Introduction: Female athletes have a greater incidence of non-contact anterior cruciate ligament (ACL) injuries than their male counterparts. Hormonal gender differences have been thought to present a possible explanation for this discrepancy. Several laboratories have demonstrated the presence of sex steroid hormone receptors in fibroblasts of human and animal ACLs by immunohistochemistry and reverse transcription-polymerase chain reaction (RT-PCR). In vitro study demonstrated that fibroblast proliferation and type 1 procollagen synthesis in human ACL fibroblasts were reduced with increasing estradiol concentration. However, to our knowledge, there have not yet been any in vivo studies to elucidate the influence of sex steroid hormones on ACL histologically. We hypothesized sex steroid hormones have some effects on the composition of extracellular matrix in ACL.

Materials and Methods: Thirty-four Wistar female rats aged 12 weeks were used. By the ovariectomy (OVX) and hormone replacement, 5 groups of animal models were prepared. The hormone replacement was performed by the subcutaneous implantation of a sustained-release pellet (Innovative Research of America) containing β-estradiol 3-benzoate (E2) or progesterone (P4). Animals were divided into OVX + E2 group (n=8), OVX + P4 group (n=5), OVX + E2 and P4 group (n=5), OVX group (n=8), and sham operation group (n=8). After 30 days, knee joints were rapidly frozen and undecalcified fresh-frozen sagittal sections tightly attached to the adhesive film (Cryofilm®, Finetec, Japan) were cut at 14μm on Cryostat (CM3050S, Leica, Germany). The sections were stained with 0.05% toluidine blue for the histological evaluation of ACL. Following sections were used for the immunofluorescence stainings of estrogen receptor α (ERα), ERβ, and type 1 and 3 collagen. Images in the insertion site to femur and the midsubstance of ACL were captured under identical conditions using a confocal laser scanning microscope (LSM 510 META, ZEISS, Germany). The mean optical density (OD) of collagen-immunoreactive area was measured and normalized to the OD of the sham operation group using ImageJ. Additionally, plasma concentrations of estradiol (E2) and progesterone (P4) before sacrifice were measured using enzyme immuno assay. The data on the immunoreactivity of collagens were analyzed using Student’s t-test. The data on the plasma concentration of E2 and P4 were analyzed using one-way ANOVA.

Results: ACL insertion consisted of fibrochondrocyte cells, and ACL midsubstance consisted of fibroblasts and dense collagen fibers (Fig.1). In all groups, the fibrochondrocyte cells in the insertion and fibroblasts in the midsubstance were stained with both ERα and ERβ. The immunoreactivity of ERα was much higher than ERβ. By E2 replacement, the immunoreactivities of both ERα and ERβ were elevated compared to the OVX group, while there were no significant changes by P4 replacement (Fig.2)

In the insertion, the immunoreactivities of type 1 collagen by E2 replacement, and type 3 collagen by P4 replacement, were significantly reduced compared to the OVX group. In the midsubstance, the immunoreactivity of type 3 collagen was significantly elevated by E2 or combined E2 and P4 replacement compared to the OVX group (Fig.3).

The plasma concentration of E2 in E2 replacement group and the one of P4 in P4 replacement were significantly higher than the OVX group.

Discussion: We demonstrated that both ERα and ERβ co-existed in the fibrochondrocyte cells in the insertion and fibroblasts in the midsubstance, and that the expressions of both estrogen receptors were influenced by plasma levels of E2, suggesting that ACL may be an estrogen-dependent tissue. We showed that estrogen down-regulated the expressions of type 1 collagen in ACL insertion. This finding is consistent with the data of in vitro study. Furthermore we demonstrated for the first time that estrogen and progesterone have some effects on the expressions of type 3 collagen in both ACL insertion and midsubstance. Collagen fulfills the major load bearing role in ligaments, and it has been reported that in connective tissues type 1 and 3 collagen are correlated with mechanical strength and elasticity respectively. Sex steroid hormones may have some effects on the properties of ACL. The female hormonal milieu may be one of the risk factors in the pathogenesis of ACL injuries.