EMBRYO DEVELOPMENT OF HANDMADE CLONED KAZAKH ARGALI (OVIS AMMON COLLIUM) EMBRYO USING FROZEN-THAWED FIBROBLAST CELLS

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Introduction: Nuclear transfer has the potential to preserve genes from critically endangered wildlife species where few or no oocytes are available from the endangered species, and where cryopreserved cell line shave been conserved in cryobanks. The purpose of this study was to investigate the developmental ability of embryos reconstructed with transfer of cryopreserved somatic cell from the Kazakh argali to enucleated domestic sheep oocytes.

Methods: Frozen-thawed fibroblasts were diluted with DMEM at a concentration of 2x 106cells mL^{-1}. Fibroblasts were incubated at 5% CO2, 37°C in DMEM + 20% (v/v) fetal bovine serum (FBS). A fibroblast monolaye after 21 to 22 days of incubation were incubated for 7 to 10min in presence of Dulbecco's phosphate buffered saline+ 0.25% trypsin, then were washed with DMEM by centrifugation at 300 g for 10min. The cumulus cells of aspirated oocytes from ovaries were removed by pipetting in 1mgmL-1 hyaluronidase in HEPES-buffered TCM-199; zonae pellucidae were removed by incubation in 2mgmL-1 pronase in HEPES-buffered TCM-199+2% cattle serum (T2) for 1 min. Bisection was performed by hand under a stereomicroscope using a microblade in 5ugmL-1 cytochalasin B in TCM-199+20% cattle serum (T20). Fusions were performed 24-28h after start of maturation with a single DC pulse of 100 V, each pulse for 9 us. One cytoplast was attached to one fibroblast in 500 ug mL-1 phytohemagglutinin dissolved in T2. In the fusion chamber, covered with fusion medium (0.3M mannitol, 0.1mM MgSO4, 0.05 mM CaCl2, and 0.01% PVA). Successfully fused embryos were activated 1 h after the end of fusion by incubation in 2 uM calciumionophore (Sigma) in T20 for 5 min followed by -h incubation in microdrops of culture medium containing 2mM 6-dimethylaminopurine. After successful reconstruction, 79 nuclear transferred and activated embryos were cultured with WOW's in trigas (5% O2, 5% CO2, 90% N2) in Submarine incubation system for 7 days.

Results: All except for 15 embryos cleaved; 35 (44.3%) developed to compacted morula, and 15 (18.9%) of them to the blastocyst stage.

Conclusion: Argali embryos developed from reconstruction using their frozen-thawed fibroblasts combined with domestic sheep's cytoplasts, however, in vitro developmental ability to the blastocyst stage was limited.