IN VITRO CELL CULTURE FROM THE BLACK RHINOCEROS (*DICEROS BICORNIS MINOR*): A NOVEL APPROACH TO GENETIC CONSERVATION

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The Black Rhinoceros (*Diceros bicornis*) is critically endangered primarily due to poaching for horns. While access to animals and success in gamete collection and assisted breeding is limited at this stage, opportunities for gene banking in cases of routine veterinary procedures, fieldwork and animal death are being lost. In this study, we investigated the feasibility of in vitro cell culture as an alternative strategy to genetically preserve the gene pool of this species in light of emerging reproductive technologies such as somatic cell cloning and cross species nuclear transfer. Three aspects were assessed: 1) time of initiation and rate of growth of primary cell cultures, 2) quantitative harvest at early passages (P), and 3) maintenance of health and vigor in vitro. Three 1.5cm^3 skin biopsies were taken from a $6\frac{1}{2}$-year-old male Southern Black Rhinoceros (*Diceros bicornis minor*), divided into epidermis (skin) and dermis (under layer), then mechanically minced. Subsequently, selected groups were enzymatically digested with 200U/ml crude collagenase type 1A (Sigma, Australia) or 0.25% trypsin/1mM EDTA (Life Technologies, Australia) over 4h, 16h or 24h, to produce primary cultures of fibroblast cells. Fibroblasts were grown until confluency whereafter, most cells were cryopreserved for future use in 90% Foetal Bovine Serum (FBS) +10% DMSO at -1°C/min until -80°C and then stored. Remaining cells were seeded at 10^4cells/ml and growth rates (% confluence, % viability, viable cell concentration and total viable cell yield) were monitored for up to 15 passages. Common Wombat (*Vombatus ursinus*) fibroblasts, routinely grown in our laboratory (Wolvekamp MCJ et al., Theriogenology 2000;53:345 abst), were cultured in parallel as control. Data were tested for significance using the appropriate t-Test with Microsoft Excel 97 software. Time of initiation and rate of growth of primary rhino cell cultures varied with tissue type, digestion time and type of digestion enzyme used. No difference in tissue type was observed after 4h collagenase digestion. However, epidermis was more sensitive than dermis after 16h collagenase digestion, having a significantly later initiation and near-significantly slower growth rate. Digestion time had no effect on dermis, but 16h collagenase digestion was harsher than 4h on epidermis having a significantly later initiation. Trypsin was harsher than collagenase after 24h digestion on dermis having significantly slower growth rate and later initiation. Early passage rhino cells (P0 and P1) had an average (±SEM) of 89.1±1.5% confluence, 94.1±0.4% viability, 103.9±4.2x10^4 viable cells/ml and 2.1±0.1x10^6 total viable cell yield. Rhino fibroblasts maintained their % confluence and % viability for the whole 15 passages up to the termination of the experiment. However, viable cell concentration and total viable cell yield decreased significantly from P1 (142.0±46.0x10^4cells/ml and 2.8±0.9x10^6cells resp.) to P3 (22.8±7.4x10^4cells/ml and 0.5±0.2x10^6cells resp.), and never recovered, remaining only 10-20 fold higher than seeded amounts. In summary, digestion using collagenase (using short times for epidermis) is the best method to establish rhinoceros primary cell cultures. Also, rapidly increasing fibroblast cell numbers should be cryopreserved at early passage number before they significantly decline. These results demonstrate a successful protocol in the black rhinoceros for the establishment of primary fibroblast cell cultures that may be applicable to other rhinoceros species.