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First attempt on somatic cell cryopreservation of critically endangered *Camelus bactrianus* of India

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ABSTRACT

The double-humped camel (*Camelus bactrianus*) of India is critically endangered since the population is around two hundred only. Immediate measures are required for its conservation so as to prevent loss of this precious germplasm forever. This is the first report on development of ear marginal tissue fibroblast cell line from Bactrian camels using primary explant technique followed by cryopreservation. The cells showed typical fusiform morphology with centrally located oval nuclei with radiating, flame like or whirlpool like migrating patterns. Four different culture media MEM, DMEM-Hi glucose, DMEM/Ham's F-12 and fibroblast specific media (HiFibroXL^{TM}) were tested with respect to the potential to support growth of camel fibroblasts. Fibroblast specific media was selected for the primary culture. Maximum cell multiplication during secondary culture was supported by the DMEM/Ham's F-12 as total cell count with same seeding (80,000 cells) and under similar culture conditions was 6,75,000, 3,05,000, 13,90,000 and 10,05,000 cells/ml for MEM, DMEM-Hi glucose, DMEM/Ham's F-12 and fibroblast specific media (HiFibroXL^{TM}), respectively. Cells followed a typical sigmoid growth curve with population doubling time of 26.13 hours. Epithelial and fibroblast cell initially grew together. However, fibroblast cells outgrew their epithelial counterparts in subsequent passages. Reverse transcriptase PCR was performed for individual samples at different passages by designing primers specific to Cytokeratin19 (CK19) and Osteopontin (OPN). Osteocytes were absent from the very beginning whereas, epithelial cells were eliminated after the first passage. Thus, the cells were cryopreserved from 4th to 6th passages. Chromosome karyotyping showed normal diploid (2n=74) number and the cells were free from microbial contaminations. Cells had more than 90% viability in the culture after one month of cryopreservation. These cells were morphologically indistinguishable from the cell stocks prior to freezing. Thus, genetic resource of Bactrian camel has been preserved at cellular level. The repository can also serves as a valuable resource for genome, post genome and somatic cell cloning research.