Table 1. Pregnancy results established from embryos of different origins

Source of embryos	No. of transferred embryos	No. of recipients	Re-oestrus after 25 d	Re-oestrus after 45 d	Re-oestrus after 65 d	Re-oestrus after 90 d
−196°C cells	45	4	2	1	1	0
Fresh digestion cells	81	8	5	2	1	0
TSA treatment cells	88	9	4	3	1	1*
TSA treatment embryos 0–60 h	138	11	5	3	1	2*
TSA treatment cells and embryos 24–60 h	104	8	3	2	1	1*,1▲

Note: 0: mean of pregnant cow 90 days, all re-oestrus. 1*: mean of pregnant cow in pregnancy from 65 to 90 days re-oestrus, abortion. 1▲: mean of pregnant cow 90 days without re-oestrus, but give birth to offspring.

(17) CELL CYCLE SYNCHRONIZATION OF BOVINE FIBROBLASTS BY *AZADIRACHTA INDICA* EXTRACTS

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One requirement for somatic cell nuclear transfer (NT) is the coordination between donor cell cycle and recipient cytoplasm. As an alternative to commercially available substances to synchronize the cell cycle in G0/G1, we tested 2 extracts, aqueous and hexane obtained from the plant *Azadirachta indica* A. Juss (popularly called Neem). Extracts from this plant have shown antiviral, antibacterial and anticancer activities, widely described in the literature (Kumar *et al.* 2009 *Invest. New Drugs* **27**, 246–252). The hexane extract was prepared in the Soxhlet apparatus until total collapse and then submitted to rotary evaporation. The aqueous extract was prepared by dynamic maceration and was subsequently lyophilized. Bovine fibroblasts collected from Gyr cows were cultured in DMEM (Sigma) supplemented with 10% fetal cow serum (FCS) and incubated at 37°C, 5% CO_2 and 95% humidity. After obtaining 70% of cell confluence, the extract was added to cells at the following concentrations: 0 $\mu g\,mL^{-1}$ (negative control), 50 $\mu g\,mL^{-1}$, 100 $\mu g\,mL^{-1}$, 200 $\mu g\,mL^{-1}$ and 300 $\mu g\,mL^{-1}$, for 12 and 24 h. Simultaneously, a group with serum starvation (positive control; cells cultured in DMEM plus 0.5% FBS for 3 days) was prepared. Three repetitions were performed in triplicate for each concentration and control groups. Cell cycle readings were performed by flow cytometry (Facs Callibur, Becton Dickinson, San José, CA, USA) and DNA histograms were analysed by WinMDI software to determine the percentage of cells in G0/G1 phase, S and G2 cell cycle, so that 10 000 cells were analysed in each reading on a flow cytometer. Data were analysed by analysis of variance and means were compared by Student-Newman-Keuls test. The percentages of cells at G0/G1 phase for aqueous extracts were lower ($P < 0.05$), regardless of the concentration and exposure time, than the 0 $\mu g\,mL^{-1}$ (83.73 ± 1.14%) and serum starvation (86.64 ± 1.44%). In contrast, the percentages of cells synchronized at G0/G1 with 50 $\mu g\,mL^{-1}$ for 12 h (84.23 ± 0.56%), 50 $\mu g\,mL^{-1}$ for 24 h (85.66 ± 0.57%), 100 $\mu g\,mL^{-1}$ for 12 h (87.85 ± 0.51%) and 200 $\mu g\,mL^{-1}$ for 12 h (85.87 ± 0.45%) using hexane extracts were higher ($P < 0.05$) than with 0 $\mu g\,mL^{-1}$ (81.44 ± 0.29%), but lower ($P < 0.05$) than the serum starvation (91.33 ± 0.31%). In conclusion, hexane extracts from the plant *Azadirachta indica* A. Juss can synchronize mammalian cell cycle at G0/G1 despite the low proportion when compared with serum starvation. Studies to evaluate efficiency of cell cycle resuming and viability after somatic cell nuclear transfer are ongoing.

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18 TOTAL PARENTERAL NUTRITION DURING NEONATAL CARE OF THE FIRST BITRANSGENIC FEMALE BOVINE CLONE

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This work describes the treatment with total parenteral nutrition (TPN) of a 37-day cloned calf after suffering ruminal acidosis by ingestion of milk. Cells for SCNT were obtained by using a bicistronic vector for human lysozyme and lactoferrin. We obtained 7 embryos and 2 pregnancies. Only one fetus was born alive weighing 47 kg and it was presented to the neonatal unit of INTA showing a deep depression, diarrhoea, dehydration (10%),