LACK OF HORMONAL INFLUENCES ON MECHANICAL PROPERTIES OF SHEEP KNEE LIGAMENTS

**Strickland, S M; *Belknap, T W (A-Dana and Clark); *Levine, R E (A-Dana, Clark, and NIH); **Turner, A S (A-NIA, B-Lilly); *Wright, T M (A-Dana and Clark); **Hannafin, J A

+*Hospital for Special Surgery, New York, NY. 535 E. 70th St./New York, NY 10021, (212) 606-1466, Fax: (212) 606-1490, Strickland@hss.edu

**Colorado State University, Dept. of Clinical Sciences, Fort Collins, CO.

Introduction The rate of anterior cruciate ligament (ACL) injury in female athletes has been reported to be four to five times that of male athletes at similar levels of athletic competition. In contrast, medial collateral ligament (MCL) injury rates were only slightly higher in women. Some have attributed this gender difference to alterations in knee laxity in women, resulting from hormones such as estrogen, progesterone, and relaxin. Instrumented measurements of ACL laxity at different phases of the menstrual cycle have suggested a correlation between estrogen and knee laxity. Other theoretical explanations for the disparity between men and women ACL injury rates include differences in intercondylar notch width and neuromuscular performance. In this experiment we hypothesized that ACL and MCL strength, stiffness, and energy to failure would be altered by administration of either estrogen or a selective estrogen receptor agonist (raloxifene). The sheep was chosen as a large animal model, because it has a hormonal milieu similar to that of women.

Methods The project received IACUC approval. Thirty-eight mature ewes, same breed/housing, were randomly divided into five, six-month duration, treatment groups: sham operated (n=6), ovariectomized (OVX; n=9), OVX and estradiol implant (OVXE; n=7), 0.02 mg/kg/day raloxifene after OVX (RAL1; n=9), and 0.1mg/kg/day raloxifene after OVX (RAL2; n=7). The raloxifene was delivered subcutaneously daily. The animals were killed and the knees were frozen at -20°C. The knees were subsequently thawed and stripped of all soft tissue aside from the MCL and the anteromedial bundle of the ACL. The origin of the MCL was osteotomized from the medial femoral condyle and potted in PMMA. All ligaments were measured at midsubstance using an area micrometer to determine cross sectional area. After potting the tibia and femur in PMMA, the bone-ACL-bone construct was positioned in 45° of knee flexion. Using a servohydraulic mechanical tester (Instron; Canton, MA), the tibia was displaced vertically at a rate of 5mm/sec until complete failure of the ligament. The bone-MCL-bone construct was then displaced axially in line with the MCL fibers. Displacements of the ligament insertions were recorded with a digital motion capture system (QUALISYS; Glastonbury, CT). Analysis of variance was utilized for statistical evaluation.

Results Due to damage during dissection or testing difficulties, the MCL sample sizes were decreased an average of 2 per group. However, the minimum MCL sample size was 3. Maximum load, stiffness, and energy to complete failure are summarized in Figures 1, 2, and 3. No differences were found between force (p=.177), stiffness (p=.565), and energy (p=.182) among the ACL treatment groups. Likewise, no differences existed between MCL treatment groups in force (p=.614), stiffness (p=.647), and energy (p=.852).

Discussion Several studies have suggested that estrogen may affect laxity of ACL’s in females, however, few have isolated ligaments for mechanical testing. A recent report isolated rabbit ACL’s and compared an ovariectomized group to an estrogen supplemented group. In the rabbits, significant reduction of load to failure was demonstrated in the estrogen treated group. However, the estrogen supplement was consistent with the knowledge that investigates the effect of a physiologic dose of estrogen or estrogen receptor agonists on knee ligaments of a large animal. Our study did not demonstrate that estrogen and estrogen receptor agonists alter the measured ligament mechanical properties in a sheep model.


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**Fig 1. Maximum Force**

- Sham
- OVX
- OVXE
- RAL1
- RAL2

**Fig 2. Stiffness**

- Sham
- OVX
- OVXE
- RAL1
- RAL2

**Fig 3. Energy to Failure**

- Sham
- OVX
- OVXE
- RAL1
- RAL2

Approximately one-third of the ACL’s failed at the distal ligament-bone interface; two-thirds failed at mid-substance. There was no statistical difference between groups in terms of mid-substance cross sectional area for the ACL (p=.728) and the MCL (p=.504).

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