Cryopreservation of stem cells derived from equine bone marrow

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Abstract

The formation of cell banks is essential to optimize the use of stem cells. Thus, the development of efficient cryopreservation techniques are important to allow cellular storage for a prolonged period., Despite being a good cryoprotector, DMSO is toxic and therefore it has some limitations for clinical use. Thus, several techniques have been studied in order to minimize the damage caused by the use of DMSO in cryopreservation, among them is the addition of FBS and disaccharides such as trehalose and sucrose to the culture media in order to decrease the concentration of DMSO. Disaccharides interact with membranes, liposomes and protein, stabilizing these biomaterials during the process of freezing and thawing, while the beneficial effect of the FBS occurs through its interaction with cellular macromolecules during the cryopreservation process, stabilizing and protecting cell membranes. The objectives of this study were to compare the use of traditional freezing media, based on low glicose DMEM/F12 media (1:1) suplemented with DMSO as cryoprotector, with the use of fetal bovine serum (FBS) based media for the cryopreservation of equine mesenchymal stem cells (MSCs). Moreover, the benefit of using trehalose as an additive to the cryopreservation medium was studied. The MSCs culture (n= 3 animals) was performed at the Laboratory of Cell Therapy, Department of Veterinary Surgery and Anesthesiology at FMVZ – UNESP, in Botucatu. After bone marrow aspiration and cell culture, five freezing media were tested, and cellular growth and viability was analyzed. The media used were: media 1 (control) – DMEM low glicose/F12 1:1 + 20% FBS, and 10% of the cryoprotector DMSO; media 2 (trehalose) – DMEM low glicose/F12 1:1 + 20% FBS, 0.5 M trehalose and 5% DMSO; media 3 (FBS) – 90% FBS and 10% DMSO; media 4 (FBS + trehalose) – 90% FBS + 0.5 M trehalose and 5% DMSO; and media 5 – commercial media Bambanker®. For the freezing curve, the samples were placed in properly identified 1 ml ampoules and taken to the -80 º C freezer for a period of 60 days. The cellular viability test was performed before cryopreservation with staining with trypan blue, used to estimate the integrity of the cell membrane; and after cryopreservation with staining with annexin V, used to evaluate the process of programmed cell death (apoptosis). In the pre-freezing analysis with trypan blue, the samples presented 84.48% of viability. After thawing, the analysis was performed by flow cytometry with Annexin V. The media 1 showed 84.2% of viability;

the media 2 71.2%; the media 3 87.4%; the media 4 57.7%; and the media 5 84.9%. Our results showed that the most effective media for cryopreservation was FBS + DMSO. However, commercial media such as Bambanker® and the control media + Trehalose presented similar maintenance of viability. It was possible to conclude that both FBS and trehalose had a significant role in the maintenance of cell viability after thawing.