Simulated Estrous Cycle Reveals Phase-Specific Regulation of Tendon Matrix Remodeling

Caitlin J. Colicchio[1], Brianne K. Connizzo[1]
[1]Boston University, Boston, MA
ccolicch@bu.edu

Disclosures: Caitlin J. Colicchio (N), Brianne K. Connizzo (N)

INTRODUCTION: Sex hormones naturally fluctuate throughout the menstrual cycle in females, creating recurring shifts in the chemical environment that can influence musculoskeletal health. However, many experimental models rely on constant hormone supplementation [1], while others use ovariectomy-induced hormone depletion to make conclusions in the absence of hormones [2]. These approaches overlook the physiologic cyclicity of estrogen and progesterone, despite evidence supporting phase-specific changes in tendon structure and function [3,4,5]. To better reflect physiologic conditions, we implemented a simulated estrous cycle that mimics hormone fluctuations to evaluate the importance of hormonal cyclicity and identify phase-specific effects. We hypothesized this would reveal distinct, phase-dependent transcriptional responses regulating extracellular matrix (ECM) turnover.

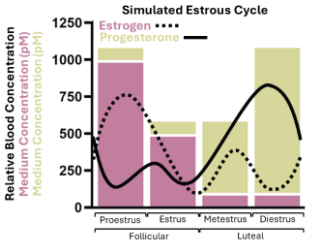

Figure 1. Simulated 4-day murine estrous cycle mimicking relative estrogen (dashed line) and progesterone (solid line) concentrations. Human follicular and luteal phase equivalents are indicated.

METHODS: Female mice were used exclusively in this study to assess estrous cycle-related hormonal effects. Young adult (4-month) female C57BL/6J mice (BU IACUC approved) were euthanized following a seven-day acclimation period to normalize for stress-induced hormonal changes associated with transportation. Flexor digitorum longus (FDL) tendon explants were harvested and then cultured at 3% static strain in a tensile-loading bioreactor [6]. For cycling experiments, explants were cultured for 12-15 days with media simulating a 4-day estrous cycle consisting of varying concentrations of estrogen and progesterone (n=3/cycle phase; Fig. 1), developed using hormone concentrations established previously [7, 8]. For constant hormone experiments, explants were cultured for 12 days in metestrus-like hormone-supplemented medium (n=3). Tendons from day 12 of the simulated cycle, representing metestrus, were compared to the constant metestrus-like treatments to assess the effect of cycling between protocols. Independent samples were also collected from each of the 4 days in the cycle (D12-15) to identify phase-specific remodeling characteristics. All samples were homogenized, and RNA was purified according to the Zymo Quick-RNA Microprep Kit protocol. Sample quality control, RNA library preparation, sequencing, and preliminary bioinformatics analysis were performed by Novogene. The resulting data was filtered to include differentially expressed genes (DEGs) with an FDR<0.01 and >2x fold change. DEGs were uploaded to EnrichR to perform gene ontology (GO) analysis. The union of all differentially expressed genes across pairwise phase comparisons was matched to corresponding FPKM expression values, which were $\log_2$-transformed and z-scored. K-means clustering was then performed on this union gene set (k = 4, correlation distance, 10 replicates) to identify shared expression patterns across estrous cycle phases. Phase-averaged, min-max normalized FPKM values were used to plot cyclic expression patterns with the 4-day phase sequence. Gene ontology analysis was performed on the matrisome subset [9] of phase-specific genes, and the top 10 enriched pathways from each GO category (Cellular Component, Molecular Function, and Biological Process) were functionally grouped. Phase-wise summaries classified functional group enrichment as low (0–10% of top pathways), moderate (10–30%), or high (>30%) for each phase.

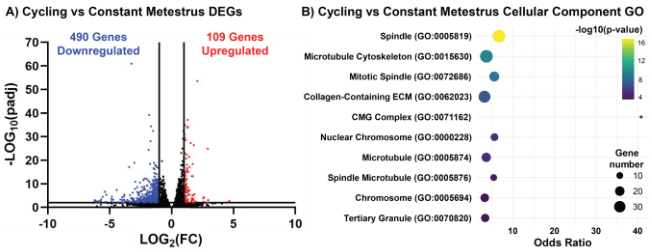

Figure 2. (A) Volcano plot of differentially expressed genes between cycling and constant metestrus-like treatments. (B) Gene ontology analysis of top 10 cellular components associated with differentially expressed genes.

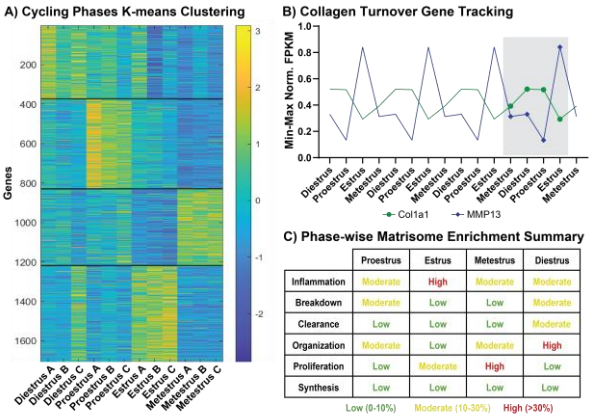

Figure 3. (A) K-means clustering of differentially expressed genes across simulated estrous cycle phases. (B) Phase-averaged, min–max normalized FPKM data used to visualize phase-dependent expression patterns of collagen I and MMP13. (C) Phase-wise matrisome enrichment highlighting enriched functional categories across estrous cycle phases (GO-based).

RESULTS: Differential expression between cycling and constant metestrus-like groups revealed a total of 599 significantly altered genes, with 109 upregulated and 490 downregulated in the cycling group (Fig. 2A). Gene ontology identified enrichment of pathways associated with microtubule and spindle organization, chromosome condensation, and collagen-containing extracellular matrix, with cycling metestrus exhibiting downregulation of proliferative and mitotic terms and mixed regulation among collagen-related genes (Fig. 2B). K-means clustering of genes across the four cycling phases demonstrated clear phase-specific transcriptomic patterns, with the four gene clusters corresponding to distinct simulated stages of the estrous cycle (Fig. 3A). Tracking of extracellular matrix–related genes revealed complementary fluctuations in collagen synthesis (Col1a1) and degradation (MMP13) across the four phases (Fig. 3B). Phase-wise analysis revealed dynamic changes in ECM-related activity across the simulated estrous cycle, with distinct shifts in inflammation, matrix remodeling, and proliferation between phases (Fig. 3C). Specifically, matrisome enrichment analysis indicated moderate inflammation, breakdown, and organization during proestrus; high inflammation and moderate proliferation during estrus; moderate inflammation and organization with high proliferation during metestrus; and moderate inflammation, breakdown, and clearance with high organization during diestrus (Fig. 3C).

DISCUSSION: Our transcriptomic analysis revealed that simulated estrous cycling drives fluctuating, phase-dependent ECM remodeling, while constant hormone conditions result in altered dynamics. Notably, tendons exposed to simulated cycling demonstrated fluctuations in collagen turnover, reflecting continuous ECM adaptation throughout the cycle. K-means clustering of cycling groups revealed distinct phase-specific transcriptomic signatures, emphasizing that each phase of the estrous cycle elicits unique transcriptomic responses. During proestrus, moderate inflammation and matrix breakdown suggest early ECM restructuring, potentially to clear damage in preparation for new deposition. In estrus, heightened inflammatory activity and the onset of increased proliferation mark an early tissue response phase. During metestrus, increased proliferation and matrix organization indicate a transition to tissue repair and reinforcement. Finally, in diestrus, peak organization and clearance indicate a phase focused on matrix stabilization and maintenance. This cyclicity could influence microdamage repair capacity or injury risk at certain phases of the cycle. Interestingly, the integration of cytoskeletal and matrix-related signaling suggests that fluctuations also regulate aspects of cellular organization and mechanotransduction. Together, these findings demonstrate that cyclic fluctuations may be essential to tendon homeostasis, highlighting the importance of physiologic hormonal supplementation models in experimental studies.

SIGNIFICANCE/CLINICAL RELEVANCE: This study highlights the critical role of physiologic hormonal cycling in maintaining tissue health and ECM remodeling, underscoring the importance of incorporating physiological hormonal cyclicity into future research on women's musculoskeletal health.

REFERENCES: [1] Hansen+2016. Adv Exp Med Biol. 920:139-149. [2] Fryhofer+2016. J Appl Physiol. 121(5):1106-1114. [3] Wojtys+2002. Am J Sports Med. 30(2):182-188. [4] Shultz+2006. J Orthop Res. 24:124–131. [5] Eiling+2007. KSSTA. 15:126–132. [6] Aggouras+2024. J. Biomech. Eng. 1-41. [7] Lee+2004. Matrix Biol. 23:323-329. [8] Tashjian+2021. J. Orthop. Res. 39:2711-2724. [9] Naba+2011. Mol Cell Proteomics. 11(4).

ACKNOWLEDGEMENTS: This work was supported by funding from the NIH NIGMS (R35-GM151127), a Wu Tsai Human Performance Alliance Agility Award, and National Science Foundation Research Traineeship (NRT) in Biological Feedback Control (NSF-DGE #2244366).