O-19 Preparation of Reconstructed Mature Oocytes by Nclear Transfer of Primordial Follicular Oocytes in Bovine

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Objective: For the breeding of specific animal species and infertility treatment of human, metaphase II (MII) oocyte is an essential phase in the oocytes to achieve the zygote by fertilization. MII oocytes can be prepared by controlled ovarian hyperstimulation or in vitro maturation (IVM) of immature oocytes in mammals. In this experiment, we investigated the possibility of the preparation of MII oocytes by nuclear transfer (NT) technique and IVM of reconstructed Germinal Vesicle (GV) stage oocytes in bovine. Also, we compared the rates of reconstruction, GVBD, and MII stage oocytes according to the usage of cytochalasin B (CB).

Materials and Methods: Full grown GV stage oocytes were collected from the antral ovarian follicles, and primordial or primary follicular oocytes were obtained from ovarian cortex using the enzymatic digestion method. GV stage oocytes were enucleated under micromanipulator in PBS supplemented with or without cytochalasin B (5 μg/ml). Nucleus from primordial follicular oocytes was inserted into the perivitelline space of enucleated GV stage oocyte, and fused by electrical stimuli. Fused oocytes were transferred into the medium (10% FBS, 0.2 mM Na-pyruvate, 0.02 IU/ml FSH, 1 μg/ml E_2 in TCM-199) and cultured for 24 hours for maturation. ICSI was performed in reconstructed MII oocytes using frozen-thawed bovine sperm, and fertilized oocytes were cultured for father development.

Results: Success rates of enucleation, reconstruction of GV stage oocytes, meiotic resumption and maturation of reconstructed GV stage oocytes were not different between two groups (Table 1). Reconstructed mature oocytes were fertilized by ICSI and cleaved to two cell embryo.

Tabel 1. Rates of enucleation, reconstruction, meiotic resumption, and maturation in bovine immature oocytes

Experimental group	Enucleated oocytes (%)	No. of Nuclear Injected oocytes (%)	No. of Fused oocytes (%)	No. of GVBD oocytes (%)	No. of matured oocytes (%)
Control group	33/ 49 (67.3)	20 (90.9)	8/22 (40.0)	8/22 (40.0)	2/22 (9.1)
CB treatment group	49/113 (43.4)	22 (66.7)	15/33 (68.2)	15/33 (68.2)	3/33 (9.1)

Conclusion: Nuclear transfer of primordial follicular oocyte into the cytoplasm of fully grown GV stage oocyte may be suggested the noble technique for the specific rare animal breeding and human ART program for perimenopause patients.