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Oral Presentations

O-14
What is the accurate culture system for in vitro culture of cryopreserved human ovarian tissue?

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Background: Nowadays ovarian tissue banks have been set up in many countries to improve chances of child bearing for cancer patients. As transplantation of cryopreserved ovary intensifies the possibility of malignant cells reintroduction, researchers are focusing more on ovarian tissue in-vitro culture methods.

Objective: In this study we pursue three goals to achieve the accurate culture system for in-vitro culture of cryopreserved human ovarian tissue.

Materials and Methods: First, comparing agar as a cultivation substrate with matrigel-coated insert in order to attain a suitable culture substrate. Afterwards, investigating the effect of basic fibroblast growth factor (bFGF) and/or kit ligand (KL) in the culture medium. Third, evaluating the effect of Phosphatase and TENsin homolog (PTEN) inhibitor (Bpv (HOpic)) and/or mTOR activators, phosphatidic acid (PA) and propranolol (PP), on the activation and subsequent development of in-situ culture of human primordial follicles. All 7-day cultures were performed with slow frozen-thawed human ovarian cortical tissues obtained from transsexual women. At first the ovarian fragments were cultured on either matrigel-coated inserts or agar-soaked substrates. In the second phase, four different groups were examined: 1) control (base medium; BM), 2) KL (BM+100 ng/ml KL), 3) bFGF (BM+100 ng/ml bFGF) and 4) bFGF+KL (BM+100 ng/ml KL+100 ng/ml bFGF). In the third phase, control (without stimulators), Bpv (100 µM BpV (HOpic)), PA (200 µM), PA+PP (50 µM), and Bpv+PA+PP groups were compared. The incubation of ovarian cortical fragments was conducted for 24 hours with different stimulators and then for 6 days without stimulators. Follicular growth, proliferative, apoptotic and developmental gene expression, hormone secretion and PI3K/mTOR pathway protein expression were evaluated.

Results: In the first phase, no significant difference was found for follicular growth. The apoptotic index was lower in the agar cultured group and *Ki67* gene expression showed a significantly higher expression in agar cultured group. In the second phase, the proportion of growing follicles had no significant difference between cultured groups. The level of estradiol hormone had significantly increased in the bFGF+KL group. The expression of *Ki67* gene indicated a significant increase in the bFGF+KL group. In the third phase, the proportion of primordial and growing follicles were not significantly different after 24 hours of incubation among experimental groups. Western blot analyses indicated a significant reduction of FOXO3a in the PA+PP or Bpv+PA+PP groups compared to the control group. After 7-days of culture, the proportion of transitional follicles were significantly higher in the PA group compared to other groups. The estradiol level was significantly higher at the last day of culture compared to day 1, except for the Bpv group. Hormonal secretion was significantly higher in the PA and PA+PP groups and lower in the Bpv and Bpv+PA+PP groups compared to the control group.

Conclusion: Agar is similar to matrigel-coated inserts for culturing human ovarian tissue and it is an inexpensive substrate too. The combination of KL and bFGF positively influences steroidogenesis in the granulosa cells without increasing the total number of growing follicles. Temporary treatment of human ovarian tissue with mTOR activators, enhance the initiation of primordial follicle development and positively influence steroidogenesis, while Bpv (HOpic) has a potentially negative effect on follicular activation and function.

Key words: *In-situ ovarian culture, Agar substrate, Kit ligand, Basic fibroblast growth factor, mTOR, PI3K pathways.*