Researchers are developing 3D approaches for *in vitro* ovarian follicle maturation, a promising therapy for infertility.

**Principal Investigator:** Lonnie D. Shea  
**Collaborator:** Teresa Woodruff (Department of Neurobiology and Physiology)

**Objective:** The *in vitro* maturation of ovarian follicles is a potential therapy for female infertility resulting from chemotherapy, polycystic ovarian syndrome, or premature ovarian failure. Cryopreservation of ovarian tissue supports the survival of the smallest ovarian follicles, which must be matured in order to achieve successful fertilization. At present, *in vitro* maturation of ovarian follicles is conducted on two-dimensional surfaces, which results in disruption of follicular architecture and communication breakdown between the oocyte (the inner part of the follicle) and the granulosa cells (the outer layers of the follicle). The Shea group is developing a novel methodology for ovarian follicle maturation, in which the follicle is encapsulated within a hydrogel designed to mimic the native three-dimensional environment of the ovary. This system preserves the follicular architecture and cell-cell interactions, while providing biochemical signals in the form of soluble (e.g., growth factors) and insoluble (e.g., adhesion sites) factors.

**Approach:** Murine ovarian follicles (day 12) are suspended in a sodium alginate solution. Alginate – a polysaccharide derived from algae – has gentle gelation to allow for cell encapsulation, exhibits minimal non-specific protein adsorption, and can be modified with peptides to provide specific cellular interactions. The solution is added dropwise to a solution of calcium chloride, which gels the alginate, to obtain beads containing the follicles. The beads are porous, allowing for transport of growth factors and nutrients to the follicle by diffusion. Beads with individual follicles are cultured for 10-days, then the follicles are extracted from the beads, and the oocytes isolated for maturation with gonadotrophins. Microscopy is employed to examine the overall health of the oocyte, and the ability to resume meiosis. These oocytes will ultimately be used in traditional IVF techniques to examine the effects of *in vitro* growth and maturation of the follicle on the final quality of the resulting embryos.

**Results:** The Shea group has demonstrated that encapsulation and culture of immature ovarian follicles within a three-dimensional gel supports oocyte maturation. The architecture of the follicle is maintained throughout the culture period, the oocytes increase in size, and the gap junctions between the granulosa cell and the oocyte are retained. In basal cultures (without serum or gonadotropins), more than 50% of the follicles survive the 10-day culture, which is significantly greater than that observed for culture on surfaces (<10%). Additionally, during the culture, the oocytes gain the ability to resume meiosis, the process by which the oocyte prepares for fertilization. The addition of gonadotrophins to the culture results in increase in follicles size, while retaining the overall 3D architecture. Alginate gels have been chemically modified with controlled densities of short peptides derived from extracellular matrix (e.g., RGD peptides) to provide sites for granulosa cell interaction with the matrix. Granulosa cells exhibit specific attachment and spreading as well as increased secretion of steroids compared to the levels observed when the cells are grown on tissue culture plastic or unmodified alginate gels. This system allows for the effects of multiple factors that affect follicle maturation to be examined either in isolation or in combination, within the context of the 3-dimensional architecture.


[Data collected by P. Kreeger](#)