C526996 : MAJOR BIOLOGICAL SCIENCE
KEY WORD: *IN VITRO* MATURATION/ *IN VITRO* FERTILIZATION/ FOLLICULAR OOCYTE/ STORAGE CONDITION/ PIG

SUNPETCH SOPHON : EFFECTS OF PORCINE FOLLICULAR OOCYTE STORAGE CONDITIONS ON MATURATION AND FERTILIZATION *IN VITRO*. THESIS ADVISOR : PROF. MANEEWAN KAMONPATANA AND ASSOC. PROF. DR. VITHAYA YODYINGYUAD, Ph.D. 92 pp.
ISBN 974-634-875-2

Porcine follicular oocytes obtained from ovaries collected from a slaughter house by aspirating the follicle 2-6 mm in diameter. M199B was used as basic medium for holding the oocytes. The oocytes were cultured in IVM medium which was M199B supplemented with follicle stimulating hormone 1 unit/ml, luteinizing hormone 1 unit/ml, oestradiol 1 microgram/ml and pig follicular fluid 10% v/v. Matured oocytes were fertilized, with sperm from fresh ejaculated semen obtained from boars, in IVF medium which was M199B supplemented with 2 mM caffeine . The oocytes were cultured and fertilized at 39^{o}C in 5% CO_2 in air atmosphere. The oocytes were cultured for the period of 12-48 h and checked the maturation of nucleus every 2 h. The result showed that oocytes developed to metaphase II around 32th h and high maturation rate started from 36th h. The ability of semen from boars were tested. Results showed that there were difference among boars on fertilization ability and the development of fertilized oocytes to reach 2-4 cell stage. The ovaries were kept before oocytes collection at 24^{o}C for 0, 8, 12, 18 or 24 h starting from ovaries were arrived in the laboratory. Oocytes maturation were not significantly different ($P > 0.05$) between oocytes collected from 0 h or 8 h storaged ovaries. Five cryoprotectants were tested for toxicity to the oocytes and it was found that glycerol (GLY), dimethylsulfoxide (DMSO), ethyleneglycol (EG), propyleneglycol (PROH) and butanediol (BUOH), at 1.5 M in M199B did not effect oocytes maturation. Effect of low temperature on oocytes maturation was tested by cooling the oocytes from 29^{o}C to 4^{o}C. Percentage of oocytes maturation was significantly decreased ($P < 0.001$) when cooling to 15^{o}C and beyond 15^{o}C to 4^{o}C the oocytes could not developed to metaphase II. Toxicity of 3 vitrification mediums (VTM), VTM 1 consisted of EG 7.5 M in medium 199 with 6% bovine serum albumin (BSA), VTM 2 consisted of EG 6.25 M, GLY 0.7 M sucrose 0.1 M and 20% feotal bovine serum (FBS) in medium 199 and VTM 3 consisted of EG 7.15 M, sucrose 0.1 M and Cytochalacin B (CB) 7.5 microgram/ ml in medium 199, were tested. The equilibrated oocytes in each VTM could mature nonsignificantly different from the unequilibrated oocytes. Cryopreservation oocytes by vitrification with each VTM could not protect the oocytes from damage. Vitrification of matured oocytes with each VTM resulted in unfertilized oocytes with damaged nucleuses after thawing. The photographs by transmission electron-microscope confirmed the damaging of oolemma in both frozen oocytes and matured oocytes.

The imformation and knowledge from this study can be used as a base for further studying. The pig ovaries were easily collected and could be used as a mean for scientists to practise the cryopreservation of oocytes that related to IVM/IVF/IVC research.

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