SUMMARY

Cumulus cells that surrounding the oocyte have important functions in protecting the oocyte, coordinating follicular development, and determining oocyte maturation. Another function of cumulus cells is providing potential donor for somatic nuclear transfer technology (SCNT). Somatic cells nuclear transfer technology facing the problem in terms of suitable donor cells. Fresh cumulus cells have been known as one of the suitable donor cells in giving a higher percentage of *in vitro* embryonic development using SCNT. A long-lasting nuclear donor cell preservation is needed to provide stock. The aim of this study is to find a suitable method for cryopreservation of bovine cumulus cells from primary cell culture.

The research had been conducted according to Complete Randomized Designed with 2 Factors. The first factor was the freezing temperature consisted of -20^{o}C, -80^{o}C, and liquid N_2 (-196^{o}C), the second factor was the cryoprotectant consisted of 10% of Ethylene Glycol (EG) in DPBS contain 20% of New Born Calf Serum (NBCS) and 10% of Dimethyl Sulfoxide (DMSO) in DPBS contain 20% of New Born Calf Serum (NBCS). The observed parameters were cell concentration efficiency, cell viability efficiency, cell attachment efficiency, confluency percentage, and morphology for post-thawing culture. The data were subjected for normality test using the Levene test and homogeneity test using the Kolmogorov-Smirnov test prior to two ways ANOVA analysis.

The two ways ANOVA results showed that there was no significant difference in cell concentration efficiency ($p>0,05$). A significant difference was shown on cell viability efficiency in which -20^{o}C freezing temperature was significantly lower than -196^{o}C ($p<0.05$). There was no significant difference in the cell viability efficiency preserved in the presence of EG nor DMSO ($p>0.05$). The highest viability efficiency was observed at -196^{o}C using DMSO 10% treatment. The cells were attached on Day-2 of post-thawing culture for treatment -80^{o}C and -196^{o}C. There was no cell attachment on post thawing culture of -20^{o}C treatment. Low cell confluency (9% - 18.75%) was shown on the post-thawing culture at day-8 culture. The highest confluency was on cell treated with -80^{o}C using EG 10% treatment. In conclusion, cumulus cell primary culture can be cryopreserved at -80^{o}C and -196^{o}C with DMSO 10% or EG 10% CPs.

Keywords: *cryopreservation, EG, DMSO, cumulus cells, cell culture, SCNT*