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

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| --- | --- | --- | --- | --- |
| **Menstrual cycle and knee function** | | | | |
| Sex differences in knee joint laxity change across the female menstrual cycle[27]**.** | | | | |
| **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. |
| Variations in varus/valgus and internal/external rotational knee laxity and stiffness across the menstrual cycle[31]**.** | | | | |
| **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. |
| Changes in serum collagen markers, IGF-I, and Knee joint laxity across the menstrual cycle[32]**.** | | | | |
| **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. |
| 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[33]**.** | | | | |
| **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** | | | | |
| The effect of the menstrual cycle on anterior cruciate ligament injuries in women as determined by hormone levels[34]**.** | | | | |
| **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. |
| Does estrogen alter the mechanical properties of the anterior cruciate ligament? An experimental study in rabbits[35]**.** | | | | |
| **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. |
| Anterior cruciate ligament elasticity and force for flexion during the menstrual cycle[36]**.** | | | | |
| **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. |
| **Estrogen and anterior cruciate ligament extracellular matrix** | | | | |
| Combined effects of estrogen and progesterone on the anterior cruciate ligament[37]**.** | | | | |
| **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. |
| *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[38]**.** | | | | |
| **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. |
| Estrogen inhibits lysyl oxidase and decreases mechanical function in engineered ligaments [29]**.** | | | | |
| **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). |