

# Peak Estradiol Concentrations Increase Collagen Degradation by Female, but Not Male, Meniscal Cells

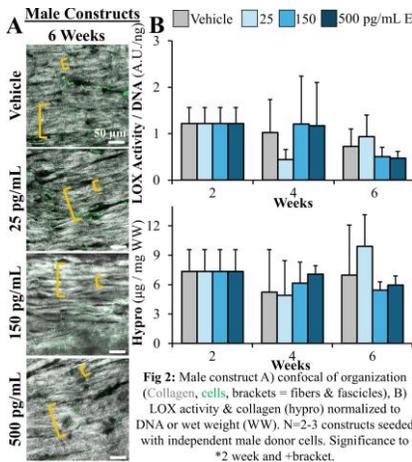
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**DISCLOSURES:** Andrea M. Frankel (N), Jennifer L. Puetzer (N)

**INTRODUCTION:** Menisci are semi-lunar wedge-shaped discs that aid in knee load distribution. They are able to distribute these loads due to a complex collagen organization, dominated by circumferentially aligned fibers.<sup>1</sup> Cells organize these fibers hierarchically, assembling them from nm-wide fibrils into larger fibers and fascicles.<sup>2</sup> Injuries disrupt these fibers resulting in pain and reduced function. When accounting for sport, adolescent females are 2-6 times more likely to tear their meniscus or anterior cruciate ligament (ACL) than males.<sup>3</sup> For the ACL, this increased injury rate has been attributed to sex-hormones, with females having increased ACL laxity and injury-rate when estradiol (E2) peaks prior to ovulation.<sup>4</sup> Due to the major role of collagen fibers in the ACL, laxity changes have been attributed to changes in collagen fibers. E2 has been shown to inhibit LOX activity in ACL fibroblasts,<sup>5</sup> suggesting peaks in E2 may reduce LOX crosslinking resulting in weaker ACLs, however the mechanism remains unknown limiting injury prevention. Further, despite similar sex-based injury rate, little work has explored effects of E2 on menisci. The objective here was to explore how E2 dose affects human meniscal fibrochondrocyte (hMFC) regulation of hierarchical fibers. To do this, we will use our established culture system which guides cells in high-density collagen gels to develop native-sized hierarchically organized collagen fibers over 6 weeks of culture (Fig.1A).<sup>2,6</sup> We hypothesize high concentrations of E2, matching peak concentration prior to ovulation, will reduce LOX activity, significantly reducing mature crosslinks and overall tissue strength in male and female hMFC-seeded constructs.

**METHODS:** Human meniscal fibrochondrocytes (hMFCs) were isolated from partial meniscectomy waste of deidentified donors (13-19 years old, N=3 per sex, IRB Exempt). Cells from each donor were expanded (PDL 12-14) and seeded individually into high-density collagen gels as previously described.<sup>2,6</sup> Briefly, hMFCs were mixed into collagen and cast into 1.5 mm thick sheet gels at 20 mg/mL collagen and  $5 \times 10^6$  cells/mL.<sup>2,6</sup> Rectangles (8x30 mm) were cut from the gels and clamped in our culture device to guide cells to form aligned fibrils by 2 weeks, and larger fibers and early-fascicles by 6 weeks (Fig.1A-B).<sup>2,6</sup> At 2 weeks, once cells had formed aligned fibrils, construct media was switched from our standard media (DMEM, 10% FBS, 1% antibiotics/antimycotic, 0.1 mM non-essential amino acids, 50 ug/mL ascorbic acid, and 0.8 mM L-proline<sup>2</sup>) to media with the same composition, only using phenol-free DMEM and charcoal stripped FBS to eliminate the effect of endogenous hormones. From weeks 2 to 6, while cells were forming larger fibers, constructs were dosed with E2 concentrations representative of low (25 pg/mL), medium (150 pg/mL), and peak (500 pg/mL) female serum concentrations during the menstrual cycle which correspond to the follicular phase, luteal phase, and pre-ovulation respectively (Fig.1C).<sup>7</sup> Vehicle controls were dosed with ethanol. Post culture, collagen organization was evaluated via confocal reflectance.<sup>2</sup> DNA, glycosaminoglycan (GAG), lysyl oxidase (LOX) activity and collagen were determined via PicoGreen, DMMB, LOX activity, and hydroxyproline (hypro) assays, respectively.<sup>2</sup> Tensile properties were analyzed by tensile tests at 0.75% strain/sec to failure.<sup>2</sup> All data are mean  $\pm$  standard deviation. Significance determined via 2-way ANOVAs with Tukey's post-hoc ( $p < 0.05$ ).

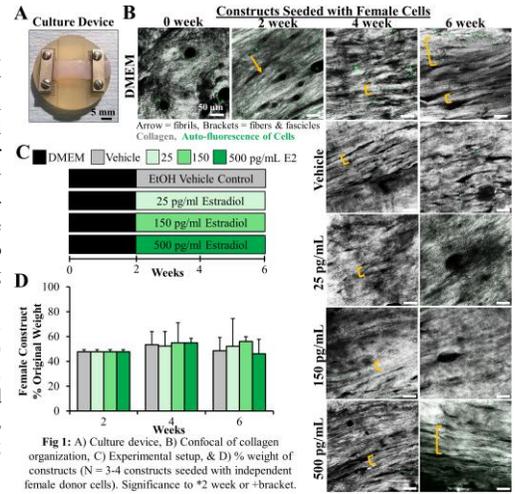


**Fig 2:** Male construct A) confocal of organization (Collagen, cells, brackets = fibers & fascicles), B) LOX activity & collagen (hypro) normalized to DNA or wet weight (WW). N=2-3 constructs seeded with independent male donor cells. Significance to \*2 week and +bracket.

confocal observations and tensile properties may suggest the peak of E2 for 2-3 days prior to ovulation may increase collagen degradation, allowing cells to produce collagen fibers quicker in our system, but reduce collagen concentration in the short-term, producing weaker tissues. If this is the case, it may suggest that E2 kinetics, rather than concentration, may drive changes in tissues. This same trend was not observed in constructs seeded with male hMFCs, which showed consistent fiber formation and no significant differences in composition between treatment groups, despite 150 and 500 pg/ml being supraphysiological for males. Ongoing work is increasing sample size, and evaluating tissue mechanics, hierarchical fiber formation, LOX crosslinking, and collagen degradation.

**SIGNIFICANCE:** Overall, we found peak E2 concentrations reduced collagen in the short-term, which may explain increased meniscal injuries in females. However, long-term exposure to high E2 increased collagen concentration and organization, suggesting that E2 kinetics, rather than concentration, may drive changes. A better understanding of how E2 affects collagen regulation by fibrochondrocytes will help reduce female meniscal injuries and inform hormone therapy.

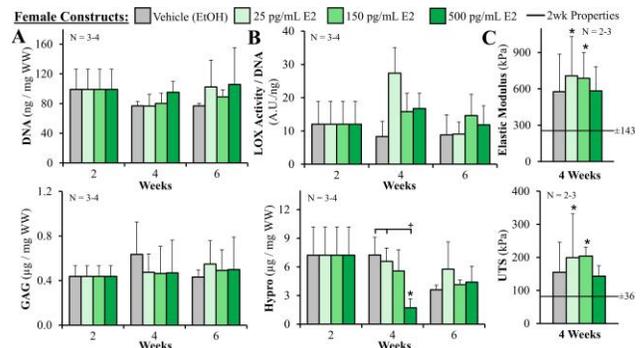
**REFERENCES:** 1.Nesbitt+*JOR* 2024; 2.Puetzer+*Biomater* 2021; 3.Hernandez+*OARS* 2024; 4.Wojtyś+*Am J Sports Med* 2002; 5.Lee+*J Appl Physiol* 2015; 6.Gouldin+*SB3C trans.* 2023. 7.Sluss+*Clinica Chimica Acta* 2008.



**Fig 1:** A) Culture device, B) Confocal of collagen organization, C) Experimental setup, and D) % weight of constructs (N=3-4 constructs seeded with independent female donor cells). Significance to \*2 week or +bracket.

**RESULTS:** Similar to previous work,<sup>2,6</sup> male and female hMFCs produced aligned collagen fibrils by 2 weeks which matured into larger fibers and early-fascicles by 6 weeks in our culture system (Fig.1B). Interestingly, in constructs seeded with female cells, the lower E2 concentrations (25 and 150 pg/mL) appeared to reduce fiber formation compared to DMEM and vehicle controls, however the high 500 pg/mL E2 group appeared to have accelerated and increased fiber formation compared to controls (Fig. 1B). Conversely, constructs seeded with male cells had consistent fiber formation by 6 weeks regardless of E2 dose (Fig. 2A) and had no significant compositional differences between treatment groups (Fig. 2B, DNA and GAG not shown). Similarly, female constructs for all treatment groups had similar contraction, percent weight (Fig. 1D), DNA, and GAG concentrations throughout culture (Fig.3A). Further, there were no significant differences in LOX activity for female constructs (Fig.3B), however the 25 pg/mL E2 group appeared to have an increase at 4 weeks, albeit non-significant. Interestingly, the 500 pg/mL E2 female group had significantly reduced collagen at 4 weeks, which later recovered by 6 weeks (Fig.3B). Mirroring reduced collagen concentrations, the 500 pg/mL E2 group did not have improvements in tensile properties at 4 weeks, while the lower E2 female groups had significant improvements compared to 2 weeks (Fig.3C).

**DISCUSSION:** Contrary to our hypothesis, there were no significant differences in LOX activity (Fig. 2B, Fig. 3B), however in female constructs the 25 pg/mL E2 group appeared to have an increase in activity at 4 weeks, which may become significant with increased donors. In female constructs, the 500 pg/mL E2 group had a significant reduction in collagen at 4 weeks, which then recovered by 6 weeks. This, combined with



**Fig 3:** A) DNA and GAG content normalized to wet weight (WW), B) LOX activity and collagen (hypro) content in constructs, and C) Tensile properties at 4 weeks. Significance to \*2 week and +bracket.