FOLLICLE OUTCOMES IN HUMAN OVARIAN TISSUE: IMPACT OF FREEZING, CULTURE AND CAM-GRAFTING

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Abstract Body

Background: Follicle burnout occurs after both in vitro culture (IVC) and xenografting of cryopreserved human ovarian tissue, but the effect of freezing on follicle loss is still poorly understood. Furthermore, the chorioallantoic membrane (CAM) has been shown to curtail follicle activation in animals, though its impact on human follicles is yet to be elucidated.

Objective: To assess the involvement of freezing, culture and CAM-grafting on follicle burnout and PI3K pathway modulation in human ovarian tissue.

Materials and methods: Fresh and frozen-thawed ovarian tissue from 10 patients was cultured and compared (fresh-IVC vs. FT-IVC). FT-IVC fragments were then examined against fragments grafted to CAM (FT-CAM). Follicle assessments performed on days 0, 1 and 6 included histology and immunohistochemistry (Ki67 [proliferation], caspase-3 [apoptosis], LC3B [autophagy], p-Akt, FOXO1 and p-rpS6 [PI3K activation]). Droplet digital PCR further investigated expression of PI3K pathway- and oocyte-related genes in tissue sections.

Results: No major differences were encountered between fresh-IVC and FT-IVC groups. While a significant drop was observed in primordial follicle proportions in case of IVC (d0 vs d6, $p<0.002$), the FT-CAM group held steady. Importantly, avian erythrocytes were already identified in 30% of implants from d1, proving rapid revascularization. Apoptotic and autophagic follicle rates increased during IVC ($p<0.008$), but remained significantly lower in the FT-CAM group ($p<0.01$). PI3K/FOXO pathway upregulation was noted in IVC groups, demonstrating primordial follicle activation, while significant pathway downregulation was detected in the FT-CAM group ($p<0.03$). ddPCR confirmed (i) oocyte growth during IVC and (ii) follicle autophagy in all groups. However, the PI3K pathway looked to be differentially modulated in tissues and follicles.

Conclusions: Freezing itself does not cause follicle burnout. CAM-grafting appears capable of preserving the follicle pool, probably thanks to rapid revascularization. Follicle autophagy was also upregulated in all groups, suggesting that it plays a key role in sustaining survival.