed ram semen quality.

**Keywords:** Cholesterol,  $\beta$ -cyclodextrin, Methyl- $\beta$ -cyclodextrin, Trehalose, Ram, Semen.