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**Ovarian function recovery after transplantation of ovarian tissue cryopreserved and stored for long-term**

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**S**tatement of the Problem: Ovarian tissue cryopreservation represents a valid strategy to preserve ovarian function in cancer patients with a high risk of premature ovarian failure due to chemo/radiotherapy. The ovarian tissue remains frozen for very long period of time (the request of tissue replanting usually occurs after at least five years from the end of therapies and this period may dragging on further in the case of diseases that require prolonged treatments or in the case of pediatric patients). The purpose of this study is to evaluate the morphology and functional activity of cryopreserved ovarian tissue stored for 18 years after thawing and transplantation. Methodology & Theoretical Orientation: Ovarian tissue of a 29 years old patient suffering from Hodgkin Lymphoma was cryopreserved at our Centre ahead of starting anticancer treatment. Eighteen years after storage her ovarian tissue was evaluated by light microscopy, transmission electron microscopy, TUNEL assay and LIVE/DEAD viability/cytotoxicity test and then heterotopically transplanted in two subcutaneous pockets of patient. Follicle development was evaluated by ultrasound examination on the graft sites. Findings: Ovarian tissue showed a good morphology, no apoptosis signs, sub-cellular integrity of follicles and interstitial oedema foci. The LIVE/DEAD assay which is performed on stromal cells, isolated from cryopreserved tissue, showed viable cells (>97%) after the 2 and 7 days of culture. The patient had the first menstruation five months after transplantation and, to date (20 months from the graft), she is regularly menstruating every 30–40 days. Follicular development is monthly witnessed by a bulge palpable beneath the skin in the graft sites. Conclusion & Significance: This study provides evidence that the storage time does not impact on tissue quality and on tissue ability to resume the ovarian function after replanting. These results can give hope especially to the cancer girls, whose tissues could remain cryopreserved for a very long time.

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