An examination of the ovarian extracellular matrix composition in Reproductive-age women

Ansen Tan[1], Andrea S.K. Jones[1], and Ariella Shikanov[1,2,3]
[1]Department of Biomedical Engineering, University of Michigan, [2]Department of Obstetrics and Gynecology, University of Michigan, and [3]Cellular and Molecular Biology Program

BIOMEDICAL ENGINEERING
UNIVERSITY OF MICHIGAN

ENGINEERING HONORS PROGRAM
UNIVERSITY OF MICHIGAN

Motivation

The mechanisms behind the quiescence and activation of ovarian follicles are still largely unknown. It has been shown that the ovarian extracellular matrix (ECM) plays a significant role in the signaling pathways and mechanical cues responsible for regulating follicle quiescence.

This study seeks to investigate the differences in ECM composition between different regions of the human ovary as well as how the composition varies with respect to age.

Immunofluorescent Staining Approach

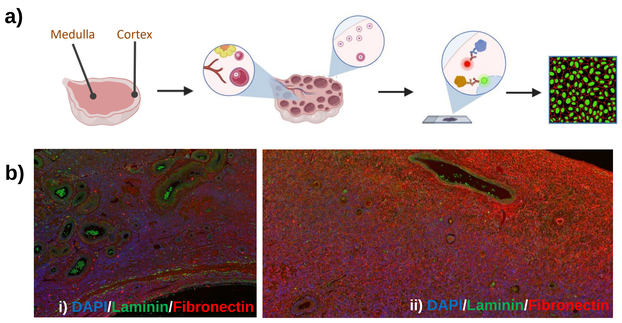

Figure 1. a) The human ovary can be divided into two regions: the cortex and the medulla. The cortex represents the thin outer layer of the ovary which contains the ovarian reserve of quiescent follicles. Upon activation, follicles migrate towards the center of the ovary, or medulla, which houses growing follicles. Ovarian tissue from five donors was harvested, fixed in PFA, embedded, sectioned, then treated with four rounds of immunofluorescent staining against six antibodies: anti-Perlecan, rat (1:100); anti-Collagen IV, rabbit (1:500); anti-Collagen VI, rabbit (1:250); anti-Fibronectin, mouse (1:200); anti-Laminin, rabbit (1:100); and anti-Collagen I, rabbit (1:250). Each round of staining consisted of two antibodies alongside DAPI as a nuclear stain and secondary antibodies (1:1000). Schematic created using biorender.com. b) Regions of the medulla (i) and cortex (ii) were then individually imaged using confocal microscopy.

Fluorescence Quantification Approach

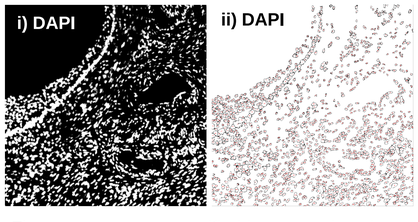

Figure 2. High resolution images acquired via confocal microscopy were exported to ImageJ where each fluorescent channel was analyzed individually.

Fluorescence was quantified by converting channels to greyscale images (i) to record the mean intensity of fluorescence and the total area of intensity across each replicate. The number of nuclei (ii) was acquired by converting the **DAPI** channel to a binary image with a >200 px size discrimination.

Characterization of ECM Proteins in the Medulla and Cortex

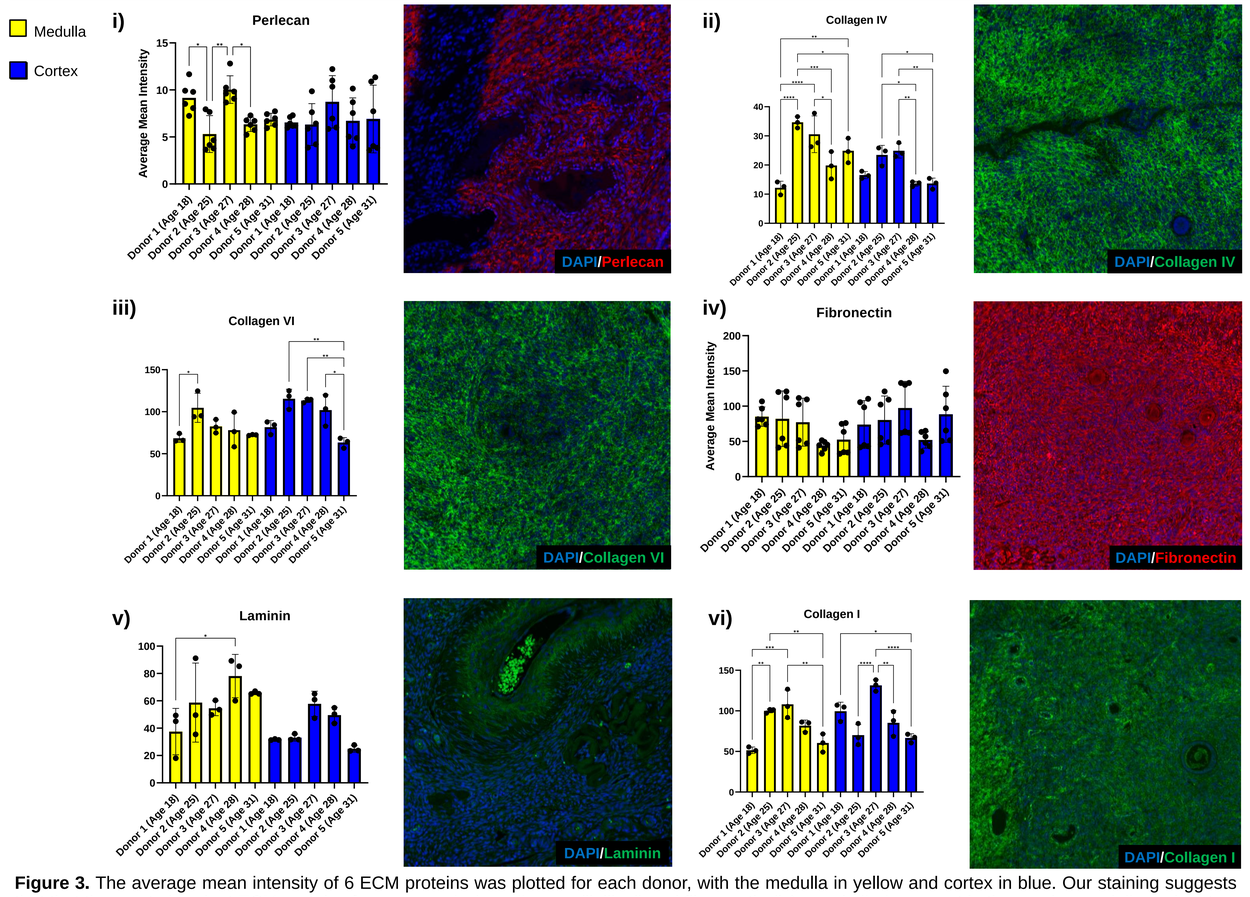

Figure 3. The average mean intensity of 6 ECM proteins was plotted for each donor, with the medulla in yellow and cortex in blue. Our staining suggests that that the most abundant ECM proteins from our analysis, collagens (ii, iii, vi) and fibronectin (iv) are similarly present in the ovarian medulla and cortex. Additionally, perlecan (i) and laminin (vi) were more prevalent in the medulla in nearly all cases. These proteins are involved in angiogenesis, mechanical stability, and cell proliferation or differentiation, and may be found in greater abundance in the center of the ovary which is highly vascularized and responsible for supporting the development of activated follicles. Proteins such as fibronectin drastically varied in intensity while others such as collagen I, IV, and VI were observed consistently throughout the cortex.

Comparing the Presence of ECM Proteins in the Medulla and Cortex

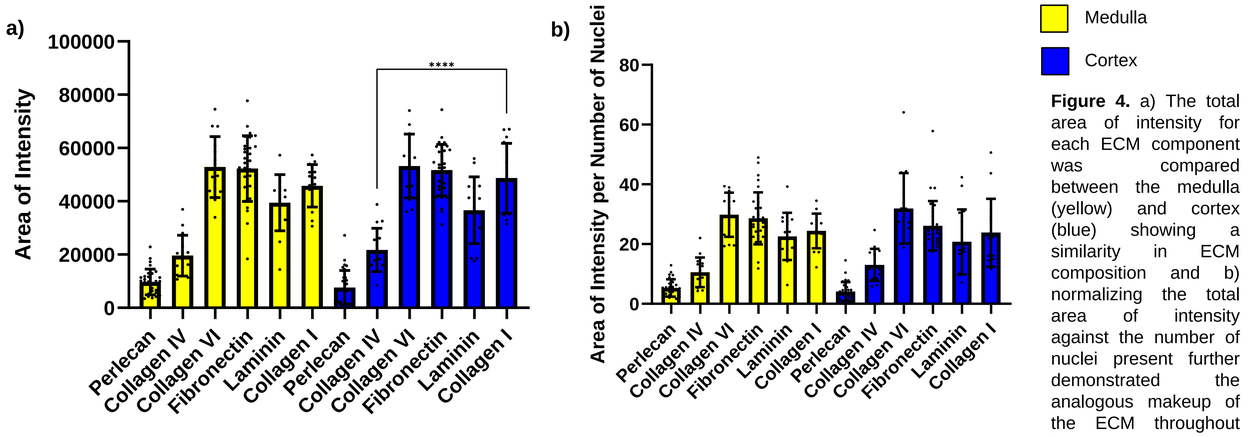

Figure 4. a) The total area of intensity for each ECM component was compared between the medulla (yellow) and cortex (blue) showing a similarity in ECM composition and b) normalizing the total area of intensity against the number of nuclei present further demonstrated the analogous makeup of the ECM throughout the ovary.

Changes in ECM Composition with Respect to Age

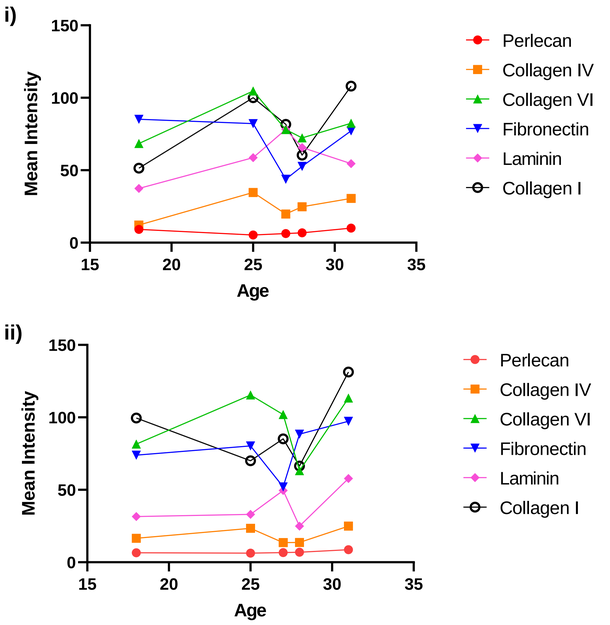

Figure 5. An examination of average mean intensity of each protein with respect to age revealed no significant changes among our donors in ECM composition in both the medulla (i) and cortex (ii).

Conclusions and Next Steps

While a comprehensive analysis of ovarian ECM composition across the reproductive lifespan has yet to be published, this investigation provides a brief insight into the differences in ECM composition between the medulla and the cortex as well as discrepancies with respect to age. Our findings showed that the medulla and cortex share relatively similar amounts of essential ECM proteins.

Despite showing no significant changes in ovarian ECM with respect to age, future studies should consider analyzing a greater age distribution given that the donors for this study ranged from ages 18 to 31. Other factors such as race and diet could also warrant further exploration.

Acknowledgements

This work was funded by NIH grants T31H-D079342 (A.J.), T32-GM70449 (D.F.H.), and F31-HD106626 (A.J.), and Chan Zuckerberg Foundation grant CZF2019-002428 (A.S., E.E.M., J.Z.L, and S.S.H).

MICHIGAN ENGINEERING
UNIVERSITY OF MICHIGAN
MEDICAL SCHOOL
UNIVERSITY OF MICHIGAN

SHIKANOV LAB