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ABSTRACT 193

HANDMADE CLONING IN GOATS

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Animal cloning technology has been the focus of interest by many research groups around the world. Despite the effort, cloning continues to be inefficient, becoming even less effective when applied to other species rather than cattle. More recently, alternative cloning methods, such as the handmade cloning (HMC), have simplified the process, also decreasing the time and cost for training of personnel and for embryo production *per se*. The aim of this study was to adapt the handmade cloning procedure to the goat, based on our procedures in cattle (Ribeiro et al., 2009, Cloning Stem Cells, 11:377-386), for the production of transgenic cloned embryos.Goat cumulus-oocyte complexes (COCs) from slaughterhouse ovaries were *in vitro*-matured in holding medium (HM: TCM199, 0.022 µg/mL sodium pyruvate, 10,000 IU penicillin, 10,000 ìg/mL streptomycin sulfate,25 ìg/mL amphotericin B) supplemented with10 µg/mL EGF, 5 µg/mL FSH, 10 µg/mL LH, 1 µg/mL 17â-estradiol and 100 µM cysteamine,for 20 h at 38.5°C, 5% CO_2 and high humidity. Subsequent to cumulus cells removal and polar body (PB) selection, matured oocytes were briefly exposed to 0.25% protease for zona pellucida removal, followed by a rinse in pure fetal calf serum (FCS) and multiple washes in HM + 10% FCS. Zona-free oocytes were hand-bisected in 2.5 µg/mL cytochalasin B and screened under UV light for selection of enucleated hemi-cytoplasts. Then, two hemi-cytoplasts briefly exposed to phytohemoagglutinin were adhered to a single fibroblast cell from primary culture cells between the 2nd and 4th passage and at high confluence (>95%), established from a skin biopsy collected from an hG-CSF transgenic goat. Reconstructed structures were electrofused by two 1.1 kV/cm DC pulses for 5 ìs (~26 h post-IVM), after a brief exposure to a 7.0 V pre-fusion AC pulse. Fused structures were activated in ionomycin/6-DMAP followed by *in vitro* culture in the WOW system in SOFaa + 2% FCS + 0.3% BSA, at 38.5°C, 5% CO_2, 5% O_2 and 90% N_2, for seven days. The maturation rate attained after 3 replications, based on PB selection, was 84.6% (549/649), with the fusion rate for reconstructed structures reaching 83.2% (139/167). Cleavage rate was 85.4% (105/123), with 17.1% of embryos developing to a transferable stage (8 compact morulae and 13 blastocysts/105) on Day 7 of development. Such preliminary results are similar to those reported by others using standard cloning procedures. In conclusion, the HMC procedure appears to be an effective alternative for the production of transgenic cloned embryos in goats. Further studies are underway for the production of live born offspring.

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EFFECT OF MITOMYCIN C ON VIABILITY AND CELL CYCLE OF BOVINE ADULT FIBROBLASTS AFTER DIFFERENT EXPOSURE TIMES

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Currently, the derivation of embryonic bovine cells (CTE-like) is a great challenge, because the culture protocols used are very divergent and inefficient. One way to improve this methodology would be to use species-specific feeder cells. Mitomycin C (MMC) is an antiproliferative agent, which has inhibitory action in the S e G2/M of cell cycle but may cause cytotoxicity. The effect of MMC on bovine fibroblasts derived from adult animals (FBA) is still little known. Thus, the aim of this study was to establish a protocol for mitotic inactivation of FBA, with intention to apply it in the derivation CTE-like. Adult bovine fibroblasts was cultured in DMEM medium supplementedwith 10% fetal calf serumand incubatedat 38,5°C, 5% CO2 and 95% of humidity. After reaching 60% of confluence, the cells were treated with MMC (10 µg/ml) at different exposure times: 0h (control), 2h, 3h, 4h e 5h. Subsequently, the fibroblasts were analyzed for flow cytometry for evaluation of the cell cycle phases (G1/G0, S, G2/M) and cell viability. For cell cycle analysis, fibroblasts were fixed (ethanol 70%) at 4 ° C for 2.5 h. After the cells were treated to RNAse A (100 ìg/mL) for 10 min, and they were g/mL) 5 min before reading. Cellmstained with propidium iodide (50 viability was evaluated in non-fixed cells stained with PI (50 ìg/mL) for 30 min at 37°C. Three repetitions with three replicate each one were performed. Statistical analysis was performed by ANOVA and means compared by Student Newman Keus.In the results presented in the cell cycle it can be observed an increase of the peaks of the phases S and G2/M and a decrease of G1 in all exposure times to MMC compared to control. No had difference (p>0,05) in cell proportion in S phase between exposure times 2 (30.64 ± 3.05%), 3 (30.85 ± 4.31%), 4 (29.79 ± 4.81%) h. The proportion of cells in G1/G0 phase in the 2 (42.42 ± 3.29%), 3 (44.82 ± 4.24%) h did not differ (p>0,05), and 3h was similar (p>0,05) to 4h (47.38 ± 5.39%). The time 5h had higher (p<0,05) proportion of cells in G1/G0 (51.342 ± 3.67%). However, o time 2h had higher amounts of cells concentrated in S and G2 (22,90±2,59%)compared with other exposure times, while the time 5h showed lower (p<0,05) proportion of cells in S (22.576 ± 3.21%).A lower ratio (p<0,05)of viable cells was observed in the times 2 (89,67±3,78%), 3 (89,87±2,07%), 4 (88,28±3,82%), 5 (87,122±2,52%) h compared ofcontrol (93,592±2,12%), but despite that there was no difference (p>0,05) between treatments. Through the results, it can be concluded that MMC inhibits the proliferation of fibroblasts and the time 2 h of exposure was most effective because it keep cells in the S and G2/M, but the MMC decreases cell viability in this concentration.