

# The Heterogeneity of Ovary-derived Hydrogels Elucidates the Matrisome Properties that may influence Follicle growth and survival *in vitro*.

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## ABSTRACT:

Premature ovarian insufficiency (POI) is characterized by a decline in ovarian function resulting in infertility and reduced ovarian hormone production. With the global prevalence of POI at approximately 3.5%, there are over 138 million women experiencing subfertility or complete infertility with disruption of sex hormones. However, there are many limitations to the current fertility preservation and restoration options that result in suboptimal outcomes. Additionally, oncofertility patients with metastatic disease within their ovary are ineligible for transplantation of tissue in its native form. This leaves a large population of individuals without desired biological children or necessary hormones. As such, future methods for fertility preservation and restoration will include isolating ovarian follicles to grow *in vitro* or transplant into a bioprosthetic ovary. These methods necessitate a deeper understanding of the ovarian microenvironment in order to define an engineered environment that best supports follicle growth and maturation.

The human ovary is compartmentalized into two regions: the cortex and the medulla. Our lab has shown that there are differentially expressed matrisome proteins across these regions. Here, we developed compartmental decellularized extracellular matrix (dECM) hydrogels by processing 10 bovine ovaries into cortical tissue (CTX) and medullary tissue (MED) then decellularizing the tissue slices to generate hydrogels. Using atomic force microscopy, we determined that CTX-dECM and MED-dECM hydrogels were similarly rigid (537Pa, 468Pa). Thus, we hypothesized that differences observed in downstream applications of these gels are due to differences in biochemical cues or protein composition and relative abundance. ECM proteins' chemical and structural properties influence cellular behavior. So, we performed second harmonic generation scanning microscopy and determined that MED-dECM hydrogels had significantly wider and longer fibers than CTX-dECM and a lower fiber angle alignment.

To evaluate the gel's ability to serve as a bioscaffold, we selected two biologically unique CTX and MED-dECM hydrogels, encapsulated primary murine follicles (~110µm), and assessed growth and survival. Interestingly, we observed increased follicle growth in CTX-dECM 1 and MED-dECM 2 when compared to our control alginate. Day 8 follicle diameter for CTX-dECM 1 was significantly larger compared to follicles grown in CTX-dECM 2 (241.75, 189.95µm). Similarly, follicles grown in MED-dECM 2 were significantly larger at day 8 compared to follicles grown in MED-dECM 1 (212.12, 173.02µm). These data indicate biological differences in the dECM hydrogels.

To investigate these differences, we performed proteomics on these four biologically unique hydrogels. A total of 604 proteins were detected. Further analysis comparing the uniquely present or absent proteins between the two selected biological replicates for each compartmental hydrogel resulted in 150 proteins unique to CTX-dECM 1, 51 in CTX-dECM 2, 70 in MED-dECM 1 and 82 unique in MED-dECM 2. Gene ontology (GO) analysis of biological processes was performed for each hydrogel based on the differentially present proteins. Interestingly, the two compartmental hydrogels that resulted in follicles with larger diameters than the control (CTX-dECM 1 and MED-dECM 2) had comparable GO biological terms with an overrepresentation of proteins involved in metabolic processes. However, further work must be undertaken to identify how this heterogeneity in the hydrogels influenced follicle growth. With further interrogation of these differences, we can engineer an environment that supports follicle growth and increases the production of functional MII oocytes. Importantly, this work aims to lay the groundwork for designing enhanced biomaterials that improve fertility restoration options for a variety of individuals.