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Lyophilization as an alternative method of human amniotic membrane preservation for ophthalmic use. Ana Beatriz Toledo Dias, Maria Cristina Martins, Acácio Alves de Souza Lima Filho, Alexandre Nakao Odashiro, Andréia CS Lourenço, José Álvaro Pereira Gomes, Miguel NN Burnier Jr and Brazilian Ocular Pharmacology and Pharmaceutical Technology Research Group
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Purpose: to compare 2 different ways to preserve human amniotic membranes (HAM), freeze-drying (FD) and fresh-freezing (FF), evaluating histological results and immunohistochemical (IH) expression of vimentin (VIM), cytokeratin 8 (CK8) and 18 (CK18) in distinct periods of time.

Methods: a total of 64 samples of HAM, obtained from 4 immediate post-partum women, were evaluated. For each amniotic membrane, 8 samples were freeze-dried and 8 were fresh-frozen. The samples submitted to lyophilization process were previously freeze-dried under a mixture of dry ice and isopropyl alcohol and then, they were dehydrated in a Boc® equipment. The time of lyophilization cycle was 24 hours. The control-group was submitted to the conventional freezing method. After 1, 7, 30 and 90 days, 2 samples from each group (I - freeze-dried and II - fresh-frozen) were analyzed by Hematoxilin & Eosin (HE) and Periodic Acid-Schiff (PAS) staining. IH expression of vimentin in the conective tissue, cytokeratin 8 and 18 in the epithelium were also performed. The samples were classified semi-quantitatively according to degree of epithelial vacuolization, autolysis, integrity of basement membrane, and the expres-sion of VIM, CK8 and CK18. To verify possible differences between distinct periods of time in each group for each defined known variable the Friedman´s test was used (or two-way analysis on ranks test), added when necessary with multiple compare-son´s test. And to verify possible differences between the 2 groups (I and II) in each defined known variable in each period of time a non-parametric test for two inde-pendent samples was used (Mann-Whitney´s test).

Results: a statistically signifi-cant difference was found in 2 situations: samples of group I for VE between 1 and 7, 30 and 90 days with better results for day 1 and for AUT in the samples of group II with better results for days 1 and 7, by Friedman´s test and multiple comparison´s test. Using Mann-Whitney´s test, the following analysis was found to be statistically significant: for the variable VE on the days 1 and 90, AUT on day 90, IMB on the days 7 and 30, for VIM on day 30, for CK8 at 1 and 90 days and for CK18 on the days 30 and 90. In these cases, the samples of group I were better preserved than the samples of group II. As a suggestion of significancy it was found AUT on day 30 and VIM on day 90.

Conclusions: freeze-drying and fresh-freezing are effective methods for HAM preservation. Upon statistical analysis of the specimens, FD proved to have a better preservation of its structure than FF.