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Award Winners

A-6

Follicular reconstruction in artificial ovary made by human isolated ovarian cells from chemotherapy-induced POF patient seeded into human ovarian decellularized ECM after xenotransplantation

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Background: Depletion of ovarian reserve due to dismiss of follicular growth in chemotherapy induced premature ovarian failure (Chemo- POF) is the main concern for oncofertility researchers who try to find a practical way to restore the ovarian function.

Objective: Artificial ovary is preparing a new niche for ovarian cells and reconstruction of follicular activity may be developed to aid infertility treatment by transplantation of engineered ovary.

Materials and Methods: Ovarian tissues were taken from 8 Chemo- POF women and 15 transsexuals. The medulla was carefully removed and the cortical tissue was cut into 5×5×5 mm3 strips and then cryopreserved. Ovarian cells were removed with NaOH as main detergent from human ovarian pieces of transsexual patients and then decellularized cortical tissue (DCT) was evaluated by DNA content analysis, hematoxylin & eosin and DAPI staining techniques. Human ovarian cortical cells (HOCCS) were finely minced and enzymatically isolated and characterized by real time PCR for IFITM3, vimentin, *FSH-R* and *KI67* genes and immunostaining of vimentin, Inhibin-α and IFITM3 markers. Then the isolated HOCCS (2 × 10^6 cells) from both Chemo- POF and transsexual ovaries were seeded into DCT by injection and spinner flask culturing for one wk (AO; artificial ovary). Also, MTT assay was performed to measure the in vitro cytocompatibility of ovarian scaffold. Then AO was xenotransplanted to NMRI mice beneath the abdominal sub-serosal fascia for two months. Finally, H&E, hormonal tests (FSH, AMH and E2), real time PCR (GDF-9, ZP3, VEGF, CD34 and KI67) and immunohistochemistry (IHC) (GDF-9) assessments were applied for the calculation of transplantation outcomes.

Results: H&E, DAPI and DNA content confirmed over 95% decellularization. Immunofluorescence showed that isolated HOCCS from transsexual and Chemo- POF ovarian tissues included 80-85% stromal, 5-10% granulosa and < 5% oogonial stem cells by expressing the vimentin, Inhibin-α and IFITM3 markers in passage one. Expression patterns of the mentioned proteins in passage two were appraised 70-75%, 5-10% and > 10%, respectively. Also, HOCCS well expressed Vimentin, *FSH-R*, *FRAGILIS*, *DDX4*, *STELLA* and *KI67* genes in real time PCR technique. One wk culture of AO in spinner flask indicated the HOCCs could penetrate not only into the exterior surfaces also to the depth of the ovarian scaffold (H&E). Histological study and quantitative evaluation of Estradiol, FSH AND AMH production after two months of AO xenotransplantation confirmed the presence of morphologically health and secretory active reconstructed human ovarian primordial and primary follicles. IHC for GDF9 confirmed the paracrine activity of oocytes within the follicles. To approve existing active follicles within AO, the real time PCR demonstrated a good expression of the follicle-related genes like GDF9 and ZP3 in both groups. Furthermore, the angiogenesis genes VEGF and CD34, in both transplanted groups showed high expression. At the end, the expression of kI67, cell proliferation and survival factor, approved the cellular multiplication and health in AO of the both groups.

Conclusion: Our results approved that ovarian follicular reconstruction and function is possible in the case of ovarian insufficiency through xenotransplantation of AO made by DCT seeded by Chemo-POF ovarian isolated cells.

Key words: *Human artificial ovary, Ovarian follicular reconstruction, Oogonial stem cells, Spinner flask.*