CRYOPRESERVATION OF SOMATIC CELLS FOR CHROMOSOMAL STUDIES IN CATTLE AND HORSE

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Tissue culture technique is widely used for cytogenetic studies for karyotyping, chromosomal analysis and reproductive biotechnology. Cryobanking of cultured cells has the advantage of preserving them for a long time; even more than hundred years. Therefore, the purpose of this study was to establish a tissue culture method to study chromosomes in cattle, horse and to preserve the cells for future use.

Tissue biopsies (1cm) were obtained from the ear of a cow and a horse. These biopsies were dipped in 70 % ethanol for 20 seconds and washed extensively in Phosphate buffer solution (PBS) and Dulbecco's modified Eagle medium (DMEM). Biopsies were sliced into small pieces and digested with 0.5 ml of 0.5 % collaginase solution at 37 ^{0}C in a CO_2 incubator for 2 -3 hours. Digested material was transferred into tissue culture bottles/ plates containing 6 ml DMEM2 [DMEM, 1 % penicillin and streptomycin and 20 % fetal bovine serum (FBS)]. The cells were fed with fresh medium in every 3rd or 4th day. Once the cells were confluent the cells were lifted by digesting with 2ml trypsin. Two passages were done and cell division was arrested by adding 40 µl colcemid. Cells were lifted and subjected to hypotonic shock with 12 ml 0.075 M KCl at 37 ^{0}C for 20 minutes. Several washing steps were done with a fixative containing methanol and glacial acetic acid (3:1). The cells were suspended in a small amount of fixative, metaphase chromosomal spreads were prepared on slides, stained with Giemsa, observed under 100x magnification to isolate chromosome spreads, photographs were taken and karyogramme was prepared.

Remaining cell cultures were harvested and transferred to cell freezing medium (DMEM, 20 % FBS, 1 % penicillin/ streptomycin and 10 % DMSO) at 4 ^{0}C , filled into 1ml cryo-vials and stored at -196 ^{0}C in liquid nitrogen. Efficacy of preserved cells was tested after one month by thawing the cells and culturing. Results revealed that the cells cryo-banked were growing successfully post thawing. Chromosomal spreads were easily prepared from these cells for cytogenetic studies. This technique can effectively be used to preserve the somatic cells of indigenous or endangered animal species in order to utilize them in future research, cloning, identification of chromosomal defects and genome conservation.

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