

## Exploring The Effect Of PRP On Tissue Vascularization And Survival Of Follicles In Human Ovarian Tissue Transplanted To Immunodeficient Mice.

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Ovarian tissue cryopreservation (OTC) is a method to preserve fertility in females undergoing gonadotoxic treatment. Despite successful outcomes in restoring endocrine function and achieving pregnancies following ovarian tissue transplantation, a substantial loss of follicles following transplantation reduces the clinical efficacy of the procedure. This study explores the potential of platelet-rich plasma (PRP), specifically human platelet lysate (hPL) and umbilical cord plasma (UCP), to enhance vascularization and follicular survival in human ovarian tissue transplanted to immunodeficient mice. After a grafting period of 3- and 6 days, vascularization was assessed using CD-31 quantification and gene expression of angiogenic markers (*VEGF/Vegf*) together with apoptosis-related genes (*BAX/BCL-2*), oxidative stress markers (*HMOX/Hmox*) and the pro-inflammatory markers (*IL-1 $\beta$ /IL-6/Tnf- $\alpha$* ) were quantitatively analysed. Follicle density was analysed in grafts after 4 weeks of transplantation. Results indicate that while there was a significant increase in the CD-31 area from day 3 post grafting to day 6, there were no significant differences between the grafts from mice treated with hPL and the control group at day 3 nor day 6, suggesting no added benefit of hPL in enhancing vascularization. Gene expression analysis revealed significant downregulation of *VEGF* and *BAX/BCL-2* from day 3 to day 6 for both hPL and control group and significant upregulation of *BAX/BCL-2* in the hPL group compared to the control. Additionally, a small pilot study exploring the suitability of ultrasound scanning of mice or graft survival and vascularization assessment was conducted. Ultrasound biomicroscopy provided valuable insights into graft morphology, necrotic areas, and blood flow, suggesting its potential as a monitoring tool. The follicle density showed a non-significant increase in

the hPL group and UCP groups compared to the control. Despite, the angiogenic properties of PRP, this study was unable to demonstrate a significant impact of hPL on vascularization nor of hPL and UCP on follicular survival in xenotransplanted human ovarian tissue. Further research is needed to elucidate the role of PRP in optimizing the outcomes of ovarian tissue transplantation outcomes.

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