Isolation, culture and cryopreservation of bovine amniotic cells with Dimethyl sulfoxide, Dimethylformamide or Glycerol

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Introduction

Adult somatic cells can undergo nuclear reprogramming after fusion with a mature oocyte, a process known as somatic cell nuclear transfer or cloning. An important requisite is the availability of cells able of undergoing reprogramming to support embryonic and fetal development after nuclear transfer (1). In this context, the bovine amniotic cells, which are relatively less differentiated, have a potential for use in nuclear transfer. Thus, this study seeking alternatives to improve the efficiency of bovine nuclear transfer aimed to evaluate the possibility of isolation and storage of amniotic cells, comparing the efficiency of three cryoprotectant solutions.

Materials and Methods

Amniotic liquid was collected from four bovine placentae ranging from 70 to 90 days of gestation. The amniotic sac was exposed and the collection of 15 mL was done with needle and syringe to isolate amniotic cells. The amniotic liquid was centrifuged for 5 minutes at 200g. Then, the cellular sediment was diluted in Amniomax medium and cultured in incubator at 5% CO_2 and 38,5°C until the cells reached confluency. Next, cells were resuspended and distributed in 3 cryopreservation solutions containing 10% Dimethyl sulfoxide (DMSO), 5% Dimethylformamide (DMF) or 7% Glycerol. Cells (2 x 10^6 cells/mL) were loaded into 0,25 mL straws and were frozen at -80°C for 24 hours and then stored in liquid nitrogen. The straws were thawed at 37°C/30s. The viability rate of cells was evaluated with *trypan blue* stain (TB). The thawed cells were re-cultivated in Amniomax medium and time to confluence was to analyzed.

Results and discussion

The cryoprotector medium with DMSO preserved 84.50 ± 9.53% of amniotic cells with intact membrane, which was superior to cryoprotector medium containing DMF, that preserved 42,00 ± 13,92% of the same cellular type (Fig 1). The cryoprotector medium supplemented with Glycerol preserved 63.00 ± 18.18% of cells and did not differ from the others treatments (P > 0.05; Fig. 1).

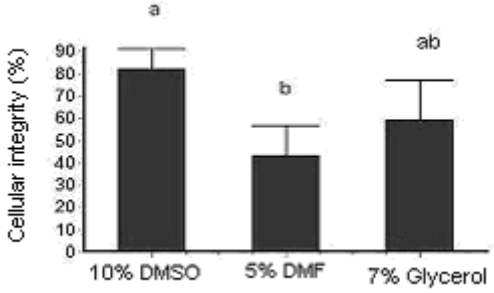

Figure 1. Integrity of the cryopreserved amniotic cells in protection solutions containing DMSO, DMF or Glycerol diluted in Amniomax medium.

After the celular re-culture, only cells frozen previously on DMSO cryprotection reached confluence in 6 days. This study demonstrated to be possibility to isolate and cryopreserve bovine amniotic cells for use in the nuclear transfer technique. The cryoprotection medium most appropriate was DMSO.

References

(1) Mastromonaco GF et al. 2006. BMC Dev Biol, 6:1-13.

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