

Poster: 20

Histological Analysis of Amnion Stored in Glycerol Sterilized with Different Doses of Ionizing Radiation

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Key words

Histology, amnion, sterilization

Objective and introduction

The radiosterilization is an alternative for ensuring quality and safety of tissues used in transplants, and other clinical applications in order to minimize the risk of contamination of the receptor tissue. Therefore, the objective of this study was to test various doses of radiation in amniotic membrane (AM) stored in glycerol using two sources of ionizing radiation: the cobalt-60 irradiator (Gamma) and Electron Beam Accelerator (E.B.).

Method

The samples of amniotic membrane were processed and stored in glycerol concentration greater than 85% for at least 30 days in the Tissue Bank. Membranes were extended on sterile filter paper, with the epithelial side up (smooth and brighter), under aseptic conditions in a laminar flow cabinet. After extended in support of filter paper, the tracks were packed in sterile transparent, sealed and stored in a refrigerator at a temperature 2-8 ° C until the time of irradiation. Each of the five membranes were divided into non-irradiated sample (control) and irradiated with cobalt-60 source and electron beam at doses of 10,15, 25 and 35 kGy.

The histological analysis was performed using sections of amniotic membrane dimension 1 x 1 cm were rehydrated for at least 30 minutes and packed in flat-bottomed container with a solution of 10% buffered formalin for 24 hours, then in 70% alcohol solution. Histologic paraffin embedded in the membranes were cut with a microtome cross-sections of 5 µm in thickness. Staining was performed in the cross-sections by hematoxylin-eosin (HE) on glass slides in order to observe the changes of the layers of the amniotic membranes.

Results and discussion

The samples irradiated in Gamma dose of 15 kGy present fragmentation of the epithelial layer similar to the dose of 10 kGy to the same source, with apparent preservation of the remaining layers of the amnion. But the samples irradiated in E.B., with a dose of 15 kGy, besides presenting fragmentation epithelium, become noticeable condensation of the remaining layers of the amnion. In samples subjected to a dose of 25 kGy Gamma epithelial cells are scarce, and the other layers are shown merged together. In samples irradiated with 25 kGy, in E.B., it is observed that the epithelial layer is present with relative cell number, but slightly misshapen, and apparent condensation of the other layers. In the latter case, probably condensation of all layers to prevent fragmentation of the epithelial layer. In the sample irradiated up to a dose of 35 kGy Gamma was observed condensation of the layers, including the presence of epithelium. The same applies to the samples irradiated in E.B., which are more evident in the changes mentioned above.

Therefore, in the optical microscopy, it was observed that the degree of condensation of the underlying layers of AM is directly related with increase of radiation's dose.

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