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Two-step transplantation with adipose tissue-derived stem cells increases follicle survival by enhancing vascularization in xenografted frozen-thawed human ovarian tissue

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Context
Ovarian tissue transplantation for fertility preservation has been gaining popularity over the past decade thanks to increasingly successful outcomes. However, follicle loss in the early post-grafting period limits the life span of grafts. Improving the grafting procedure has been the main focus of research aiming to increase follicle survival rates.
Objective
To evaluate the short-term effects of a novel two-step ovarian tissue transplantation approach using adipose tissue-derived stem cells (ASCs) to enhance graft vascularization and ultimately increase follicle survival rates.
Methods
A total of 15 severe combined immunodeficient mice were intraperitoneally grafted with frozen-thawed human ovarian tissue. Levels of partial pressure of oxygen (pO2) were monitored in vivo by electron paramagnetic resonance (EPR) oximetry on post-grafting days 3 (n=15) and 7 (n=15). Samples for histology and immunohistochemistry were collected after euthanizing the mice on day 7. One piece of ovarian tissue per patient served as non-grafted controls.
Patients
Ovarian tissue from 5 patients (aged <35).
Interventions
A peritoneal grafting site had been previously prepared in a first step using either empty fibrin (Fi+OT group [n=5]) or ASC-loaded fibrin (Fi/ASCs+OT group [n=5]) for 14 days prior to ovarian tissue grafting. Five mice were grafted using the standard one-step transplantation procedure and served as controls (OT group).
Main outcome measures
Graft pO2 levels measured by EPR oximetry on post-grafting days 3 and 7. On day 7: follicle density (hematoxylin and eosin); graft vascularization (double anti-human/mouse CD34); follicle growth (anti-human Ki67); follicle apoptosis (TUNEL assay); and fibrosis (Masson's trichrome).
Results
Significantly higher pO2 levels were observed in the Fi/ASCs+OT group compared to the OT group (p=0.028). Total CD34-positive vessel area was significantly greater in the Fi/ASCs+OT group (p<0.05). Significantly increased follicle survival rates and significantly fewer apoptotic follicles were found in the Fi/ASCs+OT group (p<0.05). Significantly higher numbers of Ki67-positive primordial follicles were

encountered in all grafted groups ($p<0.01$). No difference was observed in fibrosis.

Conclusion

Our two-step transplantation approach using ASCs evidenced higher rates of oxygenation and vascularization in grafted ovarian tissue in the early post-grafting period, leading to decreased apoptosis and increased follicle survival rates.

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