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Poster Presentations

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Evaluation of the fundamental factors, involved in successful culture of human ovarian cells, follicles and tissues: A preliminary step for assembling an artificial ovary

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Background: Todays, the new infertility treatment methods try to mimic the ovarian natural micro-environment by tissue engineering. An artificial ovary tries to preserve fertilization, even with producing oocytes or with releasing steroid hormones. Producing oocytes can be obtained even by follicle culture or by ovarian tissue culture. Many physical and chemical factors are involved in ovarian cells and follicular culture. Based on the current studies, no study has reported the best mediums for ovarian cells, follicles and tissue culture.

Objective: This study aimed to uncover the appropriate media and supplements for in vitro culture of ovarian and cumulus cells (CCs).

Materials and Methods: Cortical, medullar, and hilar cells of human ovary were cultured and their conditioned medium (CMs) were collected. The expression of GDF9 was detected in all the cells. Also, CCs were collected from healthy women, who referred due to male factor infertility. To choose the optimum basal medium, a mixture of ovarian cells was cultured with basal mediums, supplemented with various concentrations of fetal bovine serum (FBS) and human serum albumin (HSA). The cocktails were as follows: [Serum free mediums], [mediums + 10% FBS], [mediums + 20% FBS], [mediums + 1% Alb], [mediums + 2% Alb], [mediums + 10% FBS + 1% Alb], [mediums + 10% FBS + 2% Alb], [mediums + 20% FBS + 1% Alb] and [mediums + 20% FBS + 2% Alb]. The same process was repeated for CCs. Because the CCs need some supplementation, we cultured them with various concentrations of some supplements to choose the best concentration. So, CCs were cultured with various concentrations of L-Glutamine, bovine serum albumin (BSA), HSA, insulin transferrin selenium (ITS), Follitropin alfa® and Pregnyl®. Also, CCs were treated with various concentrations of follicular fluids (FFs) and CMs, too. CMs were collected from ovarian, testicular, adipose and amniotic derived and ovarian carcinoma cells. Then, CCs morphology and proliferation were evaluated.

Results: All the ovarian cells expressed GDF9, as a key factor for ovarian follicular growth. Alfa MEM + 20% FBS and DMEM F12 + 20% FBS were the most suitable cocktails for ovarian and CCs culture, respectively. 20% FBS was superior to 10% for both ovarian and CCs. Also, HSA could not support the growth of ovarian and CCs, alone. The cocktails of mediums with 20% FBS and (mediums+FBS+HSA) were superior to the others. The CMs of ovarian cortical and hilar+medullar cells could lead to higher CCs growth. 17 mM/l L-Glutamine, 24 mg/ml BSA, 20 mg/ml HSA, 10 ng/ml ITS, 300 mIU/ml Follitropin alfa and 3.5 IU/ml Pregnyl led to higher proliferation of CCs.

Conclusion: Ovarian chemical micro-environment is very complex and ovarian follicle growth needs many known and unknown elements like growth factors, which are expensive. We concluded that CMs and serums can support the follicular growth alongside with basal mediums, supplemented with hormones, ITS and L-Glutamine, which are cheaper and more accessible.

Key words: *Artificial ovary, In vitro culture, Ovarian cells, Cumulus cells, Conditioned mediums.*