Anterior Cruciate Ligament Size is Associated with Timing of Puberty Onset in the Skeletally Immature Porcine Knee Joint

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INTRODUCTION: The incidence of anterior cruciate ligament (ACL) injuries is rising rapidly, with over 200,000 cases reported annually in the United States [1]. Among adolescents, female athletes are 3 to 4 more times likely than male athletes to experience an ACL tear in sports that involve frequent cutting and jumping, such as soccer and basketball [2]. Smaller ACL size, such as volume and cross-sectional area (CSA), has been linked to increased injury risk [3,4], and indeed, females have smaller ACLs compared to males, on average [3,5]. Puberty represents a critical window during which physiological and hormonal changes may influence injury risk. Specifically, studies have shown that significant sex differences in ACL size begin to emerge during late puberty [3]. In females, the onset of puberty typically occurs between 10 and 14 years with the onset of menarche [2]. However, irregular and anovulatory menstrual cycles are common post menarche. Notably, early onset of puberty shortens the interval between menarche and cycle regularity by a factor of 3 to 4, suggesting increased early exposure to estrogen for those with early puberty onset [3,4]. The ACL expresses receptors for several sex hormones, including estradiol (E2), progesterone (P4) [6], and testosterone (T) in females [7], indicating potential sensitivity to hormonal fluctuations. However, the relationship between the timing of puberty onset, early hormone exposure, and ACL development remains poorly understood. To begin addressing this gap, this study utilizes a porcine model, as pigs exhibit an estrus cycle similar in duration (3 weeks) to humans [8]. Furthermore, the porcine model preserves both the growth-related and sex-based differences in ACL size observed in humans [9,10]. Therefore, this study aims to evaluate the association between the timing of puberty onset and ACL size in a skeletally immature porcine model.

METHODS: For this study, female Yucatan minipigs (n=12) were longitudinally monitored from ~4 months of age (pre-pubescent) to ~10 months of age (mid-adolescence) (Fig. 1). Throughout the study period, serum samples were collected under sedation weekly from each animal, with increased sampling frequency (three times per week) before magnetic resonance imaging (MRI) and euthanasia. Body weight was recorded biweekly. Progesterone (P4) was quantified using a custom enzyme-linked immunosorbent assay (ELISA), while estradiol (E2) levels were measured via radioimmunoassay (RIA). MRI scans were performed at 10 months of age with a T2 susceptibility weighted imaging sequence and were timed to occur seven days prior to the time of estrus (when E2 is low and P4 is at its peak). From MRI scans, the ACL was manually segmented to assess volumetric and cross-sectional area (CSA) metrics [5]. Associations between puberty onset and ACL morphology were assessed using a linear regression (α=0.05). Correlation strength was measured with R (range: -1 to 1), where $|R| < 0.3$ indicates no association, 0.3–0.5 weak, 0.5–0.7 moderate, and >0.7 strong. This study exclusively used female animals, since the objective was to examine the relationship between puberty onset (and related changes in female sex hormones) and ACL morphology.

RESULTS: In this study, progesterone (P4) peaks lasted longer than estrogen peaks, making them easier to detect via weekly blood draws to assess when the pigs began puberty and to monitor their cycles throughout maturation (Fig. 2A). Progesterone levels remained below 0.2 ng/mL until the onset of puberty and then peaked every ~3 weeks. Puberty onset, defined by the first progesterone peak, occurred at an average age of 6.3 ± 0.6 months and ranged from 5.19-7.30 months (Fig. 2B). Earlier puberty onset was moderately associated with greater ACL size, as measured by both volume and CSA (Fig. 3A and 3B). The timing of puberty onset was moderately associated with final body weight at 10 months (Fig. 3C). Additionally, the weight of the pigs at 10 months was strongly associated with both ACL volume and ACL CSA (Fig. 3D and 3E). When normalized to the final weight there was a weak association with ACL volume and puberty onset (R=0.48, p=0.11) and no association between ACL CSA and puberty onset (Fig. 3F).

DISCUSSION: This study demonstrates that earlier puberty onset is associated with both increased ACL size and higher body weight. These findings are consistent with existing literature showing that girls who experience menarche earlier often have greater fat mass, weight, and BMI [11,12]. However, some of these associations may be due to heavier individuals reaching menarche earlier and remaining heavier into adulthood. The long-term effects of pubertal timing remain largely unknown in humans. Human studies have linked smaller ACL size to a higher risk of injury [3], and the current data suggest that pubertal timing influences ACL size. However, these associations are no longer significant when accounting for body weight, so the role of timing of puberty onset on ACL injury risk remains unclear and further work is needed. In ongoing work, a larger set of animals as well as biomechanical and biochemical changes in the ACL over longer time periods will be assessed. This will allow for a comprehensive analysis on how the timing of puberty onset can impact ACL morphology and function throughout maturation.

SIGNIFICANCE/CLINICAL RELEVANCE: This study determines the onset of puberty in a porcine model and associates early hormone exposure with increased ACL size, while looking at long term impacts with injury risk.

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ACKNOWLEDGEMENTS: We would like to acknowledge the NCSU College of Veterinary Medicine and Laboratory Animal Resource as well as the Clinical Endocrinology Laboratory at UC Davis School of Veterinary Medicine for all their help.

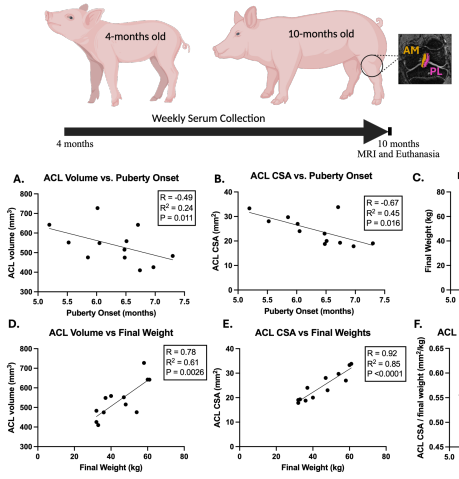

Figure 1. Overview of study design over the 6-month period.

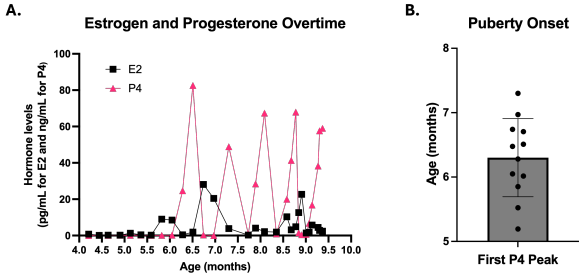

Figure 2. (A) Typical weekly estrogen (E2) and progesterone (P4) levels for a single animal across 7 months. (B) Average age of puberty onset (n=12) based off the first P4 peak.

Figure 3. Associations between puberty onset and (A) ACL volume, (B) ACL CSA, and (C) final weight. Additional associations between final weight and (D) ACL volume and (E) ACL CSA. (F) Lack of association between ACL CSA normalized by final weight and puberty onset.