Elucidating the role of inflammation and hormones on sex differences in acute post-ACL tear outcomes

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DISCLOSURES: Kaneda G (4, Intuitive Surgical, AbbVie, Pfizer, Eli Lilly & Co.), Zila L (N), Sheyn J (N), Huang D (N), Huo L (N), Trentacosta N (N), Schimmoeller N (N), Mandelbaum B (3C, Arthrex, Apex Biologics), Jefferies C (N), Tawackoli W (N), Metzger M (5, Arthrex, ConMed, ATEC; 8, JSES, AJSM), Sheyn D (N).

INTRODUCTION: Anterior cruciate ligament tears (ACLT) are among the most common musculoskeletal injuries in the US, with an estimated of 100,000–200,000 cases annually. Women are at particularly high risk for both primary ACL injury and re-tear, exhibiting a 2- to 8-fold increased risk of ACLT compared to men and a 30% higher likelihood of graft re-rupture. This heightened vulnerability is believed to result from a combination of factors, including increased joint laxity, anatomical differences in hip and knee alignment, smaller ACL size, and hormonal fluctuations. In this study, we aim to elucidate the role of hormones and inflammation on ACL tear and healing by employing a multi-omic approach to inform future sex specific treatments and prevent post-traumatic osteoarthritis (PTOA).

METHODS: All animal and patient studies were approved by the institutional IACUC and IRB respectively. Blood and biomechanic measures from female athletes on and off hormonal contraceptives (HC, Non-HC n=32, HC n=40, total n=72) were analyzed via ELISA and motion capture analysis. ACL tissue and synovial fluid from patients with a confirmed ACLT were also collected. Cells isolated from digested ACLs (n=7 per sex, total n=14), undigested ACL tissue (male n=20, female n=12, total n=32), and synovial fluid (male n=11, female n=10, total n=21) then underwent bulk proteomic profiling, which was analyzed using MetaboAnalyst and Qiagen Ingenuity Pathway Analysis (IPA) to identify sex-specific differences in protein expression and associated biological pathways. To validate and extend our human findings in a controlled model, we induced an ACLT in male and female rats (n=8 per sex for ACLT, n=4 per sex for sham, total n=24) and performed proteomic analysis of their synovium at two weeks post-ACLT. Single cell transcriptomics (scRNA-seq) analysis using the Seurat and CellChat packages was performed on a publicly available dataset (GSE280537) of synovium from three male and three females undergoing ACL reconstruction following ACLT to identify cell identity and evaluate intercellular communication between sexes. In addition, IPA was used to identify enriched signaling pathways between sexes within a single cell population.

RESULTS: Analysis of female athletes on and off HC demonstrated significant differences in systemic sex hormone concentration (**Fig. 1A**). Estrogen levels were found to be significantly correlated with multiple inflammatory cytokines while progesterone was found to be significantly correlated with multiple biomechanical outcomes including hip flexion and extension at moment of initial contact, max ground reaction force and max knee flexion (**Fig. 1B**). Proteomic analysis of ACL derived cells (**Fig. 1C**), fresh ACL tissue and synovial fluid revealed significant sex differences across all sample types. IPA pathway analysis indicated that immune-related pathways were consistently upregulated in females across all sample types, while only ACL derived cells exhibited downregulation of extracellular matrix (ECM)-associated pathways (**Fig. 1D**). Proteomic analysis of synovium from rats with and without ACLT showed distinct sex dependent response at 2 weeks post-injury (**Fig. 1E**). ScRNA-seq analysis identified 11 distinct cell types within the human synovium with males having relatively higher proportions of B-cells, dendritic cells, and T-cells but no difference in total macrophages (**Fig. 1F**). However, when macrophage population was broken down to subtypes, females exhibited elevated levels of pro-inflammatory macrophages **(Fig. 1G**). CellChat predicted increased signaling in females related to immune activation, inflammation, tissue repair, and fibrosis, while males demonstrated enhanced pathways associated with ECM remodeling, cellular growth, and antigen presentation (**Fig. 1H**). IPA analysis of synovium scRNA-seq samples predicted increased immune pathway activation in females and upregulation of protein translation pathways in males (**Fig. 1I**).

SIGNIFICANCE / CLINICAL RELEVANCE: Together, these results suggest that across multiple ACL-associated tissues, females mount a more activated and inflammatory immune response post-injury, despite males having a greater abundance of immune cell populations. Conversely, males exhibit a molecular profile favoring structural remodeling. These sex-specific molecular signatures in the ACL, synovium, and synovial fluid highlight divergent biological responses to injury. Females display enhanced immune activation and chronic stress signaling, whereas males emphasize tissue repair and regulatory mechanisms. These findings emphasize the importance of incorporating sex as a biological variable in developing targeted therapies to mitigate ACL re-tear and PTOA risks following ACL injury and reconstruction.

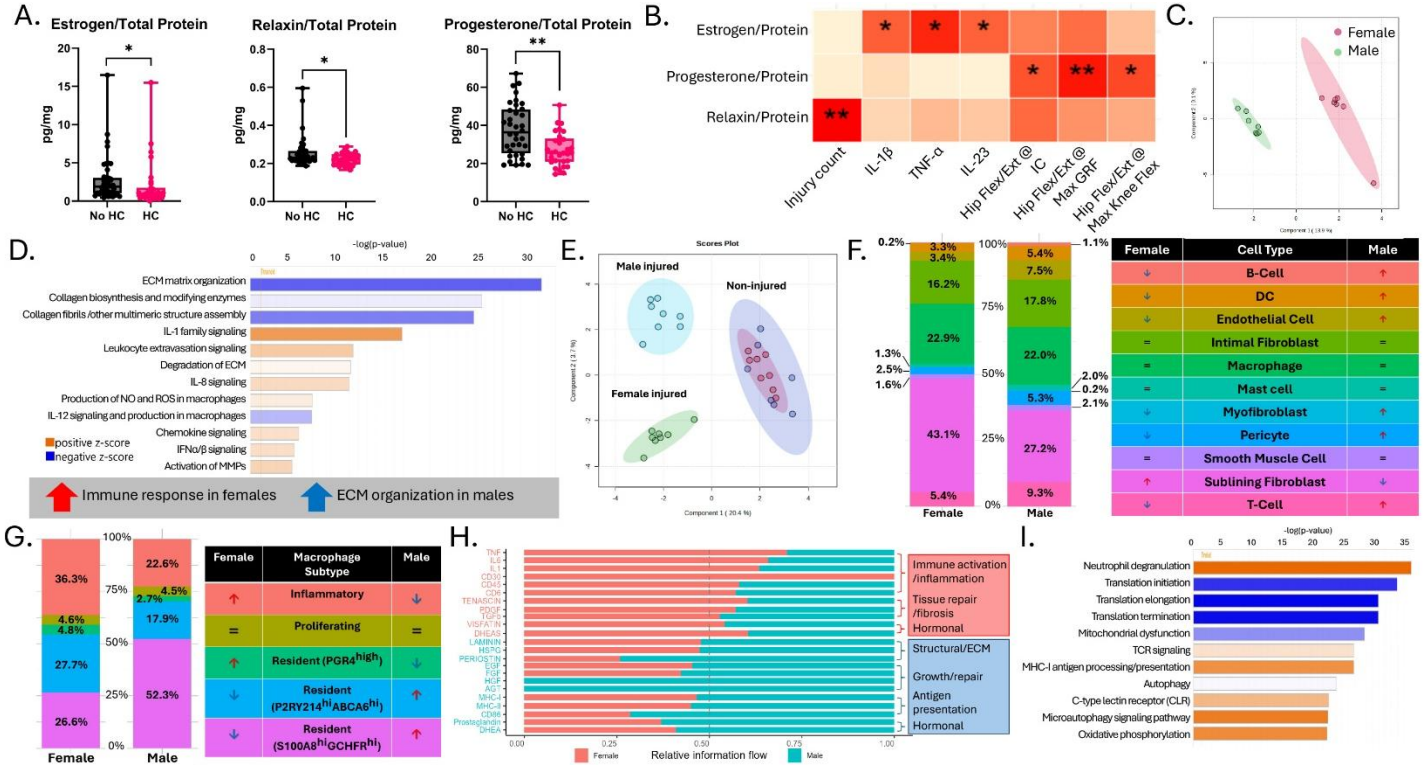

Figure 1: Sex differences in immune response and tissue composition observed following ACL tears. (A). Systemic estrogen (Left), relaxin (middle), and progesterone (right) levels in female athletes on and off hormonal contraceptives. **(B)**. Correlation of sex hormones with injury count, select inflammatory cytokines, and select biomechanic measures. **(C).** sPLS of male and female ACL-derived cells proteomic samples. (D). IPA Pathway analysis of male and female ACL derived cells, **(E).** sPLS of synovium proteomic samples from male and female rats with ACL tear or sham, **(F).** Proportion of cells identified in male and female synovium, (G). Proportion of different macrophage types identified in male and female synovium, (H). Intercellular communication analysis of male and female synovium (I) IPA Pathway analysis of male and female synovium. *p<0.05, **p<0.01