ULTRASTRUCTURAL CHANGES IN IN-VITRO MATURED BOVINE OOCYTES CRYOPRESERVED IN ETHYLENE GLYCOL BASED SOLUTION BY CONVENTIONAL METHOD

SHAHARA KHATUN

**A THESIS SUBMITTED IN THE PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE (ANATOMY)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
1998**

ISBN NO 974-661-112-7

3937571 SCAN/ M : MAJOR : ANATOMY ; M. Sc. (ANATOMY)
KEY WORD : IN-VITRO MATURATION / CRYOPROTECTANT/ FREEZING
SHAHARA KHATUN : ULTRASTRUCTURAL CHANGES IN IN-VITRO MATURED BOVINE OOCYTES CRYOPRESERVED IN ETHYLENE GLYCOL BASED SOLUTION BY CONVENTIONAL METHOD.THESIS ADVISOR : KANOK PAVASUTHIPAISIT, M.D.,Ph..D., REON SOMANA, M.D.,Ph.D.; YINDEE KITIYANANT, D.V.M., M.Sc., 68_p ISBN 974-661-112-7

In the backdrop of reduced fertilization and subsequent embryonic developmental rates of frozen-thawed oocytes, and an increasing need for safe and effective oocytes cryostorage in the treatment of human infertility and livestock breeding, the present study was undertaken to investigate the structural alteration in in-vitro matured bovine oocytes brought about by cryopreservation with 1.5 M ethylene glycol solution. Abattoir derived bovine oocytes were cultured in TCM 199 supplemented with HTFCS, GnH, and gentamycin. Matured oocytes in the control group were directly processed for transmission electron microscopy. Oocytes in the experimental group were frozen-thawed by the conventional method of freezing and stored in liquid N_2 for 1 day. TEM observation revealed absence of suboolemmal CG, loss of cristae from the majority of mitochondria, loss of microvilli and presence of peripheral large irregular vesicles in all F/T oocytes. An intact oolemma and few normal mitochondria were evident in all the F/T oocytes. It is proposed that the absence of CGs could be due to their centripetal redistribution in the ooplasm. The changes in the position of CGs, disappearance of microvilli and maintenance of oolemmal integrity might be attributed to the solvent action of EG on the cytoskeletal component of the oocytes. It is concluded that IVM bovine oocytes retained their structural viability after slow cooling preservation in 1.5 M EG solution. Further investigations are suggested on the fertilizability and subsequent developmental capacity as well as the cytoskeletal alteration of IVM bovine oocytes after freezing and thawing by the same protocol used in the present study.