

Optimizing Cryopreservation of Human Testicular Tissues

Valli-Pulaski, H.,^{1,2} Sukhwani, M.,² Peters, K.A.² and Orwig, K.E.^{1,2}

¹Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh School of Medicine, Pittsburgh PA 15213

²Magee-Womens Research Institute, Pittsburgh PA 15213

Objective: Chemotherapy and radiation treatments for cancer or other conditions can cause permanent infertility. While adolescent and adult men have the option to cryopreserve sperm prior to treatment, this is not an option for prepubertal boys who are not yet producing sperm. Several centers in the US and abroad are preserving testicular biopsies for prepubertal male patients with anticipation that spermatogonial stem cells (SSCs) in the tissue can be used to achieve fertility in the future. In order to maximize the use of the tissue in the future, we compared cryopreservation of cell suspension to intact piece of tissue to discover the optimal cryopreservation methods.

Design: Laboratory study using human tissue.

Materials and Methods: Human testicular tissues were cryopreserved either as an intact piece of tissue by slow freezing, or as a cell suspension. The efficiency of each technique was analyzed by immunocytochemistry (ICC) for spermatogonia marker UTF1 and human-to-nude mouse xenotransplantation.

Results: The average UTF1 positive cells per gram of tissue was highest in the in fresh tissue (15.52 ± 2.5 UTF1 positive cells/gram of tissue) and it was significantly higher than any other group ($p < 0.005$). From the cryopreserved groups, large tissue pieces and small tissue pieces had similar number of UTF1 positive cells per gram of tissue (9.78 ± 1.8 and 11.36 ± 4.5 , respectively). There was no statistical significance between these two groups ($p = 0.8$). Cell suspension had the least UTF1 positive cells per gram of tissue (2.76 ± 1.0) and was significantly worse than cryopreserved intact tissue pieces ($p < 0.001$).

Conclusions: Based on the results from ICC and human-to-nude mouse xenotransplants, slow freezing of small piece of tissue is the most efficient technique to cryopreserve human testicular tissue. These studies are important because they will maximize the use of cryopreserved undifferentiated spermatogonia for future use. Intact tissue pieces have the advantage that they can be used for tissue based or cell based approaches; whereas a cell suspension can only be used for cell culture or SSC transplantation. In case organ culture or testicular tissue grafts are a viable option to restore male fertility in the future, an optimal cryopreservation technique needs to be established.

Disclosures: None.

Funding: This work was supported by NIH grants HD055475 and HD061289, Magee-Womens Research Institute, Foundation and the Richard King Mellon Foundation and the United States-Israel Binational Science Foundation.