

**Matrix Stiffness Regulates Antrum Formation in a Three-Dimensional Ovarian Follicle Culture System**

Formation of fluid-filled antral cavity has been a defining, yet not well understood characteristic of ovarian follicles maturation. It was hypothesized that antrum facilitates oxygen transport in follicle growth, and recent studies found antrum formation to be aided by aquaporin proteins. Using a novel 3-dimensional follicle culture system, we are able to regulate antrum formation via the physical properties of the culture matrix, and study aquaporin and hypoxia-inducible factor alpha (Hif-1 $\alpha$ ) gene expression in the system. In this system, multilayered secondary follicles isolated from mouse ovaries were placed in droplets of alginate, which were then allowed to cross-link in calcium chloride solution. Studies were performed in 0.5% and 1.5% alginate concentrations, which have significantly different stiffness. RNA was isolated from the follicles after 2-8 days of culture, for RT-PCR analysis of aquaporin and Hif-1 $\alpha$  gene expression. Only 5.5% of follicles cultured in 1.5% matrix formed antral cavity, but the follicles showed high expression of aquaporin and Hif-1 $\alpha$  genes. Follicles cultured in 0.5% less stiff alginate matrix formed antra in over 85% of follicles, but showed low expressions of aquaporin and Hif-1 $\alpha$  genes. It is thus proposed that stiffness of alginate matrix used in 3D *in vitro* culture system has regulating effects on antrum-regulating gene expression, due to induced hypoxic effect in follicles. Follicles cultured in stiffer alginate matrices are unable to expand in the growth process, and thus antrum unable to form, due to the external pressure of the stiffer alginate matrix. However, because of increasing tissue mass within the follicle, oxygen transportation into the follicle is impeded, causing hypoxic conditions within the follicle. Aquaporin proteins, particularly aquaporin 7 and 8, are then expressed to alleviate the hypoxic effect, thus describing the regulation of alginate matrix stiffness in gene expression related to antrum formation in *in vitro* cultured ovarian follicles.

(300 Words)