567d Alginate Hydrogel Mechanics Regulate Follicle Growth in a Three-Dimensional *in Vitro* Culture System

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In vitro ovarian follicle culture provides a means to study the regulation of follicle development, and could eventually provide reproductive options for the treatment of disease-related or chemotherapy-induced sterility. Three-dimensional culture systems are able to maintain the follicular structure and provide both soluble and insoluble signals, which are vital for follicle maturation. The alginate hydrogel must allow for expansion of the follicle during the culture period, and we have investigated the effects of the mechanical properties of alginate on follicle growth and oocyte quality. The mechanical properties of the hydrogel were manipulated through the molecular weight of the polymer, with higher molecular weights producing stronger hydrogels. Alginate was exposed to a 5 Mrad dose of radiation to decrease molecular weight. The viscosity of 1.5% alginate solutions was 0.50 Pa•s for 100% non-irradiated alginate, and a blend of 50% irradiated/50% non-irradiated alginate had a viscosity of 0.078 Pa•s. The elastic modulus of 1.5% alginate gels decreased from 1602±214 Pa to 912±32 Pa by blending alginate with 50% irradiated alginate ($P<0.05$). Follicle survival decreased with increased molecular weight hydrogels (42% for lower molecular weight hydrogels vs. 50% for higher molecular weight hydrogels). Encapsulation of follicles in gels containing 50% irradiated alginate resulted in increased follicle growth over eight days (105%) vs. follicles cultured in non-irradiated gels (58%), an indication of growth inhibition possibly due to compression of the follicle by the gel. The developmental status of the oocyte was assessed by its ability to resume meiosis. Preliminary results indicate increased progression to the GVBD stage in oocytes isolated from follicles cultured in gels containing 50% irradiated alginate vs. those cultured in 100% non-irradiated alginate. Optimization of our culture system to incorporate ideal mechanical properties and both soluble and bound signaling factors will result in a biomimetic three-dimensional culture system to increase follicle and oocyte growth and viability. Supported by NIH U54HD41857-01A1