O-083

Towards an artificial ovary: Grafting preantral follicles on decellularized human ovarian tissue

S. Pors[1], S.G. Kristensen[2], K. Lundsgaard[2], M. Ramløse[2], C.Y. Andersen[2]
[1]Rigshospitalet, Laboratory of Reproductive Biology, KØBENHAVN Ø, Denmark
[2]Rigshospitalet, Laboratory of Reproductive Biology, Copenhagen, Denmark

Study question:

Can decellularized human ovarian tissue support development of follicles and become a biocompatible and biofunctional artificial ovary ?.

Summary answer:

We have demonstrated an effective protocol for decellularization of human ovarian tissues and successful recellularization with isolated preantral follicles.

What is known already:

Decellularization of ovarian tissue entails the removal of cells, including possible malignancies. The physiological extracellular matrix (ECM) left behind offers the complex milieu, which facilitates the necessary interaction between ovarian follicles and their surroundings to ensure their growth and development. A bioengineered ovary would thus facilitate the growth and development of reseeded frozen-thawed early stage follicles, free of malignancies. Decellularized tissue (DCT) also benefits from its biocompatibility due to clearance of immunogenic substances. Further research in developing a bioengineered ovary entails thorough studies into its biocompatibility and biofunctionality.

Study design, size, duration:

Donated human ovarian tissue and isolated preantral follicles from women undergoing ovarian tissue cryopreservation for fertility preservation. Biofunctionality of the decellularized human ovarian tissue was evaluated by in vitro and in vivo studies. Furthermore, scaffolds were transplanted to immunocompetent mice and evaluated for inflammatory reactions.

Participants/materials, setting, methods:

Ovarian cortical and medullary tissue were decellularized (0.1% SDS for 3, 6, 24 hours; followed by 24 hours DNase (1mg/mL)). Decellularization and preservation of composition were characterized by DNA and collagen quantification, Periodic Acid-Schiff (PAS) staining, and immunoflourescence for collagen I and DNA (4',6-diamidino-2-phenylindole (DAPI)). Human granulosa cells (GCs) were reseeded on the DCT and cultured in vitro. Murine and human preantral follicles were isolated, then reseeded on the DCT and grafted subcutaneously to immunodeficient mice.

Main results and the role of chance:

Incubation in 0.1% SDS for 6-24 hours adequately decellularized both human ovarian cortical and medullary tissue by eliminating all cells and leaving the ECM intact. DNA quantities in the DCT were significantly lower compared to matched native samples. Histological examination using PAS staining confirmed that the cortical and medullary tissues were completely decellularized, and no visible nuclear material was found within the decellularized sections. DCT also stained positive for

collagen I and collagen quantities in DCT constituted 88-98% of the individual baselines for native samples (n=4). Mature human GCs were able to recellularize the DCT in vitro by successfully repopulating and migrating into the scaffold. Xenotransplantation experiments showed that the DCT was able to support survival of isolated human follicles and growth of isolated murine follicles of which several grew to antral stages. The follicular recovery rates after 3 weeks grafting were similar for both human (25%) and murine follicles (26-32.5%). The average diameter of isolated murine follicles increased from 114 ±26µm to 195 ±143µm following 3 weeks grafting.

Limitations, reasons for caution:

Further studies are needed to increase recovery and survival of the reseeded follicles. Furthermore, longer grafting periods should be evaluated for observing development of follicles. Survival of the follicles might be impaired by the lack of stroma cells. This challenge must be overcome to advance the bioprosthetic ovary further.

Wider implications of the findings:

This is the first time that isolated human follicles have survived in a decellularized human scaffold. Therefore, this proof-of-concept could be a potential new strategy, to eliminate the risk of malignant cell re-occurrence in former cancer patients having cryopreserved ovarian tissue transplanted for fertility restoration

Trial registration number:

Not applicable

No
Funding by hospital/clinic(s)