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REVIEW ARTICLE

The Effect of Estrogen on Anterior Cruciate Ligament Structure and Function: A Systematic Review

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Abstract

Background: There is a widely acknowledged biological sex difference in anterior cruciate ligament (ACL) injuries, with females at a significantly higher risk compared to males. Due to the sex difference, the influence of the sex hormone estrogen has been investigated. Consequently, the aim of this study through reviewing current literature was to explore the association fluctuations in estrogen concentration during the menstrual cycle and altered ACL extracellular matrix (ECM) structure and subsequent function.

Methodology: Scopus and PubMed search engines were selected to perform a series of searches in April and May 2022. An exclusion criterion was implemented, and each article was critically analysed. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) structure was utilised to outline how the literature selection was made.

Results: Estrogen surges were concurrent with increases in knee laxity. A time delay of 2-3 days could be identified between the estrogen surges and increases in knee laxity, indicating the days that could hold the highest risk of injury due to ligaments being unable to tolerate normal loads. Moreover, specific to ACLs, increasing estrogen concentrations are coupled with reduced ultimate tensile stress and linear stiffness. The mechanisms by which estrogen impacts ACL function are still unclear; however, it has been suggested that estrogen causes alterations to ECM structure either by decreasing type I collagen production and ACL fibroblast proliferation, or by reducing collagen crosslinking via lysyl oxidase inhibition.

Conclusions: The findings of this review highlight that increases in estrogen concentration are associated with structural and cellular changes within ACLs, specifically decreased type I collagen content and crosslinking. Subsequent increases in knee laxity indicate estrogen should be considered a potential risk factor for ACL injuries in female athletes.

Keywords

Estrogen, Sporting knee injuries, Anterior cruciate ligament, Knee laxity, Extracellular matrix

Introduction

Knee injuries account for 41% of all sporting injuries, with Anterior Cruciate Ligament (ACL) injuries, accounting for one fifth of such knee injuries [1]. Moreover, ACL injuries impact over two million people worldwide annually with an incidence of 68.6 per 100,000 person-years, thus, demonstrating the large scale impact [2-4]. A crucial risk factor of ACL injury is biological sex, with females having a 3-6 times higher risk of experiencing an ACL injury compared to males [5,6]. This review explores the impact of estrogen on the observed sex difference.

ACL injuries and resulting consequences

ACL injuries occur due to one of three main mechanisms: Direct, indirect, and non-contact, with non-contact mechanisms forming the majority (60-70%) of ACL injuries [7,8]. Typically associated with sporting activities although not exclusively, ACL tears account for 64% of knee injuries in cutting and pivoting sports such as football [7]. The short-term impacts of ACL injuries include reconstructive surgery and rehabilitation, which are associated with prolonged periods of

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reduced activity and quality of life, and significant financial cost [9]. Based on a conservative estimate that 15,000 primary ACL reconstructive surgeries are performed by the UK each year, the minimum cost of ACL reconstructive surgeries on the UK equates to £63 million per year [10,11]. Furthermore, the percentage of athletes who have had ACL reconstructive surgery and returned to their reinjure level is just 65%, with only 55% returning to competitive sport, indicating the deleterious effect of ACL injuries [12].

Additionally, the long-term effects include an increased risk of developing osteoarthritis (OA), with approximately 50% of patients with ACL injuries developing OA within 5-15 years of the initial injury [13]. Resulting from ACL injury, damage to the articular cartilage and underlying subchondral bone within the knee joint can occur. Kinematic changes at the knee joint, increased shear and compressive forces, as well as increased inflammation, are all factors which can contribute to such damage [13]. Risk factors for ACL injury have been separated into intrinsic and extrinsic factors; extrinsic factors include environmental conditions, playing surfaces and footwear whereas intrinsic factors include influences such as genetics, anatomic variables and biological sex [14].

Biological sex and ACL injury risk

As the majority of ACL injuries are non-contact, arguably the intrinsic differences between males and females could explain the reasons for the discrepancy [15]. Intrinsic risk factors of ACL injury have been categorized as neuromuscular, biomechanical, anatomic and hormonal [16]. As a result, this has led to the hypothesis that there is a hormonal effect which impacts ACL injury [17].

Female sex hormones, progesterone and estradiol (estrogen), follow a rhythmic pattern within the menstrual cycle, which is comprised of two phases (follicular and luteal) [18]. At days 1-6 of the menstrual cycle, levels of estradiol and progesterone are at their lowest, with estradiol concentration peaking at days 12-14, just prior to ovulation [17]. In addition, in the luteal phase (days 20-24), there is a second, lower peak in estradiol levels [17]. At days 19-24 (the mid-luteal phase), progesterone levels are at their highest having risen gradually from the late follicular phase just prior to ovulation [17].

The effects of hormones on the musculoskeletal system have been investigated, and receptors for estrogen, progesterone and relaxin have been found in human ACL tissue, possessing varying roles in ligament metabolism [19-21]. The association between ACL laxity, a term denoting ligament flexibility and thus the ability of the ligament to withstand loads, and fluctuating hormone levels has been examined, identifying the phases of the menstrual cycle where a female could have a higher risk of injury [22,23]. Some have suggested that the pre-ovulatory stage of the follicular phase, when estrogen levels peak, is the period of highest risk of ACL injury due to increased ACL laxity [24].

Estrogen and altered knee function

The steroid hormone, estrogen, is found in three major physiological forms: Oestrone, estradiol and estriol, with estradiol the most abundant during reproductive years and most potent [18,25]. In addition to being essential for reproductive tissues, estrogen possesses roles in the skeletal, cardiovascular and central nervous system. Within bone remodelling, estrogen plays a key role in the balance between bone resorption by osteoclasts and bone formation by osteoblasts via the expression of certain cytokines and growth factors [26].

Cyclical raises in serum levels of estradiol have been identified as being responsible for increased knee laxity [27]. Moreover, fluctuations in estrogen concentration have been associated with changes to the Extracellular Matrix (ECM) composition of the ACL. ACLs are a band of dense connective tissue, comprised of collagen bundles (predominantly type I), elastin, glycosaminoglycans and proteoglycans. Raised estrogen has been linked with decreased lysyl oxidase (LOX) activity which catalyses collagen and elastin cross-linking, thus decreasing the stiffness of the ligament [28,29]. Therefore, understanding the mechanisms by which estrogen alters ACL structure and function is important when considering the increased ACL injury risk in females.

Thus, this review aims to elucidate the influence of estrogenon ACL ECM composition and subsequent functional changes.

Methodology

Selection of databases

Literature searches were performed during April and May of 2022. Scopus and PubMed were selected to conduct the literature searches (Figure 1). The combination of Scopus and PubMed ensured the most up-to-date and relevant research articles were identified, through the usage of advanced search criteria and exclusion functions.

Literature searches

Keywords were identified to conduct relevant literature searches surrounding the topic of estrogen and sporting knee injuries (Table 1 and Figure 1). This produced a total of 205 hits. The advanced search tool and exclusion function were then utilised to include only primary research articles, removing publications such as reviews, book chapters and conferences, leaving a total of 164 hits (Figure 1). End Note enabled publications to be exported into a Microsoft Excel; the duplications were identified via the Excel search tool, “Find and

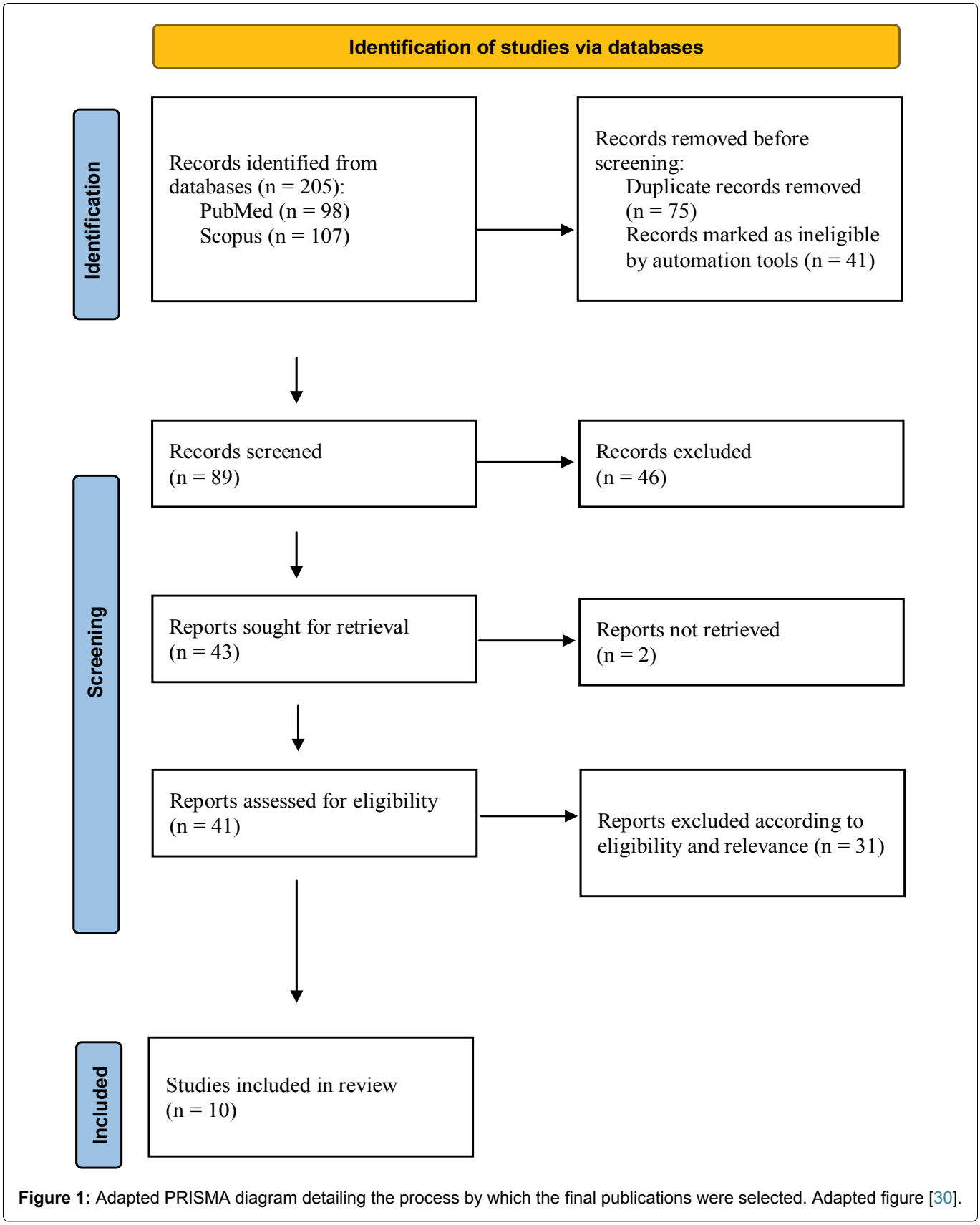

Figure 1: Adapted PRISMA diagram detailing the process by which the final publications were selected. Adapted figure [30].

Table 1: Literature searches performed. The numbers represent the order in which the searches were performed.

Number	Search performed
1	"Menstrual cycle" (title) AND "knee laxity" (title)"
2	"Estrogen/oestrogen" (title) AND "Ligament (title) AND "Human" (keyword)"
3	"Estrogen/oestrogen" (title) AND "Anterior cruciate ligament" (title)"
4	"Menstrual cycle" (title) AND "Anterior cruciate ligament" (title)"

Select" and then "Find" and removed leaving a final total of 89 hits (Figure 1).

Shortlisting of papers

Articles were excluded due to their focus on periodontal, uterosacral ligaments and spinal ligaments, as opposed to ACLs (n = 31). If the paper focussed on the impact of ACL reconstruction as opposed to pre-injury or the time of injury itself (n = 2) these were also excluded. Additionally, articles published prior to 2000 having been superseded and those conducted via a questionnaire as opposed to an experimental laboratory design were excluded (n = 13). If full access to a paper could not be reached due to the requirement of costly membership, then the publication was excluded (n = 2). Of the remaining 41 articles, 10 articles were shortlisted (Table 2), demonstrating greatest relevance to this study, indicating specific interest in estrogen, and meeting the requirements of the exclusion criteria. A challenge when conducting the literature search was the relatively small amount of literature currently available.

Menstrual cycle and knee function

Within the literature, it is widely recognised that fluctuations in female sex hormones, particularly estrogen, correspond with changes in knee laxity. This is important with regards to sporting injuries, given the implications laxity has on knee stability and the ability to withstand load. However, Belanger, et al. (2004) and Hertel, et al. (2006) found there was no significant correlation between changing hormone concentrations in the menstrual cycle and knee laxity and neuromuscular control, thus, there are differing research findings as to the effect estrogen has on knee laxity [39,40]. Additionally, challenges are created when comparing and critically analysing articles due to differing descriptions utilised to distinguish the phases of the menstrual cycle.

Both Shultz, et al. (2005) and Shagawa, et al. (2021) assessed the hormonal influence on knee joint function throughout the menstrual cycle in humans, and concluded that higher estradiol concentrations are concomitant with increased knee laxity [27,33]. Shagawa, et al. (2021) utilised a range of measurements: Anterior knee laxity, stiffness, genu recurvatum and general joint laxity, to identify the fore mentioned relationship [33]. Shultz, et al. (2005), in addition to identifying the link between hormone fluctuations and altered knee function also demonstrated the sex difference in knee function and hormone concentration fluctuations [27].

Shultz, et al. (2005) utilised an inclusion criterion which excluded those who had external hormonal intervention that could impact estrogen levels, such as through oral contraceptives or other hormone stimulating medication [27]. Moreover, those with prior knee joint injury or medical condition affecting the connective tissue of the joint such as Ehlers-Danlos disease (joint hypermobility syndrome) were also excluded, as anterior knee laxity may have been compromised by such conditions. This left 22 eumenorrheic (normally menstruating) females (23 ± 3.5 years-old), and with an adapted inclusion criterion and20 males (23.3 ± 2 years-old). 5-7 cm of venous blood was withdrawn at the same time of day to account for diurnal hormonal fluctuations, from females over the course of 20 days and from males over 4 test days. In females, beginning and end data collection was decided either as ovulation, via the detection of luteinizing hormone, or at menses onset, via self-report. Assays detecting estradiol, progesterone and testosterone were performed and in males, over the 4 days there was no significant difference in estradiol ($p = 0.98$), progesterone ($p = 0.385$) and testosterone ($p = 0.143$), thus an average was taken from the 4 measurements and utilised as a comparison to the 20 measurements from females. Estradiol levels were significantly greater ($p < 0.0001$) in females compared to males with said differences being cycle dependent. Due to males producing a lower and constant amount of estradiol, the influence on knee function provides an effective comparison when identifying the impacts of fluctuating estradiol concentrations in females. The phases that females had significantly higher estradiol concentrations were the days near ovulation representing the estradiol surge, the early luteal phase, and the late luteal phase.

Utilising the KT-2000 knee arthrometer, anterior knee laxity was measured and demonstrated comparable patterns to estradiol concentrations throughout the menstrual cycle, with females having significantly higher knee laxity than males ($p = 0.023$) and laxity values gradually rising following the estradiol surge. The differences in knee laxity that were significant between males and females throughout the cycle were similar to those significant differences in estradiol concentration: Days 3-5 near ovulation, early luteal days 1-4 and days 1, 2, 4 and 5 of the late luteal phase. Arguably, the data demonstrates a 2-3 day delay in response to estradiol concentrations, which may be important when considering times when individuals will be most prone to sporting knee injuries.

Estrogen and ACL function

The ACL is critical in maintaining stability within the knee as it provides 87% of the total restraining force against anterior tibial translation [41]. Thus, alterations to ACL structure and function will have potentially deleterious implications to knee joint function. Wojtys, et al. (2002) identified that susceptibility to ACL injury in females was higher in the ovulatory phase of the menstrual cycle where, through urine samples, a peak of estrogen was detected [34]. The relationship between estrogen concentration and tensile load of the ACL was originally elucidated by Slauterbeck, et al. (1999), who

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Table 2: Table containing a synopsis of the 10 shortlisted publications.

Menstrual cycle and knee function				
Aim:	**Methodology:**	**Key findings:**	**Conclusion:**	**Limitations:**
Assess the hormonal influence on anterior knee joint laxity and stiffness.	• Males and females were tested for serum levels of estradiol, progesterone, and testosterone. • Anterior knee joint laxity and stiffness were measured utilising the KT 2000 knee arthrometer. • Anterior directed loads of 46N, 89N and 133N were utilised to compare knee laxities. • Anterior knee joint laxity and stiffness measurements were repeated 5 times each day of testing.	• Males had no significant differences across the 4 test days in estradiol, progesterone, testosterone, knee laxity or stiffness values. • In females, estradiol was significantly higher on days 1-5 prior to ovulation, days 1-5 of early luteal phase and days 1-4 of the late luteal phase than in males. • Females had significantly greater knee laxity than males with variances in the differences being on day 5 of menses, days 3-5 prior to ovulation, days 1-4 of the early luteal phase and days 1, 2, 4 and 5 of the late luteal phases.	Sex differences in knee laxity are cycle dependent with the greatest difference between males and females at the early luteal phase of the menstrual cycle. **Relevance to this study:** The sex difference in knee laxity could be suggested as the mechanism for the sex difference in sporting knee injuries. Moreover, Shultz, et al. (2005) has demonstrated the potential role of female sex hormones (particularly estrogen (estradiol)) in said sex difference in knee laxity.	• Shultz, et al. (2005) does not investigate the consequences of the hormone-mediated increases in knee laxity/alterations in knee function. Thus, although providing context to this study, it does not necessarily address the exact question of the role of estrogen in sporting knee injuries. • Shultz, et al. (2005) did not identify the hormonal effect on specific tissues (ACLs) within the knee joint.
Aims:	**Methodology:**	**Key findings:**	**Conclusions:**	**Limitations:**
Identify the days where anterior knee laxity was at the maximum and minimum values in females and compare varus-valgus (VV) and internal-external rotational (IER) laxities and stiffnesses in males and females.	• Two time points (during menses (T1) and early luteal phase (T2)) were used for measuring laxity variables. • During a biomechanical testing session, VV and IER laxity and stiffness (via the Vermont Knee laxity device), anterior knee laxity (via the KT-2000 arthrometer), genu recurvatum (amount of hyperextension), and general joint laxity (according to the Beighton and Horan Joint Mobility Index) were measured.	• In females but not males, anterior knee laxity, genu recurvatum and general joint laxity increased between T1 and T2. • Anterior knee laxity and genu recurvatum were greater in females at T2 only. • A sex by time interaction was observed for VV stiffness, in females there was a decrease in stiffness between T1 and T2, whereas in males there was an increase.	Females experience larger cyclic variations in anterior knee laxity, genu recurvatum and general knee laxity compared to VV and IER laxity and stiffness. Laxity across different planes of the joint does not change equivalently across the menstrual cycle. **Relevance to this study:** Shultz, et al. (2011) indicates the possible role of cyclic changes of hormone concentrations within the menstrual cycle in knee laxity and it can therefore be inferred that estrogen is important in these changes in knee function and could explain risk of sporting knee injuries.	• Shultz, et al. (2011) does not address ligament-specific responses to cyclic hormone changes, thus there is not an explanation for the differing laxity behaviours of the different planes of the knee. • The measurements were only taken at two points, thus potential the true maximum and minimum results may not have been captured. • Shultz, et al. (2011) does not specifically measure estrogen levels and thus only addresses the aims of this study indirectly.

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Aim:	Methodology:	Key findings:	Conclusions:	Limitations:
To identify whether variations in hormone concentrations throughout the menstrual cycle can alter knee joint laxity and collagen metabolism.	• From a previous study, serum samples and anterior knee laxity data taken daily over 1 complete menstrual cycle was accessed from eumenorrheic females. Measurements were compared to oral contraceptive users. • Assays were used to measure estradiol, testosterone, progesterone, insulin growth factor-1 (IGF-1), collagen production marker, C-terminal propeptide of collagen type-I (CICP) and collagen degradation marker, Carboxyterminal telopeptide of type I collagen (ICTP) levels. • Anterior knee laxity was measured with the KT-2000 knee arthrometer. • To identify whether daily change in collagen metabolism could be utilised to predict change in anterior knee laxity, stepwise removal linear regression analysis was utilised.	• Significant differences in daily changes in ICTP, IGF-1 and anterior knee laxity between eumenorrheic and oral contraceptive females. • ICTP levels decreased in eumenorrheic females near ovulation, whereas in oral contraceptive users, levels remained stable and were generally higher. • IGF-1 concentrations decreased in the early and late luteal phases in oral contraceptive users but remained stable in eumenorrheic females. • Eumenorrheic females had generally lower CICP concentrations than oral contraceptive females. • Anterior knee laxity was stable in oral contraceptive females, but in eumenorrheic females, anterior knee laxity increased in the first 3 days of the early luteal phase and days 2, 4 and 5 of the late luteal phase. • Decreases in CICP and increases in IGF-1 could be used to predict increases in anterior knee laxity in both groups of females across the 20 days of the menstrual cycle.	Collagen metabolism is altered by the changes in sex hormone concentrations across the menstrual cycle and could be associated with altered knee structure and function. **Relevance to this study:** Shultz, et al. (2012) demonstrated the role of varying sex hormone concentrations throughout the menstrual cycle in collagen metabolism and that collagen metabolism could be utilised to predict knee laxity levels. The results of Shultz, et al. (2012) could therefore indicate that perhaps estrogen plays a role in collagen metabolism which could be part of the mechanism of increased risk sporting knee injuries, as indicated by the changes in knee laxity.	• Shultz, et al. (2012) utilised serum markers for collagen metabolism which may not represent the collagen metabolism *in situ* within the knee joint. • Only type 1 collagen metabolism was measured, where there are other known collagen types that impact ligament structure and function. • There was a suggested 3-5 day time lag with regards to changes in sex hormones; thus, the results may not be wholly representative of the complex mechanisms linking collagen metabolism and knee structure and function.

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Aim:	**Methodology:**	**Key findings:**	**Conclusions:**	**Limitations:**
Assess variations in anterior knee laxity, stiffness, genu recurvatum and general joint laxity during the late follicular phase compared to the ovulation phase of the menstrual cycle.	• Basal body temperature was utilised to estimate the day of ovulation of the female subjects. • Estradiol concentration was measured via a saliva test. • Anterior knee laxity was measured as anterior tibial displacement after 44, 89 and 133N was applied to the tibia. • Stiffness was measured as Δforce/ Δdisplacement at loads between 44 and 89N and between 89 and 133N. • Genu recurvatum (via a goniometer) and general joint laxity (by the University of Tokyo joint laxity test) were evaluated. • Each measurement was made 3 times each during the follicular phase (2nd to 5th day after the end of menstruation) and the ovulatory phase (2nd to 5th day after the day when the ovulation kit gave a positive result).	• In the ovulatory phase, estradiol concentrations were significantly higher compared to the late follicular phase. • There was no significant difference in anterior knee laxity between the phases. • Genu recurvatum and general joint laxity were significantly raised in the ovulatory phase compared to in the late follicular phase.	Concentrations of estradiol which vary across the menstrual cycle may affect genu recurvatum and general joint laxity. **Relevance to this study:** Shagawa, et al. (2021) demonstrated the potential link between differing estradiol levels and altered knee function, thus it could be inferred that the hormone estrogen could have an impact when considering sporting knee injuries.	• Due to the restricted time periods where the measurements were taken from in Shagawa, et al. (2021), differences in anterior knee laxity according to the menstrual cycle may not have been seen. • Specific tissue types within the knee joint were not examined. • Of the recruited subjects, only 25% were included in the data analysis. • Shagawa, et al. (2021) did not consider the impact of other female hormones.
Estrogen and anterior cruciate ligament function and injury				
Aims:	**Methodology:**	**Key findings:**	**Conclusions:**	**Limitations:**
To assess whether there is a correlation between the distribution of ACL injuries in female athletes and the menstrual cycle phase. In addition, confirm whether self-reported menstrual cycle phases are as accurate as laboratory urine sample measurements.	• Recruitment of females who sustained acute noncontact ACL injuries within 24 hrs of the injury. • A questionnaire was completed indicating history of menstrual cycle, mechanism of ACL injury and whether the injury was contact or noncontact, • Two urine samples were produced by each subject, one within 24 hrs of the injury and one within 24 hrs of her next menstrual cycle. • The samples were analysed for the metabolites of estrogen, progesterone, luteinizing hormone and creatinine (which normalized the hormone concentrations).	• A greater proportion of ACL injuries were found during the ovulation phase (where there was a peak of estrogen) and a smaller proportion was found in the luteal phase. • The association between the menstrual phase and ACL injury was removed by oral contraceptive usage. • There were discrepancies between the two methods of questionnaire and urine sample measurements.	Females have an increased susceptibility to ACL injury in the ovulatory phase of the menstrual cycle and use of oral contraceptives can diminish this susceptibility at certain times of the menstrual cycle. **Relevance to this study:** The link between the ovulation phase which is associated with peaked estrogen levels and ACL injuries is important with regards to this current study. Also, Woitys, et al. (2002) suggests that oral contraceptives could be utilised as preventative measures with regards to reducing the hormonal risk in sporting knee injuries.	• There were a low number of subjects using oral contraceptives and as such the statistical power was too low to utilise any statistical analysis from this group. • Specific tissue types were not examined. • Urine samples may be representative of systemic hormone levels as opposed to levels within the knee joint.

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Aims:	Methodology:	Key findings:	Conclusions:	Limitations:
Identify the relationship between serum estrogen levels and ACL mechanical properties in rabbits, to understand whether such estrogen levels play a role in the sex difference in ACL injuries.	• 32-week-old female Japanese White rabbits were ovariectomized via an intramuscular injection of ketamine hydrochloride and xylazine. • Via intramuscular injection, estradiol was delivered. Four concentrations correlated to 4 groups: low estradiol (50 µg/kg), medium estradiol (100 µg/kg), high estradiol (500 µg/kg) and control. • Rabbits were killed 5 weeks after the ovariectomy and the ACL from the right hind-limb was exposed. • ACLs were separated into lateral and medial portions; the lateral portion was resected, and the femur-mACL-tibia complex was used for mechanical testing. • Via a table-top Instron material testing machine the tensile load to failure was applied, from which the ultimate tensile stress was derived.	• In the control group, serum estradiol concentrations were significantly lower in the 4th and 5th week. • There was a significant difference in the ultimate tensile stress between the low and medium, and the high estradiol groups with the level being lower in the high group. • There was a significant difference in linear stiffness between the low and high serum estrogen groups. Linear stiffness was lower in the high serum estrogen groups. • There was a positive correlation between linear stiffness and ultimate tensile stress.	High serum estrogen levels may be one of the risk factors contributing to ACL rupture as demonstrated by the decreased ultimate tensile stress and linear stiffness. **Relevance to this study:** Komatsuda, et al. (2006) look specifically at how estrogen impacts ACL function which could therefore indicate the role of estrogen as a risk factor for knee injuries. Furthermore, given that Komatsuda, et al. (2006) demonstrates that high estrogen levels are associated with decreased function; this could be used to predict the phases of the menstrual cycle when female athletes would be most at risk of sporting knee injuries.	• There were a small number of animals in each estradiol group. • A short observation period was utilised. • Rabbits were utilised instead of humans thus perhaps limiting the clinical relevance of the paper. • The impact of other female sex hormones was not acknowledged. • Ligament structure and function may have been altered due to the division and resection.

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Aim:	Methodology:	Key findings:	Conclusions:	Limitations:
To assess the relationship between serum estradiol concentration and ACL elasticity and how this associates with tissue temperature throughout the menstrual cycle in females.	• Non-athletic young healthy females were utilised for this study. • Estradiol serum concentrations were taken from the antecubital area and assessed using the TOHSO estradiol ST AIA assay. • Using the Forward Looking Infrared 660 IR camera, leg skin temperature was measured prior to ACL laxity and quadriceps elasticity measurements and after when the heat pads at 38°C were removed. • The KT-2000 knee arthrometer measured the knee elasticity of the ACL. • The continuous passive motion device measured muscle and tendon flexibility providing the value of force needed to flex the knee. • An electric goniometer was utilised to measure knee flexion-extension hysteresis.	• Estradiol concentrations varied significantly over the menstrual cycle, with the lowest concentrations found during menstruation and the highest during ovulation. • Significant difference in knee skin temperature between the early follicular and the early luteal phase with the temperature being higher in the early luteal phase. Similarly, this was seen in the quadriceps skin temperature. • Anterior knee ligament elasticity was greatest during ovulation when estradiol peaked under both ambient temperature and after 38 °C. • Force to flex the knee decreased between menstruations to ovulation, this difference was not seen at 38 °C. • Knee flexion-extension hysteresis increased significantly from ovulation to menstruation and from ovulation to early luteal/middle luteal phases when at ambient temperature. These changes were not identified at 38 °C warming.	Serum estradiol concentration and temperature impacts ACL elasticity, force to flex the knee and knee flexion-extension hysteresis during the menstrual cycle. With regards to ACL laxity, serum estradiol concentration had more of an impact than temperature. **Relevance to this study:** Lee, et al. (2013) demonstrated raised estradiol (estrogen) concentrations during the menstrual cycle contribute to altered knee function through raised ACL elasticity. Although sporting knee injuries are not specifically reported, Lee, et al. (2013) infers estrogen could be a risk factor. Lee, et al. (2013) looked at effect of temperature to replicate when females play sports in hot environments.	• The effect of other female sex hormones was not considered. • The mechanism of how estrogen impacts knee function was not elucidated. • The sample size was small. • Non-athletic females were utilised which may not provide accurate insight into the conditions of the knee joint in female athletes.

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Estrogen and anterior cruciate ligament extracellular matrix				
Aim:	**Methodology:**	**Key findings:**	**Conclusions:**	**Limitations:**
To assess the effects *in vitro* of varying estrogen and progesterone concentrations on human ACL fibroblast proliferation and procollagen synthesis.	• A primary cell culture of human ACL fibroblasts was established from two patients who had differing ACL injuries. • The fibroblasts were exposed to logarithmic concentrations of estradiol and progesterone to replicate both physiologic and supraphysiologic levels for 1, 3, 5, and 7 days. • 3H-thymidine was incorporated into the cultures to monitor fibroblast proliferation. • Radioimmunoassays were utilised to measure procollagen levels. • Procollagen type I was measured using the monoclonal antibody directed against the trimeric carboxy terminal of procollagen I. Levels of procollagen type III were measured by monoclonal antibody for the amino terminal of procollagen type III. • An iodine tracer was incubated in the culture. • Western Blot analysis determined the intensity of the procollagen levels.	• A significant decrease in fibroblast proliferation was seen after day 1 of exposure to estradiol, whereas an increase was seen after progesterone exposure. • Combination exposure to estradiol and progesterone led to decrease in proliferation on days 1, 3 and 5 which was dose dependent with increasing estradiol concentrations. Increasing concentrations of progesterone attenuated this decrease. • Increasing estradiol concentrations led to a dose dependent decrease in Type I procollagen synthesis on days 1, 3 and 5. The opposite was seen with increasing progesterone concentrations. By day 7, there was no significant correlation between estradiol or progesterone concentration and procollagen Type I synthesis.	Physiologic and supraphysiologic estradiol concentrations have a dose dependent reductive effect on ACL fibroblast proliferation and Type I procollagen synthesis. **Relevance to this study:** Yu, et al. (2001) presents the possible mechanistic action of estrogen as a risk factor for sporting knee injuries. Decreased ACL fibroblast and procollagen synthesis may explain altered structural integrity of the ACL throughout the menstrual cycle. In addition, the opposing action of progesterone may be indicative of protective characteristics.	• Yu, et al. (2001) utilised an *in vitro* model which may not be replicative of *in vivo* conditions. • The correlation between procollagen synthesis, fibroblast proliferation and mechanical function of the ACL was not identified, thus further research would need to be done to connect changes in fibroblast proliferation and collagen synthesis and sporting knee injuries.

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Aim:	Methodology:	Key findings:	Conclusions:	Limitations:
To assess whether type I and III collagen and cartilage oligomeric matrix protein (COMP) expression and localisation of estrogen receptor α and β (ERα and ERβ) are linked to changes in serum concentration of estrogen and/or progesterone in rats.	• 12-week-old female Wistar rats were anesthetized and underwent bilateral ovariectomy or sham operation. • Hormone replacement via subcutaneous implantation of a sustained-release pellet containing estradiol (0.5 mg/60 days) and/or progesterone (200 mg/60 days). • Hormone replacement was conducted over 30 days to reach a steady-state level. • Blood samples were collected from the animals after being anesthetized. • An enzyme immunoassay of estradiol and progesterone was utilised to measure serum concentrations of said hormones. • Knee joints were harvested, frozen and sectioned. • Sections were stained with Haematoxylin and Eosin (H&E) and toluidine blue, and ERα, ERβ, types 1 and 3 collagen, and COMP were recognised by immunofluorescence staining by a confocal laser scanning microscope. • Rabbit polyclonal anti-ERα Immunoglobulin (Ig) G, ERβ IgG, rabbit polyclonal anti-collagen 1 and 3 antibody and rabbit polyclonal anti-COMP antibody were the primary antibodies utilised.	• In the estradiol group, immunoreactivity for ERα was higher than the ovariectomy control, whilst in the estradiol/ progesterone group; immunoreactivity for ERα was lower than the estradiol group. • In the progesterone group, ERα immunoreactivity was even lower than the estradiol/ progesterone group. • In the estradiol group, almost all the stained cells in the proximal and middle portions had immunoreactivity for ERα and ERβ. • At the proximal portion, type I collagen immunoreactivity was significantly lower in the estradiol group than in the ovariectomy control group. • Immunoreactivity of COMP was significantly lower in the estradiol group. • In the middle portion, there was no significant difference in type I collagen and COMP immunoreactivities, whilst type III collagen immunoreactivity levels were significantly higher in the estradiol group compared to the ovariectomy control group.	The ACL is estrogen receptor-dependent and elevated estradiol and progesterone impacts the ECM composition particularly at the proximal portion of ACL. **Relevance to this study:** Yoshida, et al. (2009) have elucidated how estrogen impacts the composition of the ACL and how this could be of interest clinically with regards to sporting knee injuries. Moreover, Yoshida, et al. (2009) has suggested the effect of estrogen is dependent on the part of the ACL which could be investigated with regards to position of tears.	• Rats were utilised in Yoshida, et al. (2009) which may not represent the structure and function of human ACLs. • The association between change in ECM composition and ACL function is still not fully understood, further research would be required to understand how the results from Yoshida, et al. (2009) relate to altered ligament function and subsequent injury.

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Aims:	Methodology:	Key findings:	Conclusions:	Limitations:
To identify whether there are differences between male and female donor ACL cells. To assess what the effect is of mimicking the oestrous cycle by phasic estrogen treatment in engineered ligaments with regards to function, mRNA expression and lysyl oxidase expression.	• Human ACLs were collected from ACLs discarded post ACL reconstruction surgeries. • Using Sinew constructs, ligaments were engineered from the freed ACL fibroblasts. • Mechanical testing was performed by a custom-built tensile tester, from which the ultimate tensile strength was derived. • A hydroxyproline assay was used to determine collagen content, and the data was read at 550 nm on an Epoch Microplate Spectrophotometer. • The cultures were given low (5 pg/mL), medium (50 pg/mL) and high (500 pg/mL), replicating the phases of the menstrual cycle. There was a 24 hrs group and a 48 hrs group. • Lysyl oxidase was determined using a lysyl oxidase activity kit. • Gene expression was measured according to total cellular RNA. • β-Aminopropionitrile treatment (a lysyl oxidase inhibitor) was added to the culture and subsequent analysis of mechanical properties and collagen content ensued.	• Low and medium estrogen treatment lead to significantly increased collagen production compared to the control but the mechanical properties were not significantly affected, suggesting a disconnect between increasing collagen caused by estrogen treatment and mechanical properties. • Following short term treatment of 500 pg/mL estrogen, ultimate tensile strength was significantly reduced but collagen content remained unchanged. • Ligaments treated with high estrogen levels had a significant decrease in lysyl oxidase activity. Likewise, lysyl oxidase mRNA decreased after high estrogen over 24 hrs. • Utilising the lysyl oxidase inhibitor, there was a significant decrease in the mechanical properties of the ligament without impacting the collagen content of the grafts.	High estrogen levels comparable to those 3-4 days prior to ovulation decrease lysyl oxidase activity in human ACL cells which corresponds with a decrease in ligament stiffness. **Relevance to this study:** Lee, et al. (2015) proposes that estrogen alters ligament function via inhibition of lysyl oxidase. This mechanism includes altered cross-linking of the tissue as opposed to change in ECM composition.	• Lee, et al. (2015) utilised an i*n vitro* model which may therefore not reflect *in vivo* conditions. Given the engineered nature of the ligaments, the responses of native tissue may not be captured. • The effects of other sex hormones are not captured. • The effect of estrogen on the whole joint may not have been captured by Lee, et al. (2015).

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utilised a rabbit model to demonstrate that increased estrogen concentration, reduces the tensile strength [42].

Komatsuda, et al. (2006), performed an *ex vivo* study also utilising a rabbit model, with the aim of identifying the relationship between ACL mechanical properties and estrogen concentrations to elucidate the sex difference in ACL injuries [35]. The rabbits were ovariectomized and then separated into groups according to the concentration of estradiol administered via intramuscular injection. Via mechanical testing, a statistically significant difference ($p = 0.04$) was found in the ultimate tensile strength between the medium (109 MPa) and high (69 MPa) estradiol groups which was the result of ultimate failure load divided by the cross-sectional area. Increasing serum estradiol corresponded with a reduction in ultimate tensile strength and linear stiffness, however, this association was not statistically significant ($p = 0.07$ and $p = 0.06$ respectively) and may indicate limits to the impact of estrogen on ACL mechanical properties. The clinical relevance of use of the rabbit model should be questioned, the rabbit ACL has been described as being anatomically different from human ACLs [43].

Effect of estrogen on ACL ECM structure and composition

Liu, et al. (1996), localised estrogen receptors within the synoviocytes and fibroblasts in the ACL stroma and blood vessel walls of the ACL, thus indicating the potential cellular mechanism of estrogen within ACLs [44]. Collagen, specifically type I collagen constitutes around 80% of the dry weight of ligaments and is crucial to the mechanical strength of ligaments via molecular cross linking [45]. Research particularly focuses on the impact of estrogen on ACL fibroblast proliferation and subsequent ECM composition, with specific interest in collagen production. The inverse relationship between serum estrogen concentration, fibroblast proliferation and collagen production could explain the alterations in function and increased risk of injury of the ACL on certain days of the menstrual cycle.

***In vitro* studies:** Yu, et al. (2001) utilised an *in vitro* model to identify the effects of variable estrogen and progesterone concentrations on ACL fibroblast proliferation and procollagen synthesis [37]. Precursors of collagen, known as procollagen, are markers of collagen biosynthesis. Per collagen molecule produced, one amino-terminal propeptide is cleaved from a procollagen molecule [46]**.** Hence, collagen biosynthesis can be quantified based on this assumption. Physiologic and supraphysiologic concentrations of estrogen and progesterone were replicated and ACL fibroblasts harvested from two patients suffering from different ACL injuries were exposed to varying hormone concentrations. By using 3H-thymidine to monitor actively dividing cells, fibroblast proliferation was monitored and a significant decrease in proliferation ($p < 0.01$) was seen with increasing concentrations of estradiol after day 1. This was contrasted with increasing concentrations of progesterone where fibroblast proliferation increased ($p = 0.02$). When the combination of estradiol and progesterone was administered on days 1, 3 and 5 there was a dose dependent decrease in proliferation ($p < 0.01$). However, such decrease in proliferation was prevented by increasing concentrations of progesterone and on day 7, there was no statistically significant correlation between fibroblast proliferation and estrogen or progesterone concentrations ($p = 0.37$). This suggests that progesterone possesses protective effects against estrogen induced decrease in fibroblast proliferation. This could be considered as a preventative measure to reduce incidence of knee injuries during phases of elevated estrogen levels.

Radio immunoassays were used to measure procollagen type I and III levels and such experiments revealed that type I procollagen synthesis followed similar trends to ACL fibroblast proliferation: Decreasing procollagen synthesis, with increasing estradiol concentrations. Furthermore, type I procollagen synthesis decreased significantly ($p < 0.01$) in a dose dependent manner on days 1, 3 and 5 with increasing estradiol concentrations, with such effects being attenuated by increasing progesterone concentrations. There was no significant effect on type III procollagen by either estradiol ($p = 0.38$) or progesterone ($p = 0.41$) and there is currently no evidence for its role when considering cyclical changes in knee function.

Yu, et al. (2001) demonstrates that estrogen impacts ACL fibroblast proliferation and type I procollagen synthesis in a dose dependent manner *in vitro* [37]. Moreover, such results could explain why acute changes in estrogen during the menstrual cycle such as prior to ovulation seem to result in altered knee function and thus, risk of injury. Additionally, Yu, et al. (2001) indicates that progesterone diminishes the effects of estrogen, again emphasising the potential for progesterone to counteract the effects of estrogen in increasing risk of sporting knee injuries [37].

***In vivo* studies-rats:** Yoshida, et al. (2009), performed an *in vivo* study utilising 12-week-old female Wistar rats to determine whether estrogen receptor (ER) location and expression of type I and type III collagen, and cartilage oligomeric matrix protein (COMP) in ACLs is related to changes in serum estrogen concentration and subsequent changes in ACL function [38]. The rats underwent ovariectomies and hormone replacement through subcutaneous implantation of a sustained-release pellet containing estradiol and/or progesterone. Knee joints were harvested 30 days after treatment and via immunofluorescence, ERα, ERβ, type I collagen, and COMP expression were quantified. Compared to the ovariectomy group, ERα expression was higher in the

estradiol group as demonstrated by the higher volume of ERα-immunoreactive cells than in the ovariectomy group. Type I collagen immunoreactivity was significantly reduced ($P < 0.05$) in the proximal part of the ACL, in the estradiol group. Moreover, COMP expression was also significantly reduced in the estradiol group compared to the ovariectomy group ($p < 0.05$). COMP is known to be involved in collagen secretion and fibrillogenesis and interacts with multiple ECM proteins, thus reduced expression could be associated with decreased strength [47]. In the middle portion of the ACL, no significant differences were seen in the levels of type I collagen and COMP.

Interestingly, the morphology of the fibroblasts differed in the middle part (ovoid or spindle shaped) of the ACL compared to the proximal part (round) which may highlight the observed differences in type I collagen and COMP expression discussed above. Subsequently, Yoshida, et al. (2009) perhaps demonstrates that estrogen impacts ACL ECM composition in a localised fashion which should be acknowledged when considering susceptibility of certain parts of ACLs to injury specifically, the proximal part.

As in Yu, et al. (2001), Yoshida, et al. (2009) demonstrated the protective effects of progesterone, as the combination of estradiol and progesterone resulted in a lack of significant difference in type I and COMP expression compared to the ovariectomy group [37,38].

***In vivo* studies-humans:** In a preliminary study, Shultz, et al. (2012), aimed to identify the effects of alterations in sex hormone concentrations across the menstrual cycle on both knee laxity and type I collagen metabolism [32]. They also hypothesised that cyclic changes in collagen metabolism could be associated with cyclic changes in anterior knee laxity. Healthy, eumenorrheic females were compared to females using oral contraceptives. Assays measuring sex hormones including estradiol, and serum markers of collagen synthesis and degradation (C-terminal propeptide of collagen type-I (CICP) and insulin growth factor-1 (IGF-1)), and collagen degradation marker (Carboxyterminal telopeptide of type I collagen (ICTP)) were measured. Prior to ovulation, the estrogen surge on days corresponded with decreased ICTP levels and were significantly lower ($p < 0.05$) than in the oral contraceptive users. CICP concentrations were significantly decreased ($p < 0.001$) in the early luteal and late luteal phases in both groups compared to in the days of menses and estrogen surge. In eumenorrheic females, IGF-1 levels remained stable ($p = 0.558$), whilst there was a decrease in the luteal phase ($p < 0.001$) in oral contraceptive users who therefore had lower IGF-1 levels than eumenorrheic females. Anterior knee laxity remained stable in oral contraceptive females ($p = 0.429$) but increased in the early and late luteal phase in eumenorrheic females ($p = 0.029$). Importantly, bivariate correlations, taking into consideration patterns of variability, revealed that decreases in serum CICP and increases in serum IGF-1 could predict anterior knee laxity increases in both groups ($p = 0.014$).

The results of Shultz, et al. (2012) only partially support their hypotheses that concentrations of serum collagen markers and mediators would be lower when estradiol levels are elevated [32]. Across the menstrual cycle, IGF-1 concentrations did not change in eumenorrheic females, which could suggest the interplay between estrogen and testosterone, given estradiol is known to decrease IGF-1 responsiveness and testosterone elevates IGF-1 concentration [48]. The pattern of IGF-1 seemed to follow that of testosterone concentrations in eumenorrheic females. Additionally, despite suppressed IGF-1 concentrations in oral contraceptive users, cyclic changes in anterior knee laxity were not observed. This is surprising given that low IGF-1 has been related to smaller collagen fibril diameter and diminished collagen synthesis which could be indicative of a poorer collagen and subsequent ACL structure [49].

The results of Shultz, et al. (2012) also demonstrate that decreased CICP and increased IGF-1 are predictors of increased anterior knee laxity in both groups, which perhaps suggests that CICP and IGF-1 interact, leading to structural changes in ACLs (Figure 2) [32]. This is deduced from the raised anterior knee laxity in the estradiol peak prior to ovulation in eumenorrheic females, following the suppression of CICP and increase (although not significant) in IGF-1. The lack of anterior knee laxity cyclical changes in the oral contraceptive females may be due to the constant lower levels of estradiol.

Lastly, Shultz, et al. (2012) demonstrated that fluctuations in sex hormones, particularly estrogen, across the menstrual cycle influence type I collagen metabolism and anterior knee laxity [32]. The authors begin to suggest that such change in collagen metabolism could affect ACL structure and function; although due to the complex interplay of sex hormones and mediators of collagen synthesis and degradation, this requires further research. With regards to this study, the understanding that cyclical decreases in CICP and increases in IGF-1 could predict anterior knee laxity, demonstrates that perhaps, estrogen influences collagen metabolism.

Estrogen and ACL lysyl oxidase (LOX) activity

Whilst prior research has focused on the impact of estrogen on the composition of the ACL ECM, the action of the enzyme LOX has been suggested to be important in the structural integrity and thus, function of the ACL. LOX is known to catalyse the cross linking of collagen and elastin, and is therefore crucial in ECM formation and the structure of the ACL [28]. Consequently, Lee, et al. (2015) hypothesised that estrogen alters ACL function

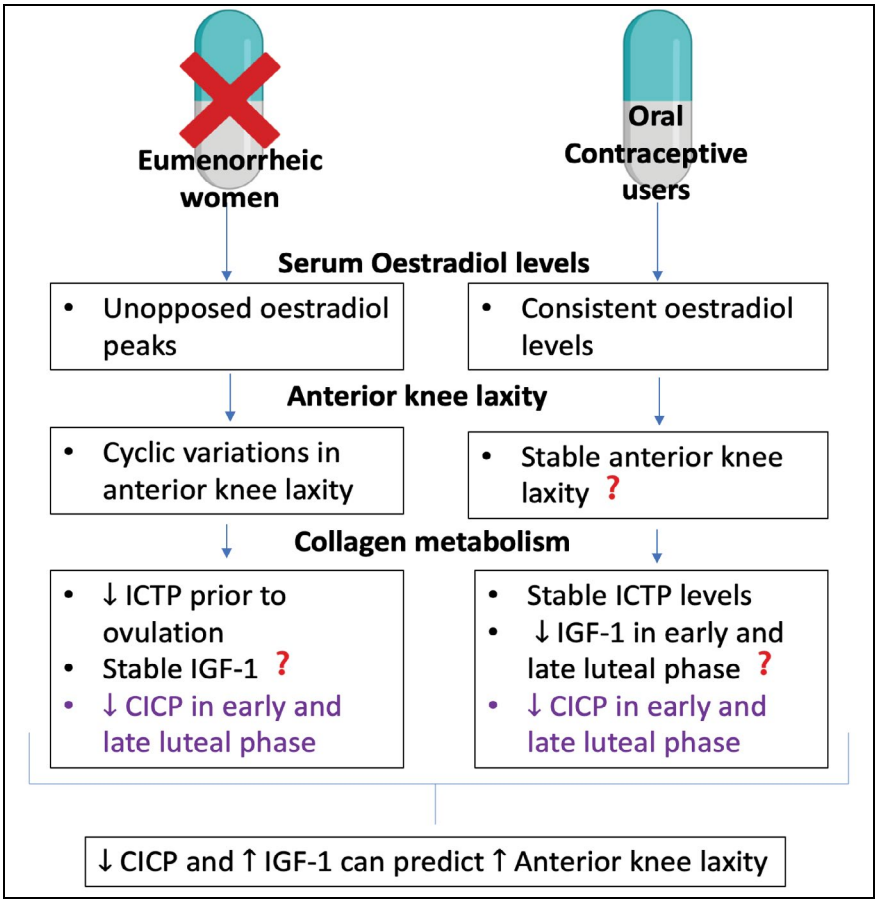

Figure 2: A summary of key results from Shultz, et al. (2012) [32]. Comparison between the results of eumenorrheic (normally menstruating) and oral contraceptive users are presented, with regards to serum estradiol levels, anterior knee laxity and collagen metabolism. From the results, bivariate correlations revealed that decreased CICP and increased IGF-1 can predict increased anterior knee laxity. Results that were not expected or went against the authors hypothesis are followed by?. In purple is a result seen in both groups. Original diagram made using Biorender.com.

by reducing LOX activity rather than altering ECM composition [29]. Research into LOX and ACLs mainly focusses on the healing capabilities of the ligament, given that LOX is also associated with wound healing. Xie, et al. (2013) demonstrated that Medial Collateral Ligament (MCL) fibroblasts had higher expressions of LOX than ACL fibroblasts which could explain the poor healing qualities of the ACL [50].

Lee, et al. (2015) hypothesised that estrogen decreases the mechanical properties of ligaments via inhibition of LOX, utilising engineered ligaments constructed from ACL cells [29]. Mechanical testing by a custom-built tensile tester revealed the maximal tensile load (MTL) and derived ultimate tensile strength (UTS) of the engineered ligaments treated with estrogen concentrations ranging from low (5 pg/ml), medium (50 pg/ml), and high (500 pg/ml). The UTS was significantly decreased ($p = 0.02$) following 48 hrs of exposure of high estrogen concentration which mimicked the peak of estrogen prior to ovulation. However, collagen content did not decrease; thus, the authors proposed the importance of differing cross-linking activity. An assay revealing LOX activity, revealed that ligaments treated with high estrogen concentration for 24 hrs resulted in a 62% decrease in LOX activity ($p = 0.0084$). Likewise, when treated for 48 hrs, there was a 77% decrease in activity ($p = 0.0021$). Total cellular RNA measurements indicated LOX gene expression decreased by 25% ($p = 0.03$) after 24 hrs of exposure to high estrogen levels. To then assess whether LOX impacts the mechanical properties of the ligaments, the ligaments were treated with the LOX inhibitor β-Aminopropionitrile (BAPN). This led to a significant decrease in MTL and UTS ($p < 0.05$). Consequently, Lee, et al. (2015) proposes an alternative mechanism of action by which estrogen alters ACL activity suggesting that it might not be the collagen content, rather the arrangement and crosslinking of the collagen that impacts mechanical function.

Discussion

The impact of estrogen on ACL function and anterior knee laxity

This review explored the association between estrogen and changes in ACL structure and function. This association is important in understanding the mechanisms behind the sex difference in ACL injuries. Although, the hormonal effect is commonly acknowledged in literature, the mechanisms by which fluctuations in estrogen and other sex hormones

impact knee joint structure and function are not fully understood. There are a limited number of preliminary studies aiming to elucidate this relationship and further research is needed to determine how estrogen can be considered a risk factor in sporting knee injuries and whether there are any preventative measures that can mitigate such effects.

Shultz, et al. (2005) indicated that increases of estrogen concentrations throughout the menstrual cycle correspond with increases in knee laxity [27]. This alteration in knee laxity may demonstrate days during the menstrual cycle when females are less able to withstand high loading through the knee, and thus, are more susceptible to sporting knee injuries. Komatsuda, et al. (2006) also demonstrated in rabbits that increased estrogen has an impact on ACL function, indicated by the significant decrease in UTS [35]. Arguably, it is the unopposed estrogen surges that present the risk factor to knee injuries, as Yu, et al. (2001) and Yoshida, et al. (2009) demonstrated both *in vitro* and *in vivo* respectively the protective effect of progesterone against estrogen mediated changes [37,38]. Shultz, et al. (2012) compared knee laxity and collagen metabolism in eumenorrheic females and oral contraceptive users [32]. This comparison presented the opportunity to observe the effect of the estrogen surges within the menstrual cycle, as those taking oral contraceptives had consistent estrogen levels without surges that were matched by consistent progesterone levels. There was a lack of cyclic variation in knee laxity in the females taking oral contraceptives, thus suggesting the negative impacts of estrogen surges in eumenorrheic females. Additionally, research indicates that the peptide hormone relaxin, acts synergistically with estrogen, exerting collagenolytic effects, thus altering ACL function and should be taken into consideration [51].

Impact of estrogen on ECM collagen content and LOX activity

Yu, et al. (2001), Yoshida, et al. (2009) and Shultz, et al. (2012) explored how estrogen alters collagen metabolism and as a result, it could be argued that estrogen exposure alters ACL function by decreasing ACL fibroblast proliferation and type I collagen production [32,37,38]. However, it could be argued that alterations in rates of collagen synthesis over the short time frame of the estrogen surges within the menstrual cycle could be of a too small a magnitude to have an overall effect on knee function. The turnover of collagen is known to be low in ligaments: Between 100 to 500 days [52]. Moreover, Smeets, et al. (2019) identified that the tissue protein synthesis rate within ACLs is 0.07 ± 0.02%/hr, which would suggest that alterations to ECM composition over the short time frame being considered may be too small to significantly raise the risk of knee injury [53]. Additionally, Seneviratne, et al. (2004) aimed to identify the effect of estrogen on ovine ACL fibroblasts and identified that there was no significant difference in ACL fibroblast proliferation or collagen synthesis [52].

Consequently, Lee, et al. (2015) proposes a mechanism by which estrogen alters ACL structural integrity without necessarily changing the protein composition that could be more plausible [29]. The enzyme LOX is crucial in catalysing collagen and elastin cross links by oxidising lysine residues, and therefore is important when considering the ECM mechanical function [54]. Lee, et al. (2015) demonstrated that estrogen exposure in engineered ligaments led to significantly decreased LOX activity and such decreased activity was associated with decreased mechanical function of the ACL [29]. This decrease in mechanical function was also demonstrated when a LOX inhibitor was added, thus indicating the importance of LOX activity when considering ACL function. In addition, Wang, et al. (2020) suggested that via the nuclear factor-kappa B pathway, LOX promotes tissue regeneration in ACL fibroblasts via the suppression of TNF-α, which suggests that LOX also modulates the inflammatory response in ACLs which is important when considering the high stresses ACLs are under in sporting environments [55]. In current literature, research into periodontal ligaments and uterosacral ligaments of those suffering from pelvic organ prolapse, concurs with the hypothesis that expression and activity of LOX relates with ligament function. Therefore, when considering risk of sporting knee injury, LOX activity and how estrogen may alter such activity should be researched into further.

Recommendations for future research

There are gaps in current understanding surrounding the role of estrogen in ACL structure and function and subsequent injuries. Current research implicates estrogen with the upregulation of osteogenic differentiation in periodontal ligaments [56]. Such alterations in cellular phenotype result in bone formation and thus structural changes to the ligament. Consequently, future research could consider if similar effects on osteogenic differentiation occur in ACLs due to changing estrogen concentrations. Additionally, this paper has highlighted the potential importance of LOX activity; however, the mechanisms by which estrogen alters such activity in ACLs remain unclear. Current literature indicates that specific micro-RNAs alter LOX activity and ER expression in females with pelvic organ prolapsed [57,58]. Thus, future research into micro-RNA content and activity in ACLs should be considered, as this could demonstrate an alternative mechanism by which individuals could be more susceptible to injury. Lastly, future research should consider potential preventative measures, with more studies needed to look at the usage of oral contraceptives by female athletes.

During the menstrual cycle, unopposed surges in estrogen occur, such increases in concentration have

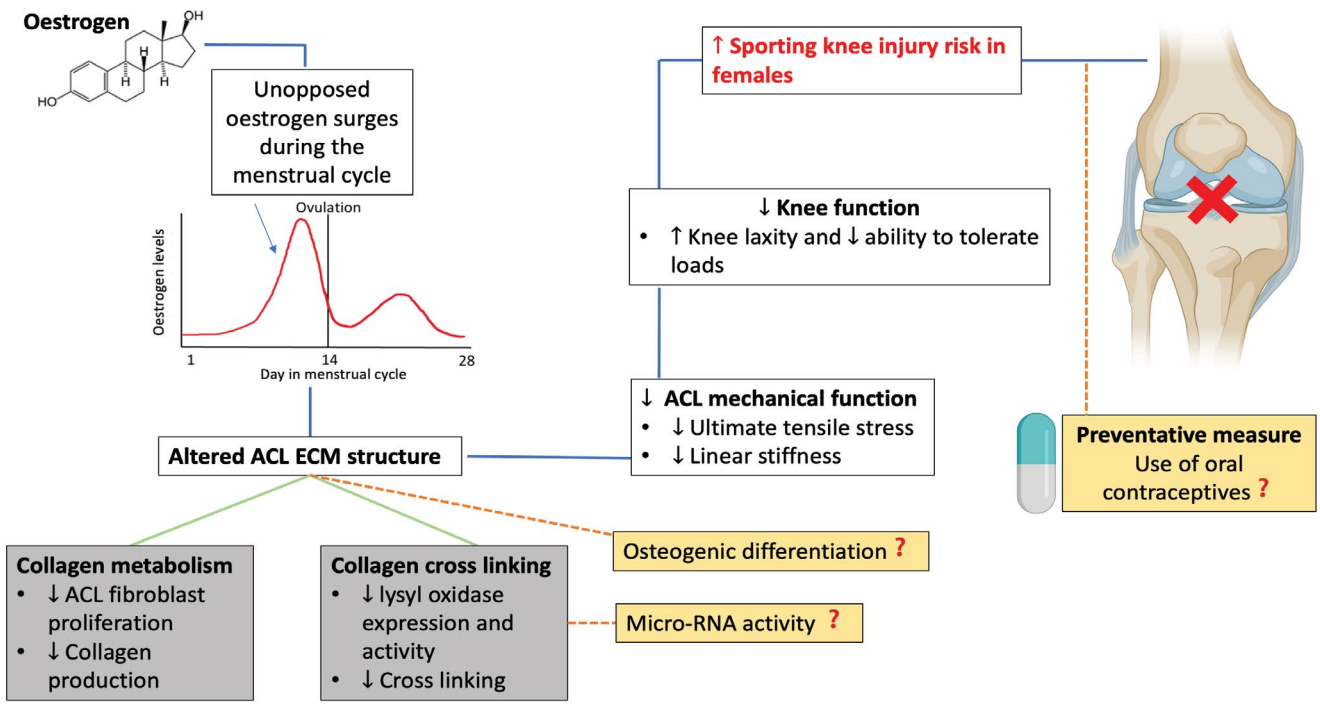

Figure 3: A schematic representation of the proposed mechanisms by which estrogen impacts knee function and thus, in females, is a risk factor in sporting knee injuries.

been linked with altered ACL ECM structure which in turn, decreases the mechanical function of the ACL. Overall, this is associated with decreased knee function due to increased knee laxityand thus potential increased risk of sporting knee injury in females.In grey are proposed mechanisms by which estrogen effects ECM structure, however the extent to which each mechanism plays in altering knee function is not conclusive. In yellow are areas that should be considered for future experiments both with regards to mechanisms of action by which estrogen impacts ACL structure, and preventative measures such as use of oral contraceptives. Original figure made using Biorender.com (Figure 3).

Conclusion

Overall, by reviewing current literature that addresses the association between estrogen and alterations in ACL structure and function, the mechanism of action appears to be involved with type I collagen metabolism and/or crosslinking. Increases in estrogen concentration throughout the menstrual cycle should therefore be considered an important risk factor for female athletes with regards to ACL injuries.

Declaration of Interest Statement

The authors report there are no competing interests to declare.

References

1. Sancheti P, Razi M, Ramanathan EBS, Yung P (2010) Injuries around The Knee-Symposium. British Journal Of Sports Medicine 44: i1-i82.

2. Gans I, Retzky JS, Jones LC, Tanaka MJ (2018) Epidemiology of recurrent anterior cruciate ligament injuries in national collegiate athletic association sports: The injury surveillance program, 2004-2014. Orthop J Sports Med 6: 2325967118777823.

3. Sanders TL, Kremers HM, Bryan AJ, Larson DR, Dahm DL, et al. (2016) Incidence of anterior cruciate ligament tears and reconstruction: A 21-year population-based study. Am J Sports Med 44: 1502-1507.

4. Renström PA (2013) Eight clinical conundrums relating to anterior cruciate ligament (ACL) injury in sport: Recent evidence and a personal reflection. Br J Sports Med 47: 367-372.

5. Lee H, Petrofsky JS, Laymon M, Yim J (2013) A greater reduction of anterior cruciate ligament elasticity in women compared to men as a result of delayed onset muscle soreness. Tohoku J Exp Med 231: 111-115.

6. Parsons JL, Coen SE, Bekker S (2021) Anterior cruciate ligament injury: Towards a gendered environmental approach. Br J Sports Med 55: 984-990.

7. Raines BT, Naclerio E, Sherman SL (2017) Management of anterior cruciate ligament injury: What's in and what's out? Indian J Orthop 51: 563-575.

8. Lin C-F, Liu H, Gros MT, Weinhold P, Garrett WE, et al. (2012) Biomechanical risk factors of non-contact ACL injuries: A stochastic biomechanical modeling study. Journal of Sport and Health Science 1: 36-42.

9. Tan CW, Hsu WH, Yu PA, Chen CL, Kuo LT, et al. (2020) Anterior cruciate ligament reconstruction in patients older than 50 Years: A systematic review and meta-analysis. Orthop J Sports Med 8: 2325967120915698.

10. Jameson SS, Dowen D, James P, Serrano-Pedraza I, Reed MR, et al. (2012) Complications following anterior cruciate ligament reconstruction in the English NHS. Knee 19: 14-19.

11. Davies L, Cook J, Leal J, Areia CM, Shirkey B, et al.

(2020) Comparison of the clinical and cost effectiveness of two management strategies (rehabilitation versus surgical reconstruction) for non-acute anterior cruciate ligament (ACL) injury: Study protocol for the ACL SNNAP randomised controlled trial. Trials 21: 405.

12. Ardern CL, Taylor NF, Feller JA, Webster KE (2014) Fifty-five per cent return to competitive sport following anterior cruciate ligament reconstruction surgery: An updated systematic review and meta-analysis including aspects of physical functioning and contextual factors. Br J Sports Med 48: 1543-1552.

13. Cheung EC, DiLallo M, Feeley BT, Lansdown DA (2020) Osteoarthritis and ACL reconstruction-myths and risks. Curr Rev Musculoskelet Med 13: 115-122.

14. Smith HC, Vacek P, Johnson RJ, Slauterbeck JR, Hashemi J, et al. (2012) Risk factors for anterior cruciate ligament injury: A review of the literature - part 1: Neuromuscular and anatomic risk. Sports Health 4: 69-78.

15. Devana SK, Solorzano C, Nwachukwu B, Jones KJ (2022) Disparities in ACL reconstruction: The influence of gender and race on incidence, treatment, and outcomes. Curr Rev Musculoskelet Med 15: 1-9.

16. Ireland ML (2002) The female ACL: Why is it more prone to injury? Orthop Clin North Am 33: 637-651.

17. Herzberg SD, Motu'apuaka ML, Lambert W, Fu R, Brady J, et al. (2017) The effect of menstrual cycle and contraceptives on acl injuries and laxity: A systematic review and meta-analysis. Orthop J Sports Med 5: 2325967117718781.

18. Draper CF, Duisters K, Weger B, Chakrabarti A, Harms AC, et al. (2018) Menstrual cycle rhythmicity: Metabolic patterns in healthy women. Sci Rep 8: 14568.

19. Sciore P, Frank CB, Hart DA (1998) Identification of sex hormone receptors in human and rabbit ligaments of the knee by reverse transcription-polymerase chain reaction: Evidence that receptors are present in tissue from both male and female subjects. J Orthop Res 16: 604-610.

20. Faryniarz DA, Bhargava M, Lajam C, Attia ET, Hannafin JA (2006) Quantitation of estrogen receptors and relaxin binding in human anterior cruciate ligament fibroblasts. In Vitro Cell Dev Biol Anim 42: 176-181.

21. Chidi-Ogbolu N, Baar K (2018) Effect of estrogen on musculoskeletal performance and injury risk. Front Physiol 9: 1834.

22. Martin D, Timmins K, Cowie C, Alty J, Mehta R, et al. (2021) Injury incidence across the menstrual cycle in international footballers. Front Sports Act Living 3: 616999.

23. Zazulak BT, Paterno M, Myer GD, Romani WA, Hewett TE (2006) The effects of the menstrual cycle on anterior knee laxity: A systematic review. Sports Med 36: 847-862.

24. Balachandar V, Marciniak JL, Wall O, Balachandar C (2017) Effects of the menstrual cycle on lower-limb biomechanics, neuromuscular control, and anterior cruciate ligament injury risk: A systematic review. Muscles Ligaments Tendons J 7: 136-146.

25. Cui J, Shen Y, Li R (2013) Estrogen synthesis and signaling pathways during aging: From periphery to brain. Trends Mol Med 19: 197-209.

26. Khosla S, Oursler MJ, Monroe DG (2012) Estrogen and the skeleton. Trends Endocrinol Metab 23: 576-581.

27. Shultz SJ, Sander TC, Kirk SE, Perrin DH (2005) Sex differences in knee joint laxity change across the female menstrual cycle. J Sports Med Phys Fitness 45: 594-603.

28. Xie J, Huang W, Jiang J, Zhang Y, Xu Y, et al. (2013) Differential expressions of lysyl oxidase family in ACL and MCL fibroblasts after mechanical injury. Injury 44: 893-900.

29. Lee CA, Lee-Barthel A, Marquino L, Sandoval N, Marcotte GR, et al. (2015) Estrogen inhibits lysyl oxidase and decreases mechanical function in engineered ligaments. J Appl Physiol 118: 1250-1257.

30. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, et al. (2021) The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ 372: n71.

31. Shultz SJ, Schmitz RJ, Beynnon BD (2011) Variations in varus/valgus and internal/external rotational knee laxity and stiffness across the menstrual cycle. J Orthop Res 29: 318-325.

32. Shultz SJ, Wideman L, Montgomery MM, Beasley KN, Nindl BC (2012) Changes in serum collagen markers, IGF-I, and knee joint laxity across the menstrual cycle. J Orthop Res 30: 1405-1412.

33. Shagawa M, Maruyama S, Sekine C, Yokota H, Hirabayashi R, et al. (2021) Comparison of anterior knee laxity, stiffness, genu recurvatum, and general joint laxity in the late follicular phase and the ovulatory phase of the menstrual cycle. BMC Musculoskelet Disord 22: 886.

34. Wojtys EM, Huston LJ, Boynton MD, Spindler KP, Lindenfeld TN (2002) The effect of the menstrual cycle on anterior cruciate ligament injuries in women as determined by hormone levels. Am J Sports Med 30: 182-188.

35. Komatsuda T, Sugita T, Sano H, Kusakabe T, Watanuki M, et al. (2006) Does estrogen alter the mechanical properties of the anterior cruciate ligament? An experimental study in rabbits. Acta Orthop 77: 973-980.

36. Lee H, Petrofsky JS, Daher N, Berk L, Laymon M, et al. (2013) Anterior cruciate ligament elasticity and force for flexion during the menstrual cycle. Med Sci Monit 19: 1080-1088.

37. Yu WD, Panossian V, Hatch JD, Liu SH, Finerman GA (2001) Combined effects of estrogen and progesterone on the anterior cruciate ligament. Clinical Orthopaedics and Related Research 383: 268-281.

38. Yoshida A, Morihara T, Kajikawa Y, Arai Y, Oshima Y, et al. (2009) In vivo effects of ovarian steroid hormones on the expressions of estrogen receptors and the composition of extracellular matrix in the anterior cruciate ligament in rats. Connect Tissue Res 50: 121-131.

39. Belanger MJ, Moore DC, Crisco JJ, Fadale PD, Hulstyn MJ, et al. (2004) Knee laxity does not vary with the menstrual cycle, before or after exercise. Am J Sports Med 32: 1150-1157.

40. Hertel J, Williams NI, Olmsted-Kramer LC, Leidy HJ, Putukian M (2006) Neuromuscular performance and knee laxity do not change across the menstrual cycle in female athletes. Knee Surg Sports Traumatol Arthrosc 14: 817-822.

41. Noyes FR (2009) The function of the human anterior cruciate ligament and analysis of single- and double-bundle graft reconstructions. Sports Health 1: 66-75.

42. Slauterbeck J, Clevenger C, Lundberg W, Burchfield DM (1999) Estrogen level alters the failure load of the rabbit anterior cruciate ligament. J Orthop Res 17: 405-408.

43. Bascuñán AL, Biedrzycki A, Banks SA, Lewis DD, Kim SE (2019) Large animal models for anterior cruciate ligament research. Front Vet Sci 6: 292.

44. Liu SH, al-Shaikh R, Panossian V, Yang RS, Nelson SD,

et al. (1996) Primary immunolocalization of estrogen and progesterone target cells in the human anterior cruciate ligament. J Orthop Res 14: 526-533.

45. Hudson DM, Archer M, Rai J, Weis M, Fernandes RJ, et al. (2021) Age-related type I collagen modifications reveal tissue-defining differences between ligament and tendon. Matrix Biol Plus 12: 100070.

46. Gäddnäs F, Koskela M, Koivukangas V, Risteli J, Oikarinen A, et al. (2009) Markers of collagen synthesis and degradation are increased in serum in severe sepsis: A longitudinal study of 44 patients. Crit Care 13: R53.

47. Posey KL, Coustry F, Hecht JT (2018) Cartilage oligomeric matrix protein: COMPopathies and beyond. Matrix Biol 71-72: 161-173.

48. Veldhuis JD, Frystyk J, Iranmanesh A, Ørskov H (2005) Testosterone and estradiol regulate free insulin-like growth factor I (IGF-I), IGF binding protein 1 (IGFBP-1), and dimeric IGF-I/IGFBP-1 concentrations. J Clin Endocrinol Metab 90: 2941-2947.

49. Hansen M, Miller BF, Holm L, Doessing S, Petersen SG, et al. (1985) Effect of administration of oral contraceptives in vivo on collagen synthesis in tendon and muscle connective tissue in young women. J Appl Physiol 106: 1435-1443.

50. Xie J, Jiang J, Huang W, Zhang Y, Xu C, et al. (2013) TNF-α induced down-regulation of lysyl oxidase family in anterior cruciate ligament and medial collateral ligament fibroblasts. The Knee 21: 47-53.

51. Dragoo JL, Padrez K, Workman R, Lindsey DP (2009) The effect of relaxin on the female anterior cruciate ligament: Analysis of mechanical properties in an animal model. Knee 16: 69-72.

52. Seneviratne A, Attia E, Williams RJ, Rodeo SA, Hannafin JA (2004) The effect of estrogen on ovine anterior cruciate ligament fibroblasts: Cell proliferation and collagen synthesis. Am J Sports Med. 32: 1613-1618.

53. Smeets JSJ, Horstman AMH, Vles GF, Emans PJ, Goessens JPB, et al. (2019) Protein synthesis rates of muscle, tendon, ligament, cartilage, and bone tissue in vivo in humans. PLoS One 14: e0224745.

54. Kumari S, Panda TK, Pradhan T (2017) Lysyl oxidase: Its diversity in health and diseases. Indian J Clin Biochem 32: 134-141.

55. Wang C, Sha Y, Wang S, Chi Q, Sung KLP, et al. (2020) Lysyl oxidase suppresses the inflammatory response in anterior cruciate ligament fibroblasts and promotes tissue regeneration by targeting myotrophin via the nuclear factor-kappa B pathway. J Tissue Eng Regen Med 14: 1063-1076.

56. Jiang B, Xu J, Zhou Y, Mao J, Guan G, et al. (2020) Estrogen enhances osteogenic differentiation of human periodontal ligament stem cells by activating the Wnt/β-catenin signaling pathway. J Craniofac Surg 31: 583-587.

57. He K, Niu G, Gao J, Liu JX, Qu H (2016) MicroRNA-92 expression may be associated with reduced estrogen receptor β1 mRNA levels in cervical portion of uterosacral ligaments in women with pelvic organ prolapse. Eur J Obstet Gynecol Reprod Biol 198: 94-99.

58. Saatci O, Kaymak A, Raza U, Ersan PG, Akbulut O, et al. (2020) Targeting lysyl oxidase (LOX) overcomes chemotherapy resistance in triple negative breast cancer. Nat Commun 11: 2416.