Cryopreservation of ovarian tissue from girls and women prior to treatment of a malignant disease

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Summary

The Ph.D.-thesis includes a review and five papers. The project was carried out at the Fertility Clinic and the Laboratory of Reproductive Biology, Rigshospitalet during the period 2001 to 2004.

The aim of the project was to develop a way of preserving the fertility in young female cancer patients by cryopreserving and autotransplanting some of their ovarian tissue and by in vitro maturing small pieces of ovarian tissue with AMH and testosterone. Additionally, we wanted to assess the ovarian function in the women after cryopreservation and chemotherapy.
A total of 30 girls and 48 women had cortical tissue from an entire ovary cryopreserved in the period from August 1999 to October 2004. Additionally, 19 women donated a small piece of ovarian tissue in connection with gynaecological laparotomy for benign reasons.
Three women with chemotherapy-induced POF had some of the cryopreserved ovarian tissue autotransplanted, which resulted in return of ovarian function and menstrual bleedings in all three. IVF was performed in two of these women resulting in aspiration of mature MPII oocytes, which were subsequently fertilized and developed into embryos.
The accuracy in letting a single ovarian biopsy, taken in conjunction with the cryopreservation procedure, be representative of the ovarian reserve of the whole ovary was refuted by analysing the follicular density in three entire ovaries. We found that primordial follicles were unevenly distributed throughout the ovarian cortex, and that the density thus varied considerably from one fragment to another within the same ovary.
Women, who had their ovary removed at a different hospital than where the cryopreservation took place, were found to have surviving follicles in their ovarian tissue after 4 hours transportation cooled on ice, and furthermore, the follicles were able to grow after a 4-week culture period under the skin of immunodeficient mice.
After a 4-week culture period in vitro of small pieces of ovarian tissue with AMH and/or testosterone, we found significantly more primary follicles that were significantly larger in the tissue cultured with AMH as compared to control medium only, suggesting that AMH enhances recruitment, survival and/or growth of human ovarian tissue in culture.
Nine patients out of 22 experienced POF after chemotherapy; all of these had received either BMT or high-dose chemotherapy with alkylating agents and only one patient first-line chemotherapy for their cancer disease. Ten patients had a seemingly normal ovarian function as assessed by ultrasonography and hormonally and three patients had an apparently impaired ovarian function. Surprisingly, all breast cancer survivors kept their ovarian function despite of treatment.
In conclusion, cryopreservation of ovarian tissue is now a possible way of preserving the fertility in young female cancer patients with a high risk of POF due to the anti-neoplastic treatment, although our results show that a lot of these patients seem to keep an intact ovarian function after treatment.