Semen was collected from 10 three-yellow cocks (Gallus domesticus) by a massage technique. Pooled semen was treated to determine the effect of extender and cryoprotectant (experiment 1) and the effect of freezing protocol and cryoprotectant (experiment 2) on frozen/thawed semen quality. The examined extenders were Beltsville poultry semen extender (BPSE) and modified Tyrode's medium (TALP). The cryoprotectants were 8% dimethyl sulfoxide (DMSO) and 6% dimethyl acetamide (DMA). In experiment 1, semen was diluted and frozen in 4 treatments: BPSE + 8% DMSO, BPSE + 6% DMA, TALP + 8% DMSO and TALP + 6% DMA. The superior result (p<0.05) as followed was obtained by using TALP + 8% DMSO: percentages (mean±SD) of live (57.32±0.40), morphological normal (56.67±1.10), motile (48.50±3.40), progressive motile (10.50±3.40) sperm, and sustenance of sperm motility at 37 °C in an incubator (5% CO₂ in air) for 5 h. In experiment 2, TALP was used as a basis extender. Semen was diluted and frozen in 4 treatments: one-step + 8% DMSO, one-step + 6% DMA, two-step + 8% DMSO and two-step + 6% DMA. The one-step method gave similar results to the two-step method in the case of using 8% DMSO, but not that of 6% DMA, in which the one-step method was superior (p<0.05).

This study indicated that from in vitro analysis, TALP with 8% DMSO was the preferred freezing solution for three-yellow cock sperm and this could be carried out by a one-step freezing protocol.