

## **The Effect of Vitrification on Bovine Ear and Muscle Tissues Extirpated After Death and Maintained at Different Time Periods.**

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Cryopreservation of livestock ear and skin tissue can be applied in cloning, transplantation and endangered biodiversity conservation ex situ. In this study we investigated the effect of stored postmortem cartilage and muscle tissues at +4 °C for 5, 24, 48, 72, 96 and 216 hours on vitrification and post thawed vitrified and fresh tissues preparation of primary tissue on culture obtained somatic cells. Postmortem tissues were maintained in PBS+1% PSA. Vitrification procedure were performed by exposing 1mm<sup>3</sup> tissues to 3,58M ethyleneglycol, 2,82M DMSO in PBS supplemented with FCS%20. Then tissues were plunged into LN<sub>2</sub> after transferring to 0,5 mm payets. Warming and dilution of tissues from cryoprotectants was performed by washing 0,5M and 0,25 M Sucrose for 5 min respectively. Vitrified and fresh tissues were seeded in a 35mm petri dishes containing DMEM/F12 supplemented with 20%(v/v) FCS and incubated 5% CO<sub>2</sub> in air at 95% relative humidity and at 37 °C. The culture was started to changed after 7 days and changed every 2 days for a 25 days maximum. After obtained the cells from vitrified and fresh tissues; H-E staining, population doubling time and MTT analyzes were done. As a result; we obtained the healthy cell population from all vitrified tissues but the cartilage cells were more quickly grown than the fibroblast cells which obtained from muscle tissues. After warming of the tissues; the mitotic activity of derived cells were decreased than the cells from fresh tissues. However, there is no significant differences between the all experimental groups. The producing cells established until 216 hours from fresh cartilage tissues that stored at +4 °C. The similar results were found after warming the same tissue samples. According to these results; it is possible to obtain cell lines from the vitrified and fresh tissues until at +4 °C and 216 hours. This study was supported by a grant from TUBITAK KAMAG, Turkey (106G005). Correspondence; S. ARAT, [sezen.arat@mam.gov.tr](mailto:sezen.arat@mam.gov.tr)