**SENSITIVE AND SPECIFIC DETECTION OF EWING SARCOMA MINIMAL RESIDUAL DISEASE IN OVARIAN AND TESTICULAR TISSUES**

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**Abstract Body**

Ewing sarcoma is a solid tumor with high metastatic potential. Due to the toxic effects of cancer treatments on reproductive functions, cryopreservation of ovarian or testicular tissue is recommended to preserve fertility. However, the risk of reintroducing residual metastatic tumor cells should be evaluated before fertility restoration. The aim of our study was to validate a sensitive and specific approach for Ewing sarcoma minimal residual disease (MRD) detection in germinal tissues using *in vitro* models.

Thawed ovarian tissue which surrounded ovarian cyst from women with benign cyst (n=12) and testicular tissue from non-obstructive azoospermia men (n=14) were co-cultured *in vitro* with increasing amounts of human Ewing sarcoma cells (RD-ES cell line). *EWS-FLI1* tumor-specific transcript was subsequently quantified by RT-qPCR in both tissues. All co-cultured samples were found to be positive for *EWS-FLI1* mARN, with a strong correlation between its level and RD-ES cell number both in ovarian ($r=0.93$) and testicular ($r=0.96$) tissues ($p<0.001$). No transcript was detected in uncontaminated control samples. The invasive potential of Ewing cells was also evaluated during these experiments. After co-cultures, tumor cells were detected in both ovarian and testicular tissues by histology, immunostaining, FISH and RT-qPCR methods. In addition, 5 ovarian and 3 testicular tissue samples from children with metastatic Ewing sarcoma were tested, and no MRD was found by RT-qPCR and histology and immunostaining.

In conclusion, our *in vitro* model allowed us to validate an accurate method to detect MRD of Ewing sarcoma in ovarian and testicular tissues by RT-qPCR, which will enable us to select the adequate method to restore fertility.