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Conferences papers

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32nd Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

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A248 Cloning, Transgenesis and Stem Cells

Viability of dog stem cells maintained at room temperature for 50 hours

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The use of mesenchymal stem cells (MSC) in the treatment of diseases has shown significant growth in veterinary medicine, since it is a therapy that can be used to accelerate wound healing, decrease inflammation and modulate the immune system, due to the release of cytokines and growth factors. MSC can be obtained from several adult tissues, however, the most commonly used source has been adipose tissue. Due to the emergency nature of some situations, as well as the need to send MSC to several Brazilian regions, an option for the immediate use of the cells in therapy emerged: the creation of cryopreservation banks of allogeneic cells. In this context, the objective of this study was to evaluate the period of time in which it is possible to maintain viable MSC at room temperature stored in a specific transport medium produced by BioCell® commercial laboratory. For this, MSC obtained from the adipose tissue of four dogs was used, each animal being considered a biological replicate. The cell line used was previously tested, by means of immunophenotyping, on the markers already described in the literature that guarantee to be a lineage of MSC. After culturing and confluence, the cells were cryopreserved and maintained in N2 until the time of the evaluations. Initially, cryopreserved samples were thawed and adjusted to $1x10^6$ / mL, protected from light and stored at room temperature in insulin syringes in the total volume of 0.5 mL of BioCell® transport medium. Samples were stained by the Alexa Fluor® 488 Annexin V / Dead Cell Apoptosis Kit (Molecular Probes) and, after 15 min incubation, the samples were evaluated in FlowSight® image flow cytometry (AMNIS, Seattle, WA). The evaluations were started 2 h after thawing and performed every 2 h for a period of 36 h, with a final evaluation at 50 h after thawing. Approximately 30,000 cells were acquired per sample and the results were analyzed using IDEAS V6.0 analysis software (AMNIS). The results were analyzed by GraphPad (Prism 6) through the analysis of variance test and the means compared by the Tukey test. It was possible to observe that, only after 30 hours in storage at room temperature, the quality of the cells changed to that observed in 2 hours of storage. To the moment of 28 hours the average of viable cells was of 78.5 ± 9.2. Based on the results, it was possible to conclude that when kept in specific transport medium at a concentration of $1x10^6$ / mL, MSC obtained from dogs can be maintained for up to 30 hours at room temperature without compromising viability.