



Tanta University  
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# **Effect of Cryopreservation and cryoprotectants agents of semen Extender on Mitochondrial function of Holstein bulls Spermatozoa.**

By

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## **THESIS**

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## **Abstract**

The goal of this study was to assess effect of three Tris-extender types, of egg yolk (E1), soy lecithin (E2), and coconut milk (E3), on sperm characteristics of Holstein bull semen at freezing stages. Semen was collected and pooled on each collection day. Semen should have good mass motility  $\geq 70\%$ . The pooled semen was divided into three divisions; E1: extended by E1: Tris-citric with 15% egg yolk; E2 and E3: diluted with 0.5% soy-lecithin and 15% coconut milk at a rate of 1:15, respectively. Semen with each extender was diluted and cooled at 5°C for 4 hours, as equilibration period. Then, semen was packaged in 0.25 ml French straws and frozen in Liquid nitrogen (-196 °C) for one month. Results showed that E2 showed higher positive impact on sperm characteristics than E1 and E3 after dilution and equilibration, and thawing in terms of livability, plasma membrane integrity, motility, intact acrosome of spermatozoa, but the differences were not significant. In post-thawed semen -E2 was showed higher ( $P < 0.05$ ) progressive motility, and livability than other semen extenders. Also, E2 showed ( $P < 0.05$ ) higher sperm plasma membrane integrity, and intact acrosome, but the differences were not significant. The percentage of sperm abnormalities was similar in all extenders. In conclusion, this study recommended that 0.5% soy bean lecithin can be used instead of 15% egg yolk or 15% coconut milk for dilution and cryopreservation of bull semen.

**Keywords: Bovine semen, dilution, cryopreservation, sperm characteristics.**

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## **LIST OF ABBREVIATIONS**

**AI-artificial insemination**

**SE-standard error LSM-least square mean**

**Cyto Cox - cytochrome c oxidase**

**CS-citrate synthase**

**IOMM -integrity outer mitochondrial membrane**

**IIMM - integrity inner mitochondrial membrane**

**RNA- ribonucleic acid**

**mRNA-messenger RNA**

**tRNA-transfer RNA**

**DNA-deoxyribonucleic acid**

**MtDNA-mitochondrial DNA**

**nDNA-nuclear DNA**

**OXPHOS-oxidative phosphorylation**

**ETC-electron transfer chain**

**ATP-adenosine triphosphate**

**GTP-guanidine triphosphate**

**ADP-adenosine diphosphate**

**Pi-phosphate**

**NAD-nicotinamide adenine dinucleotide**

**FAD-flavin adenine dinucleotide**

**acyl-CoA-Acetyl-coenzyme A**

**ETF-electron transferring flavoprotein**

**LDL-low density lipoprotein**

**TM-total motility**



**PM-progressive motility**

**-polymerase chain reaction**

**GAPDH-glyceraldehyde-phosphate dehydrogenase**

**AA-amino acid**

**ND1-NADH dehydrogenase subunit 1**