604: Establishment of in vitro culture condition for ovarian primordial follicles

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Objective
A decrease in the number of hormone-responsive follicles) is a hurdle for infertility treatment. According to women's aging, these follicles are decreased but the primordial follicles still remain. Primordial follicles are non-responsive to gonadotropins, therefore, women with diminished ovarian reserve (e.g. age factor, POI, cancer survivor, poor responder) are not ideal for current therapeutic strategy.
Design
In this study, we tried to establish in vitro culture condition of primordial follicles using a mouse model.
Materials and Methods
Ovaries of two-week-old C57BL/6 female mice were collected and dissociated into single follicles mechanically. Isolated, single primordial follicles were cultured in media drop covered with mineral oil. The media consisted of either MEMα or DMEM/F12 supplemented with bFGF. After 14 days of in vitro culture, the seeded follicles expanded to the secondary follicles then 200 IU/L FSH (Gonal-F), 100 IU/L LH (Luveris) were added. At day 20, the follicles were matured and the ovulation was induced by treatment with hCG and EGF. Distribution of tubulin was confirmed by immunostaining and the expression of Figla and Nobox was evaluated by qRT-PCR.
Results
In vitro development of primordial follicles was achieved with a higher efficiency in the group MEMα. The ovulation rate of in vitro developed oocytes was 20% and 8%, respectively. The ovulated oocytes were at M II stage and the cytoskeletal structure demonstrated normal distribution. The primordial follicle-derived oocytes expressed oocyte development-related genes, Figla and Nobox, as compatible to naturally developed oocytes.
Conclusions
Taken together, we established in vitro culture condition of primordial follicles using paracrine factors. The condition should be further optimized in order to get a higher efficiency. The fertile ability of primordial follicle-derived oocytes should be confirmed in the future.
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Disclosure
None