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1. Abstract title (25):

Impact of first-line chemotherapy prior to ovarian tissue cryopreservation on primordial follicle activation and survival in pre-pubertal and young adult patients.

2. Study question (25):

How chemotherapeutic regimen administered prior to ovarian tissue cryopreservation affect the signaling pathways regulating the ovarian reserve and follicular survival in pre-pubertal and adult patients?

3. Summary answer (25):

Chemotherapy induces key signaling processes of follicle activation and increases apoptosis in quiescent follicle. However, damages were different according to the age of the patient.

4. What is known already (100):

Therapeutic regimen can damage the ovarian reserve leading to infertility of cancer patient survivors. Among fertility preservation options, cortical tissue banking appears to be an attractive alternative for patients who cannot delay their treatment or have already started chemotherapy. Though previous studies showed that first-line chemotherapy may induce follicular damages, the impact on follicle activation signaling pathways in human remains poorly understood. Deciphering the signaling modifications under chemotherapy is critical to have a better understanding of the follicle depletion process. Moreover, only few studies on cryopreserved tissue were conducted in child whereas follicles distribution differs compared to post-pubertal women.

5. Study design, size, duration (75):

Cryopreserved ovarian tissue from young adult (16-27 years old, n=6) and pre-pubertal (3-10 years old, n=6) cancer patients were used as model. Fragments were thawed and cultured for 24 hours after size homogenization (4x2x0.5 mm). Patients who received chemotherapy before ovarian tissue cryopreservation were compared to non-exposed patients. PI3K/AKT/mTOR and Hippo pathways, as well as follicles and stroma survival, were assessed among the different groups at thawing and after culture.

6. Participants/materials, setting, methods (75):

The impact of previous chemotherapy exposure on follicle activation on the PI3K/AKT/mTOR and Hippo pathways was assessed at thawing and after 24 hours of culture by protein analyses (immunostaining and western blot). Histological analyses (follicular counting, immunostaining and TUNEL staining) were performed at the two timepoints to assess follicle distribution, morphology, stroma structure and apoptosis.

7. Main results and the role of chance (200):

The damage of chemotherapeutic regimen prior to cryopreservation was observed specifically on quiescent follicles after thawing by TUNEL staining in both adult and pre-pubertal patients. Surprisingly, apoptosis occurred more specifically in oocytes and in granulosa cells in pre-pubertal and adult treated tissue, respectively. After culture, apoptosis was observed in the stroma but healthy follicles were observed in all conditions. Atretic follicles were observed similarly in pre-pubertal and adult cortex previously exposed to chemotherapy while not in the unexposed tissue. Protein analyses showed a higher expression of PI3K and Hippo proteins

among all groups at thawing compared to cultured groups while difference was observed between pre-pubertal and adult cortex. At thawing, cortical tissues previously exposed to chemotherapy had a higher expression of phosphorylated forms of AKT and RPS6 compared to untreated groups, irrespective to the age. Moreover, immunostainings showed an oocyte-specific localization of p-AKT while p-RPS6 was more pronounced into the granulosa cells, suggesting early process of follicles activation.

8. Limitations, reasons for caution (50):

This study was limited to the evaluation of two major signaling pathways, PI3K/AKT/mTOR and Hippo. Moreover, considering the scarcity and the heterogeneity of our model, the number of patients included in this study is limited and the results should be interpreted with caution.

9. Wider implications of the findings (50):

Our results highlight the involvement of age and previous chemotherapeutic treatment in the regulation of signaling pathways regulating follicular activation, growth, and survival. Besides sustaining the chemotherapy-induced “burn out effect” theory, it opens perspectives to regulate the deleterious impacts of chemotherapy on follicles through molecular control of the altered pathways.

10. Study funding/competing interest(s):

This work was funded by The Excellence of Science (EOS) Grant. No competing interests.

11. Trial registration number (25):

N/A

Keywords: human/ ovary / follicular activation / PI3K / Hippo

Topic category: Basic Science

Topic: Male and female fertility preservation