



217 Cloning, Transgenesis and Stem Cells

Reversibility of cell cycle inhibition induced by extracts of *azadirachta indica* in bovine fibroblasts**N.C. Rabelo¹, C.C. Quintão¹, S.G. Brito¹, N.B.R. Raposo², E.D. Souza¹, J.H.M. Viana¹, L.S.A. Camargo¹**¹Embrapa Gado de Leite, Juiz de Fora, MG, Brasil; ²Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brasil.**Keywords:** cellular inhibition, gene reprogramming, SCNT.

Among the numerous challenges found in the technique of SCNT, gene reprogramming is a critical point of the process, because the nucleus must assume a gene expression pattern of a newly fertilized embryo. The use of nuclei donor cells stationed in G0/G1 stages of cell cycle is important to a correct reprogramming. Extracts of the *Azadirachta indica* (Neem) have a potential to inhibit the cell cycle of bovine fibroblasts; however, the reversibility of such inhibition is needed to resume the mitosis of zygotes reconstructed by SCNT. The objective of this study was to evaluate the reversibility of the inhibition effect caused by the extracts of this plant. Bovine fibroblast were cultured and exposed to concentrations of the extracts that have high inhibitory potential, previously evaluated (Rabelo et al., 2011. Acta Sci. Vet. 39[Supl.]:S338), as follow: 50µg/mL, 100µg/mL and 200µg/mL for 24h, for ethanol extract, and 50µg/mL, 100µg/mL and 200µg/mL for 12 h and 50µg/mL, 100µg/mL for 24 h, for hexane extract. These repetitions were performed in triplicate for each treatment. Simultaneously, a serum-starvation group was used as a control (absence of extract, cells cultured with 0,5% serum for three days). The reversibility of inhibition of the cell cycle was evaluated by Flow Cytometry (Facs Callibur, Becton Dickinson, San Jose, CA, USA) through determining the percentage of cells in each stage of the cycle, 0, 12, 24 and 36 h after removal of the extracts. Resumption of the cell cycle was considered when the proportion of cells stationed at G0/G1 stage was reduced after removing the extracts. The histograms were evaluated using the WinMDI software to determine the percentage of cells in each stage of the cell cycle (G0/G1, S and G2). Statistical analysis was performed by analysis of variance, and the averages were compared by Student Newman Keuls test. P<0.05 values were considered significant. There was a reduction in the proportion of cells in G0/G1 stage 12h after removing them from ethanol extract at a concentration of 100µg/mL, showing resumption of the cell cycle. The serum-starvation also showed a decrease in the proportion of cells in G0/G1, however, this happened 24 h after removing this condition. The other treatments, including those with hexane extract, did not affect the percentage of cells in G0/G1 at 0, 12, 24 and 36 h after removing the extracts. In conclusion, the inhibition of cell cycle with 100µg/mL ethanolic extract of Neem can be reversed at 12 h after removing the extract, which is faster than serum-starvation, and, therefore, it is the concentration indicated among those tested for bovine fibroblasts.