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Assisted reproductive techniques has been applied to rare, endangered and valuable species with the aim of increasing the population size and genetic management. Artificial insemination (AI) has been purposed as a tool for conservation of endangered Asian elephant (Elephas maximus) in Thailand. The quality of semen after freezing and thawing is an important factor for the success of AI. The objective of this study was to evaluate effect of freezing media on semen quality of post-thaw using fluorescent and electron microscopy. Semen samples were collected from four adult elephant bulls by manual stimulation on the ampulla gland. Semen samples which had progressive motility >60% and concentration >50x10⁶ cell/ml were either frozen in TEST+glycerol or HEPT+DMSO and storage for >7 days. The frozen samples were thawed and evaluated for progressive motility, plasma membrane integrity, acrosome integrity and mitochondria activity. The remaining semen was fixed in 2.5% glutaraldehyde for electron microscopy evaluation. The percentage of progressive motility, (49.0±4.2 vs 32.0±2.7), plasma membrane integrity (49.1±9.2 vs 30.9±3.9), acrosome integrity (53.7±4.9 vs 35.8±6.1) and mitochondria activity (57.0±7.2 vs 42.0±5.0) were significantly better when spermatozoa frozen in TEST+glycerol than (P<0.01) those frozen in HEPT+DMSO. Plasma membrane damaged of post-thaw spermatozoa was observed under SEM. Various types of plasma membrane and acrosome damaged of frozen–thawed spermatozoa were observed under TEM including plasma membrane disruption but acrosome intact, disruption of plasma membrane and acrosome, swell and distention of acrosome and complete loss of plasma membrane and acrosome. The results indicate that the quality of frozen–thawed spermatozoa was better when frozen in TEST+glycerol compare to HEPT+DMSO as evaluated under fluorescent markers and electron microscopy