SUMPARS KHUNSOOK : SPERM CAPACITATION AND STUDY OF SPERM CHROMOSOME OF SWAMP BUFFALO (BUBALUS BUBALIS LINN.) THESIS ADVISER : PROF. MANEEWAN KAMONPATANA AND ASSO. PROF. CHAN APASATAYA, M.S. 126 pp.

Attempts have been made to assess optimum condition for fertilization rate of sperm from swamp buffalo frozen semen to penetrate hamster free zona pellucida. Optimum heparin concentration was varried in serial dilution of 100, 50, 25, 12.5 and 10 µg/ml accordingly to the variation of incubation time in each concentration for 0, 15, 30, 45 and 60 mins. The findings suggested that heparin concentration of 10, 12.5 and 25 µg/ml for 15-45 mins incubation time inwhich the highest sperm penetration rate were achieved (69.2-73.3%). The procedure of such assessment was illustrated to prepare the sperm before insemination, frozen semen was thawed and dipped into 37 C incubated water, then washed once with a percoll gradient solution (30% and 45% percoll in BWW medium). Sedimented spermatozoa were resuspended in 5 ml BWW (0.5 mM Hypotaurine, 10 mM Cafeine, 0.6% BSA, pH 7.4) and centrifuged at 500 g 10 min. The sperm pellet was diluted with BWW and capacitated in vitro with heparin dosage 10, 25, 50 and 100 µg/ml for 15, 30, 45 and 60 min in 5% CO_2 in air at 38°C. Sperm capacitation and acrosome reaction were tested by using zona-free hamster eggs fertilized with treated sperm in BWW for 2 hr in 5% CO_2 in air at 38°C and subsequent cultured in Ham's F-10 (10% FCS) for 8 hr. The total 6,949 hamster eggs were examined th the pronuclei formation for checking fertilization rate. It was also found that the pattern of dropping culture media due to the space and volume per oocyte was influenced the success penetration rate. It may be a pattern of 5 oocytes per 25 µl of media in six drops of radius angle to allow equally equilibrated space to 5% CO_2 atmosphere was the optimum condition.

After 8 hr of incubation at 38°C in 5% CO_2 in air 0.06 µg/ml podophyllotoxin and vinblastine were added and continue cultured for 12 hr. When-finished incubation the eggs were put into hypotonic solution (1% trisodium citrate) for 3-6 min at room temperature. The eggs were fixed in a cold fixative (ethanol : glacial acetic acid; 3:1) on a slide and stained with 20% Giemsa solution for 10 min. A total of 3,722 hamster eggs were examined the chromosome, only 2 eggs (0.05%) can be seen the swamp buffalo sperm chromosome but could not indentify because the chromosome did not spread.

This preliminary report of swamp buffalo sperm capacitation suggesting further research in finding the procedure of sperm capacitation for in vitro ferlization. The method used to prepare chromosome of buffalo sperm in zona-free hamster egg in this study was poorly success. It might due to the mechanical approach to develop the testing materials was not fine enough and irregularable procedure. Research further may be needed in this area of buffalo biology.