Sex Influences the Biomechanical Outcomes of Anterior Cruciate Ligament Reconstruction in a Pre-Clinical Large Animal Model

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Introduction: Anterior cruciate ligament (ACL) injury is prevalent among young athletes, with women being at 2-10 fold greater risk than men playing the same sport [1]. Despite many advances in surgical and rehabilitation interventions, patients who have suffered ACL injury face long-term consequences that include decreased activity, a 10-25% incidence of re-injury within 5 years after surgery and a 50-100% incidence of osteoarthritis within 10-15 years of injury [1,2]. The study of sex disparities in ACL injury has emerged as a hot topic for research especially following the Institute of Medicine report in 2001 (Exploring the Biological Contributions to Human Health: Does Sex Matter?) and the 2004 combined AAOS and NIH workshop on "The Influence of Sex and Gender on Musculoskeletal Health". Despite the well-described role of sex on ACL injury risk, the role of sex on the outcomes of ACL surgery has been a topic of considerable debate over the past decade with limited and inconclusive results [3]. We have previously shown that the porcine model provides a mean to evaluate the sex-related differences in ACL surgery outcomes, as it has similar sex-specific differences in anatomy and biomechanics as to human [4]. Using this model, we are now poised to investigate the role of sex on the structural outcomes of ACL reconstruction, as the current standard of care. We hypothesized that there are sex-specific differences in the outcomes of ACL reconstruction with regards to graft structural properties, knee laxity and cartilage damage.

Methods: Following IACUC approval, 41 normal adolescent Yucatan mini-pigs (23 M, 18 F; 15±1 months) underwent unilateral ACL transection and were randomly assigned to one of 4 treatment groups [5]: conventional ACL reconstruction (n=13), or bio-enhanced ACL reconstruction with 1X (n=10), 3X (n=9) and 5X (n=9) platelet-rich plasma (PRP). ACL reconstruction was conducted using an allograft bone-patellar tendon-bone graft. The bio-enhanced ACL reconstruction was performed by adding a bio-active ECM-based scaffold (MIACH, Boston Children's Hospital, MA) around the graft after fixation. The surgical knee was randomly selected, and the contralateral ACL-intact knee served as control. Pigs were euthanized after 15 weeks, and both limbs were harvested, prepared and tested for knee anteroposterior (A-P) laxity and graft mechanical properties using a universal tensile testing machine (MTS, Eden Prairie, MN) [5]. The A-P knee laxity was measured at 30°, 60° and 90° of knee flexion through 12 cycles of ±40 N A-P shear force. The capsule and other ligaments were then dissected and graft mechanical properties were determined at 30° of knee flexion under a ramping tensile load to failure, applied to the femur-graft-tibia construct. Grafts were then dissected and prepared for histology and then stained using H&E and α-SMA antibodies [6]. Grafts were histologically scored using the Ligament Maturity Index based on three sub-scores evaluating the cellular, collagen and vascular organization [6]. Finally, macroscopic cartilage damage was assessed by measuring the area of all visible lesions across the tibiofemoral cartilage using India ink and calipers [5]. Cartilage samples were also
scored using a common macroscopic scoring method with a five-point scale ranging from 0 (no damage) to four (lesions with exposed bone >10 % of the lesion area) [5]. Measurements were conducted by two independent examiners, blinded to the treatment and sex, and averaged values were used. The graft structural measures were normalized by dividing by the value of the intact ACL of the contralateral unoperated knee and reported as the % intact. The side-to-side difference in A-P knee laxity was defined as the difference in treated knee laxity and the contralateral intact side at each flexion angle. A two-factor ANOVA was used to investigate the effect of sex on all the measured outcomes after adjusting for the treatment effect (p≤0.05).

**Results:** All animals recovered well from surgery and survived the full 15-week follow-up term with no signs of infections or other complications. Full weight-bearing status was achieved within 48 to 72 hours post-surgery. Females had a significantly lower normalized graft yield and linear stiffness (p<0.05) and a marginally significant normalized graft maximum load (p=0.058; Fig 1). Females had a greater side-to-side difference in A-P knee laxity compared to their male counterparts (Fig 1). These differences were statistically significant at 30° and 90° (p<0.05). Female pigs had a greater degree of cartilage damage after conventional ACL reconstruction than their male counterparts (Fig 3). These differences were statistically significant for the area of cartilage damage on the medial femoral condyle (p=0.014) and total cartilage score for the entire knee (p=0.043) with differences in damaged area for the entire knee and the medial femoral cartilage score approaching statistical significance (p= 0.059 and p=0.066 respectively). Bio-enhanced ACL reconstruction resulted in significantly less cartilage damage (p<0.05).

**Discussion:** To our knowledge, this is the first time that sex has been demonstrated to significantly alter the outcome of ACL surgery in a large animal preclinical model. We have previously validated the porcine large animal model as a sex-specific model with similar sex differences in knee anatomy, laxity, and ACL biomechanics as the human knee [4]. Moreover, the pig knee has been shown to be the closest surrogate model for the human knee based on the kinematics and wound healing characteristics [1]. These support the current model as a valid approach to study the sex-specific differences in human knee pathology and surgical interventions with a special focus on the ACL. In summary, the current findings support our hypothesis that sex significantly affects the graft structural properties and A-P knee laxity after ACL reconstruction, with females having weaker and less vascular grafts and looser knees. In addition, the female knees had greater cartilage damage than their male counterparts, a difference which was ameliorated with the addition of an extracellular matrix-based scaffold loaded with autologous PRP. These findings are in agreement with the findings of human clinical trials showing a higher knee laxity with increased pain frequency and intensity along with the worse knee function and patient oriented outcomes in women [7,8].

**Significance:** The current findings highlight the importance of further optimization of the current treatments to better fit each sex instead of a “one fits all” approach. This may in turn lead to improved surgical outcomes, decreased incidences of graft failure and re-injury, and a decreased risk of PTOA following ACL injury and reconstruction, especially among women.
Fig 1: Sex-specific differences in normalized graft structural properties and differences in side-to-side differences in A-P knee laxity (Mean ± SEM).

Fig 2: Sex-specific differences in graft histological properties (Mean ± SEM).
Fig 3: Sex-specific differences in macroscopic cartilage damage for animals undergone conventional ACL reconstruction (Mean ± SEM).