The present study was conducted to determine the effects of supplementing α-linolenic acid (ALA) into BioXcell® extender on post-cooling, post-thawed bovine spermatozoa and post thawed fatty acid composition. Twenty-four semen samples were collected from three bulls using an electro-ejaculator. Fresh semen samples were evaluated for general motility using computer assisted semen analyzer (CASA) whereas morphology and viability with eosin–nigrosin stain. Semen samples extended into BioXcell® were divided into five groups to which 0, 3, 5, 10 and 15 ng/ml of ALA were added, respectively. The treated samples were incubated at 37 °C for 15 min for ALA uptake by sperm cells before being cooled for 2 h at 5 °C. After evaluation, the cooled samples were packed into 0.25 ml straws and frozen in liquid nitrogen for 24 h before thawing and evaluation for semen quality. Evaluation of cooled and frozen-thawed semen showed that the percentages of all the sperm parameters improved with 5 ng/ml ALA supplement. ALA was higher in all treated groups than control groups than control group. In conclusion, 5 ng/ml ALA supplemented into BioXcell® extender improved the cooled and frozen-thawed quality of bull spermatozoa.

**Keyword:** ALA; Cryopreservation; Bull semen; BioXcell®