

EFFECTS OF MECHANICAL INJURY ON RE-VASCULARIZATION AND FOLLICLE SURVIVAL IN FROZEN/THAWED HUMAN OVARIAN CORTEX TRANSPLANTED TO IMMUNODEFICIENT MICE

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Abstract Body

The detrimental loss of follicles following transplantation of cryopreserved human ovarian tissue is hampering the procedure, and faster revascularization has been investigated as treatment. This study investigates whether needle puncturing prior to transplantation can improve revascularization and thus follicle survival in human ovarian tissue.

36 cryopreserved human ovarian cortex pieces were included in this study. 20 patients were included, where 12 were included in the long-term setup and 8 patients were included in the short term. Each piece from one patient was divided into two, where one half functioned as the untreated control and the other as the needle group: 150-200 needle punctures through the tissue piece with a needle. The tissue was transplanted to immunodeficient mice for 3, 6 and 10 days and for 4 weeks. Vascularization and apoptosis were analysed in tissue transplanted for 3, 6 and 10 days by qPCR (Vegf, Ang1, Ang2, Angptl4 and BAX/BCL2 ratio) and murine CD31 immunohistochemical analysis, whereas follicle density and morphology were determined after 4 weeks xenotransplantation.

CD31 area increased from 0.02-0.05% on day 3 to 2.8-3% on day 10, but there was no difference between needle group compared to control ($p > 0.1$). Vegf gene expression had a tendency toward being increased in the needle group compared to control on day 6 ($p = 0.0501$). Expression of Ang1 and Ang2 increased from day 3 to day 10 in the control group, however in the needle group this increase was not observed on day 10 and the difference was significant for Ang2 ($p = 0.02708$). The expression of murine Angptl4 was similar on day 3 and 6, but there was a decrease from day 6 to day 10 in both control and needle group and the decrease was significant for the needle group ($p = 0.0430$). The ratio of human BAX/BCL2 was similar at all time points and there was no difference between the treatment and control groups. Follicle density (follicles/mm³, mean \pm SEM) were higher in the needle group (5.18 ± 2.24) compared to control (2.36 ± 0.67) after 4-week xenografting, where 7 out of 12 patients had higher density compared to control. There was a lower percentage of necrotic follicles in the needle group (18.8%) compared to control (36%) ($p = 0.045$).

Mechanical injury in the form of needle puncturing had did not increase revascularization of human ovarian tissue. However, needle treatment improved follicle morphology and a slight increase in follicle density was observed.