Z-VAD-FMK TREATMENT PREVENTS GRANULOSA CELLS APOPTOSIS INDUCED BY ETOPOSIDE BUT NOT BY HYPOXIA AND SERUM STARVATION IN-VITRO
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Removal, cryopreservation and subsequent graft of ovarian strips after cancer treatment have been successfully used to re-establish female fertility. The major problem after transplantation is follicular loss due to ischemic reperfusion injury. Since granulosa cells are known to be essential for the overall survival of the oocyte, we hypothesized that adding anti-apoptotic drugs to these cells could help to prevent follicular loss.

GC1a and HGL5 cell lines were treated with antiapoptotic drugs. Etoposide was used as an apoptotic inducer. To reproduce the in-vivo early ischemic stage of the graft, cells were cultured without serum and under hypoxia (1% O2), with or without Imatinib, Nilotinib or Z-VAD-FMK.

For both cell lines, Z-VAD-FMK is the most efficient drug against etoposide induced apoptosis, as indicated by metabolic activity test (WST-1), Imatinib and Nilotinib having either lower or no effect. Flow cytometry analysis after annexin V-FITC and propidium iodide double staining shows that cells co-treated with etoposide and Z-VAD-FMK display a higher percentage of viable cells as compared to etoposide alone. Under hypoxia and serum starvation, the metabolic activity of the treated cells is similar to the control one and cell viability is not improved by Z-VAD-FMK treatment.

In conclusion, our results suggest that, in-vitro; the use of Z-VAD-FMK could be efficient in granulosa cells submitted to etoposide induced-apoptosis. However, ovarian cortical strips culture and grafting studies are still required to confirm the potential efficacy of this drug to improve tissue viability and primordial follicles preservation after transplantation.