**Review article**

# Effects of the menstrual cycle on lower-limb biomechanics, neuromuscular control, and anterior cruciate ligament injury risk: a systematic review

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## Summary

***Objective:*** **Anterior cruciate ligament (ACL) injury has a devastating impact on physical and psychological disability. Rates of ACL rupture are significantly greater in females than males during the same sports. Hormonal mechanisms have been proposed but are complex and poorly understood. This systematic review evaluates the effects of menstrual cycle on: 1) lower-limb biomechanics, 2) neuromuscular control, and 3) ACL injury risk.**

***Methods*****: The MEDLINE, CINAHL, SPORTSDiscus, Web of Science, and Google Scholar databases were searched from inception to August 2016 for studies investigating the effects of the menstrual cycle on lower-limb biomechanics, neuromuscular control, and ACL injury risk in females. Three independent reviewers assessed each paper for inclusion and two assessed for quality.**

***Results*****: Seventeen studies were identified. There is strong evidence that: 1) greatest risk of ACL injury is within the pre-ovulatory phase of the menstrual cycle, and 2) females with greater ACL laxity in the pre-ovulatory phase experience greater knee valgus and greater tibial external rotation during functional activity.**

***Conclusion*****: Females are at greatest risk of ACL injury during the pre-ovulatory phase of the menstrual cycle through a combination of greater ACL laxity, greater knee valgus, and greater tibial external rotation during functional activity.**

**Level of evidence: Ib.**

*KEY WORDS: biomechanics, neuromuscular, cruciate, ligament, menstrual.*

## Introduction

Anterior cruciate ligament injury has severe negative consequences on mobility, athletic performance, occupational capacity and personal finances[1,2]. Many of these injuries require extensive surgical and rehabilitative interventions, and regardless of treatment strategy osteoarthritis occurs at 10 times the normal rate in ACL injured patients[3,4].

The anterior cruciate ligament (ACL) is a major stablising intracapsular ligament in the knee joint. Extrinsic and intrinsic factors are thought to contribute to non-contact ACL injury and are well studied[4-6]. It is suggested that greater hip adduction, greater hip internal rotation, knee valgus, and knee internal rotation during functional tasks is the primary biomechanical intrinsic risk factor for non-contact ACL injury[4,5,7-10].

The risk of ACL injury in female athletes is 4-6 times higher than males during the same sports[11-14]. Furthermore, return to previous level of play is reported at 65 at 5-years[15,16], and is significantly lower in females than males in the same sports[16]. Abnormal lower-limb anatomy[4,5], and abnormal neuromuscular control[4,5,10,17,18] have been considered as important risk factors for non-contact ACL injury in females. Quatman et al.'s[19] systematic review reported increased greater knee valgus contributes to increased ACL injury risk in females. As a result, conservative interventions targeting lower-limb biomechanics such as physical therapy and neuromuscular training form a core component in evidence-based rehabilitation programmes. Quatman[19] also suggested the menstrual cycle represented an intrinsic factor that may confer a greater risk of non-contact ACL injury in females[19]. Of some concern, the effects of hormonal mechanisms on lower limb biomechanics and ACL injury risk are poorly understood and rarely addressed in rehabilitation. The menstrual cycle is controlled by the pituitary-hypothalamic-ovarian axis and mainly involves the interaction of oestrogen and progesterone[20]. Typically, each menstrual cycle spans 28 days. Each cycle begins with the follicular phase from days 1-9 during which oestrogen predominates, followed by the ovulatory phase spanning days 10-14, where oestrogen continues to prevail and reaches its peak[20,21]. The cycle ends with the luteal phase extending from days 15-28 during which time progesterone levels surpass that of oestrogen[20,21].

Considering the change in hormone levels throughout the menstrual cycle, its effect on material structure and mechanical properties of tendons and ligaments have been well investigated. A very recent systematic review identified 102 published *in vitro* and *in vivo* articles investigating the hormonal influence on tendinopathies[22]. Oliva et al. reported that female tendons had a lower rate of new connective tissue formation, respond less to mechanical loading, and exhibit lower mechanical strength[22]. However, the review reported no significant difference in tendon mechanical properties between phases of the menstrual cycle[22]. As a result, the Authors hypothesise this effect could be due to the continuous hormonal changes that negatively influence tendon activities by inhibiting fibroblast proliferative rate.

Similar theories have been proposed for female ligaments and most notably the female ACL. The ACL is composed of tightly knit collagen fibers, an increase of which results in greater ACL strength, integrity, and ability to withstand higher loads[20,21]. However, the presence of 17-b estradiol receptors in ACLs have been identified. One common theory suggests that exposure to increasing levels of estrogen reduces type I collagen synthesis in the ACL[20,21]. In fact, recent studies indicate a dose dependent decrease in cellular proliferation and Type 1 collagen synthesis in female human ACL fibroblasts during the pre-ovulatory phase of the menstrual cycle following exposure to increasing levels of estrogen[20,21].

Similarly, the oral contraceptive pill (OCP), an exogenous source of steroid hormones has been used to increase estrogen concentration, prevent ovulation, and potentially improve female ACL synthesis, structure, and biomechanical properties. A recent study reported significantly greater ACL laxity in non-OCP users than OCP users[21]. Furthermore, at ambient temperature, ACL laxity was significantly greater in non-OCP users than OCP users[21]. Consequently, it is believed that female athletes not on the contraceptive pill display greater ACL laxity during the pre-ovulatory phase of the menstrual cycle following an increase in estrogen concentration[20,21].

Previous systematic reviews report significantly greater incidence of ACL injuries during the pre-ovulatory phase (days 1-14) of the menstrual cycle[23]. Considering this, the effects of menstrual cycle on the ACL has been increasingly investigated, and a recent systematic review by Belanger et al.[24] reported significantly greater ACL laxity during the pre-ovulatory phase in female athletes. However, these systematic reviews[23,24], including previous attempts[18] to evaluate the effects of the menstrual cycle on lower-limb biomechanics and ACL injury risk in females present limited evidence based on low quality studies. In 2016 these reviews have at least three limitations. First, all are now dated and therefore do not benefit from important recent trials concerning biomechanical analysis and ACL injury[25-27]. Second, two of the reviews focused on the effects of menstrual cycle on ACL injury risk in female athletes only[18,23], and the other reviewed the effects of menstrual cycle on ACL laxity in female athletes only[24]. Third, no review has combined changes in lower-limb biomechanics and ACL injury risk during the menstrual cycle to better understand its significance behind mechanisms of ACL injury risk. Our aims included: 1) evaluation of the effects of menstrual cycle of lower-limb biomechanics and neuromuscular control, and 2) the effects of menstrual cycle on ACL injury risk. To our knowledge, no systematic review has evaluated this evidence to guide clinical practice.

## Methods

This systematic review meets the ethical standards of the Journal as recommended by a recent publication[28].

### Search Strategy

MEDLINE, CINAHL, SPORTSDiscus (SD), Web of Science (WoS), and Google Scholar databases were searched from inception until August 2016. Reference lists of included studies were screened and key words searched and summary of results are shown in Table I.

### Inclusion and Exclusion Criteria

Studies evaluating the effects of the menstrual cycle on lower-limb kinematics, kinetics, and neuromuscular control during weight-bearing functional activities, and non-contact ACL injury risk in females (18-45) were included (Tab. II). Unpublished studies, case-reports, non-peer reviewed publications, studies not involving humans, reviews, letters, and opinion articles were excluded. Studies including participants with other knee conditions such as patellar tendinopathy were excluded. Studies including participants with previous oral contraceptive pill (OCP) use were excluded unless non-OCP participant groups were separated. Following electronic searches, references of included studies were screened for additional relevant studies.

### Review process

All retrieved studies were downloaded to Endnote version X4 (Thomson Reuters Philadelphia, PA). Results were cross-referenced and duplicated studies were deleted. Relevant titles were highlighted, with abstracts and full texts reviewed independently for inclusion by three Authors (VB, JM, and OW).

### Quality assessment of studies

All included studies were assessed by two reviewers (VB and JM) for quality using a modified Down and Black quality assessment scale (Tab. III)[29]. Consensus was reached between the two Authors (VB and JM) without the need for third party intervention.

Table I. Search strategy.

| | Keywords | MEDLINE |
|---|---|---|
| #1 | Anterior AND cruciate AND ligament | 12499 |
| #2 | ACL | 8587 |
| #3 | Combine #1 OR #2 | 15128 |
| #4 | Biomechanics OR biomechanical OR kinetic OR kinematic OR neuromuscular | 292236 |
| #5 | Menstrual OR menstruation | 50534 |
| #5 | Combine #3 AND #5 | 65 |
| #6 | Combine #4 AND #5 | 133 |
| #7 | Combine #5 OR #6 | 179 |

Studies scoring ≥ 12/14 were considered of very high quality, 7-11/14 high, and ≤ 6/14 low.

### *Data Extraction and Analysis*

Data was extracted from each included study to assist with interpretation of findings and included the study design, population, controls, protocol, and results (Tab. IV).

The strength of evidence supporting each outcome was determined by the number and quality of studies supporting that finding using predetermined criteria similar to that proposed by van Tulder et al.[30].*Strong evidence* was based on results derived from multiple studies, including a minimum of two high quality studies. *Moderate evidence* was based on results derived from multiple studies, including at least two high quality, or from multiple low quality studies. *Limited evidence* included results from multiple low quality studies) or from one high quality study. *Very limited evidence* was based on results from one low quality study. *Conflicting evidence* included insignificant results from multiple studies, of which some show statistical significance individually.

Where homogeneity between studies was adequate (i.e., similar inclusion/exclusion criteria, participant population, and outcome measures), we pooled those results. Additionally, when identifying 'levels of evidence' for each comparison, heterogeneity has been considered, with lower levels of evidence allocated where heterogeneity exists.

## Results

### *Search results*

The initial search produced 179 citations. Following application of the selection criteria to titles and abstracts this was reduced to 28, and after viewing full texts the final number was 17. The primary reasons for exclusion were studies evaluating non-weight bearing methods only such as knee arthrometry, evaluation of animal populations only, and where findings from non-OCP participants could not be identified from OCP participants.

Results of the diagnostic checklist and modified Down's and Black scale are shown in Table II and III respectively. Table IV summarises the main methodological criteria and results for the included studies. Seven studies in this review investigated lower-limb biomechanics[26,27,31-35], five neuromuscular control[26,27,33,34,36], and ten ACL injury risk[25,27-45]. The studies were separated into four main outcome measure categories for review: I) lower-limb kinematics; II) lower-limb kinetics; III) neuromuscular control; IV) ACL injury risk.

### *Effects of menstrual cycle on lower-limb kinematics*

Six studies investigated the effects of the menstrual cycle on lower-limb kinematics in female athletes during functional cutting[31-33] and drop jumping[26,27,31,34,35]. Strong evidence from three high-quality studies reported a sub-group of female participants with significantly greater anterior knee laxity in the follicular compared to luteal phase who experienced significantly greater knee valgus during cutting and drop jump movements[27,31,32]. Limited evidence from one high quality study reported significantly greater knee valgus in female participants in the follicular phase compared to luteal phase during single-leg drop landing tasks[26]. Limited evidence from one high quality study reported significantly greater femoral internal rotation in female participants in the follicular phase compared to luteal phase during drop jump movements[27]. Strong evidence from five mixed-quality studies reported no significant changes in knee valgus in female participants in the follicular phase compared to luteal phase during cutting and drop jump movements[27,31-33,35]. Strong evidence from three high quality studies reported no significant differences in peak knee flexion and knee flexion at footstrike between phases of the menstrual cycle[34,35].

### *Effects of menstrual cycle on lower-limb kinetics*

Four studies investigated the effects of the menstrual cycle on lower-limb kinetics in female athletes during functional cutting[32] and drop jumping[27,31,35]. Strong evidence from three high quality studies reported a

**Table II. Inclusion and exclusion criteria quality assessment scale results, inter-rater reliability for each item, and total score.**

| Paper | Inclusion item (1-3) | | | Exclusion item (4-7) | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1) Female only group | (2) Menstrual age | (3) Non-contact ACL injury or healthy | (4) Previous knee surgery | (5) Irregular menstrual cycle history | (6) Suspected pregnancy or pregnancy within past year | (7) Used OCP or HRT or post-menopausal | Total |
| Dedrick (2008) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 7 |
| Beynnon BD | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 7 |
| Abt JP | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 7 |
| Adachi N | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 7 |
| Arendt EA | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 7 |
| Hertel J | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 7 |
| Park SK (2009a) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 7 |
| Cesar BM | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 6 |
| Chaudhari AMW | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 6 |
| Park SK (2009b) | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 6 |
| Wojtys EM (1998) | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 6 |
| Shultz SJ (2011 ) | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 6 |
| Slauterbeck (2002) | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 6 |
| Wojtys EM (2002) | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 5 |
| Myklebust G | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 4 |
| Ruedl G (2009) | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 4 |
| Ruedl G (2011) | 1 | 1 | 1 | 1 | 1 | ? | ? | 3 |

**Table III. Modified Down and Black scale.**

| Paper | 1) Clear aim/hypothesis | 2) Outcome measures clearly described | 3) Patient characteristics clearly described | 5) Confounding variables described | 6) Main findings clearly described | 10) Actual probability values provided | 11) Participants asked to participate representative of entire population | 12) Participants prepared to participate representative of entire population | 15) Blinding of outcome measurer | 16) Analysis completed was planned | 18) Appropriate statistics | 20) Valid and reliable outcome measure | 25) Adjustment made for confounders | TOTAL (/13) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Myklebust G | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 11 |
| Ruedl G (2009) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 11 |
| Ruedl G (2011) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 11 |
| Shultz SJ | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 11 |
| Wojtys EM (1998) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 11 |
| Arendt EA | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | U | 1 | 0 | 10 |
| Chaudhari AMW | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 10 |
| Hertel J | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 10 |
| Wojtys EM (2002) | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 10 |
| Abt JP | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 9 |
| Cesar BM | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 9 |
| Dedrick | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 9 |
| Slauterbeck | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 9 |
| Adachi N | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 8 |
| Beynnon BD | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 8 |
| Park SK | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 8 |
| Park SK (b) | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 8 |

sub-group of female participants with significantly greater anterior knee laxity in the follicular compared to luteal phase who experienced significantly greater knee valgus moments and knee external rotation moments during cutting and drop jump tasks[27,31,32]. Strong evidence from four high quality studies reported no significant difference in peak knee valgus moments[27,31,32,35] between menstrual cycle phases in participants during cutting and drop jump tasks. Strong evidence from two high quality studies reported no sig-

**Table IV. Summary of studies included in systematic review.**

| | Author/year | Study design | Participant details | Protocol | Results |
|---|---|---|---|---|---|
| 1 | Shultz SJ (2012) | | 49 males, 71 females | Participants measured for AKL, GR, VV, IER, and underwent biomechanical analysis of a double leg drop jump (0.45m) at two time points across their menstrual cycles | When landing from a jump, female clusters who increased both sagittal and frontal plane laxity had greater net movement toward knee valgus compared to females who did not increase both sagittal and frontal plane laxity |
| 2 | Cesar MG (2011) | | 23 female non-athletes | Single leg drop landing maneuver while 3-D knee kinematics and gluteus medius muscle onset timing were assessed throughout three distinct phases of the menstrual cycle (confirmed by blood hormone analysis) | Knee valgus angles were significantly less in the luteal phase compared to both follicular phases, while differences were not observed for gluteus medius onset timing |
| 3 | Ruedl G (2011) | | 93 female athletes with ACL injury and 93 healthy females | Self-reported questionnaire relating to intrinsic risk and extrinsic risk factors | Preovulatory phase of menstrual cycle (odds ratio, 2.59) was an independent ACL injury risk factor for female skiers |
| 4 | Park SK (2009a) | | 26 female athletes | Knee joint biomechanics were measured. Each subject was designated with low, medium, or high knee joint laxity.<br>Knee joint mechanics were compared between low, medium, and high laxity | No significant differences in knee joint mechanics. An increase in KJL was associated with higher knee joint loads during movement. A 1.3-mm increase in KJL resulted in an increase of approximately 30% in adduction impulse in a cutting maneuver, an increase of approximately 20% in knee adduction moment, and a 20 to 45% increase in external rotation loads during a jumping and stopping task |
| 5 | Park SK (2009b) | | 25 female athletes | Serum hormone concentrations were assessed and knee joint laxity at aload of 89N were measured during follicular, ovulation, and luteal phases during cutting movements | Increase in knee joint laxity was observed during ovulation compared with the luteal phase, but no significant changes in knee mechanics |
| 6 | Ruedl G (2009) | | X female athletes with ACL injury | Menstrual history, athletic activity, and injury history were collected from the athletes | Analysis revealed that recreational skiers in the preovulatory phase were significantly more likely to sustain ACL injury than skiers in postovulatory phase |
| 7 | Adachi N (2008) | C-C | 18 female athletes with ACL injury | Menstrual history, athletic activity, and injury history were collected from the athletes | 72% of subjects had premenstrual symptoms, 83% had menstrual symptoms. Significant association between the phase of the menstrual cycle and ACL injuries. There were more injuries in the ovulatory phase than expected |

*to be continued*

*continue from* **Table IV.**

| | | | | | |
|---|---|---|---|---|---|
| 8 | Dedrick GS (2008) | | 26 healthy females | Varus/valgus knee angle & EMG activity from six lower extremity muscles were recorded during three drop jumps from a 50cm platform in each phase of menstrual cycle | Semitendinosus and gluteus maximus muscles exhibited delayed onset timing during luteal phase, and early and late follicular phases during drop jumps |
| 9 | Abt JP (2007) | | 10 healthy females<br>Mean age-21.4<br>Mean height-1.67cm<br>Mean mass-59.9kg | Single-leg postural stability, fine motor coordination, knee strength, knee biomechanics, and serum estradiol and progesterone were assessed at menses, post-ovulatory, and mid-luteal phases | No significant difference between phases of the menstrual cycle for: i) fine motor coordination, ii) postural stability, iii) hamstring - quadriceps strength ratio at 60 degrees or 180 degrees, iv) knee flexion excursion, v) knee valgus excursion, vi) peak proximal tibial anterior shear force, vii) flexion moment at peak proximal tibial anterior shear force, vii) valgus moment at peak proximal tibial anterior shear force |
| 10 | Chaudhari AMW (2007) | | 12 female athletes | Horizontal and vertical jump, and drop from a 30-cm box on the left leg. Lower limb kinematics and peak externally applied moments were calculated Women were tested for each phase of the menstrual cycle as determined from serum analysis | No significant differences in moments or knee angle were observed between phases in female group |
| 11 | Beynnon BD (2006) | C-C | 45 females athletes with ACL injury and 45 healthy females<br>Groups matched for age, height, weight | Serum sample and self-reported menstrual history data immediately after injury. Both serum concentrations of progesterone and menstrual history were used to group subjects | Serum concentrations of progesterone revealed that alpine skiers in the preovulatory phase of the menstrual cycle were significantly more likely to tear their ACL than skiers in the postovulatory phase<br>Analysis of menstrual history found similar results, but the difference was not statistically significant |
| 12 | Hertel J (2006) | | 14 healthy female athletes | Measures of knee neuromuscular performance and laxity once during the mid-follicular, ovulatory, and mid-luteal stages of menstrual cycle. | No significant differences in the measures of strength, joint position sense, postural control, or laxity across the three testing sessions.<br>No significant correlations were found between changes in E3G or PdG levels and changes in the performance and laxity measures between sessions |
| 13 | Arendt EA (2002) | C-C | 58 female athletes with ACL injury | Menstrual history, athletic activity, and injury history were collected from the athletes | A significant 28-day periodicity of injuries was present in the entire population as well as in the two subgroups. High- and low-risk time intervals were associated primarily with follicular and luteal phases |
| 14 | Wojtys EM (2002) | | 69 female athletes with ACL injury | Mechanism of injury, menstrual cycle details, use of oral contraceptives, and history of previous injury were recorded. Urine samples validated menstrual cycle phase at the time of the ACL tear | Results from the hormone assays indicate that the women had a significantly greater than expected percentage of ACL injuries during midcycle (ovulatory phase) and a less than expected percentage of those injuries during the luteal phase of the menstrual cycle |
| 15 | Slauterbeck JR (2002) | | 38 female athletes with ACL injury | Female athletes with ACL injury reported days of their menstrual cycles and provided saliva samples for sex-hormone determination | Correlation between saliva and serum oestrogen were high. 10/27 athletes sustained an injury 1-2 days after the onset of menses |

*to be continued*

*continue from* **Table IV.**

| | | | | | |
|---|---|---|---|---|---|
| 16 | Myklebust G (1998) | P | 23 female athletes | Menstrual history, athletic activity, and injury history were collected from the athletes | Five of the injuries occurred in the menstrual phase, 2 in the follicular phase, 1 in the early luteal phase and 9 in the late luteal phase |
| 17 | Wojtys EM (1998) | | 28 female athletes with ACL injury | Mechanism of injury, menstrual cycle details, use of oral contraceptives, and history of previous injury were recorded.<br>Observed and expected frequencies of ACL injury based on 3 different phases of the menstrual cycle | A significant statistical association was found between the stage of the menstrual cycle and the likelihood for an ACL injury. There were more injuries than expected in the ovulatory phase of the cycle. In contrast, significantly fewer injuries occurred in the follicular phase. |

nificant difference in peak knee internal/external rotation moments[27,32] in participants between menstrual cycle phases during drop jump tasks. Strong evidence from two high quality studies reported no significant difference in peak knee flexion moments[31,32] in participants between menstrual cycle phases during cutting and drop jump tasks. Limited evidence from one high quality study reported no significant difference in peak hip adduction moments[35] and hip internal rotation moments in participants between menstrual cycle phases during drop jump tasks.

### *Effects of menstrual cycle on neuromuscular control*

Six studies investigated the effects of the menstrual cycle on neuromuscular control in female participants during cutting and drop jump tasks[26,27,33,34,36]. Limited evidence from one high quality study reported a subgroup of females with greater anterior knee laxity in the pre-ovulatory phase who also experienced significantly greater quadriceps amplitude during drop jump tasks[27]. Limited evidence from one study reported a significant delay in semitendinosus muscle onset timing in female participants in the luteal compared to follicular phase during drop jump tasks[33]. Strong evidence from three high quality studies reported no significant changes in quadriceps, hamstring, and gastrocnemius amplitude in participants between menstrual cycle phases during drop jump tasks[34-36]. Moderate evidence from two high quality studies reported no significant changes in gluteus medius[26] or gluteus maximus[33] onset timing in participants between menstrual cycle phases during single-leg drop jump tasks.

### *Effects of menstrual cycle on anterior-cruciate ligament injury risk*

Ten studies investigated ACL injury risk in female participants during the menstrual cycle[25,37-45]. Strong evidence from eight studies reported significantly greater ACL injury risk in female participants in the pre-ovulatory compared to post-ovulatory phase during sporting activity[25,37,38,40,41,43-45]. Limited evidence from one good quality study reported significantly greater ACL injury risk in female participants in the ovulatory compared to pre and post-ovulatory phases during sporting activity[37]. Limited evidence from one study reported no significant difference in ACL injury risk in female participants between phases of the menstrual cycle[42].

## Discussion

This systematic review provides a clinically relevant summary on the effects of the menstrual cycle on lower-limb biomechanics and neuromuscular control and potential hormonal mechanisms behind ACL injury risk. Our systematic review extends work by previous systematic reviews[18,23,24] by drawing on important recent trials concerning biomechanical analysis and ACL injury, and by combining literature on menstrual effects on biomechanics and ACL injury risk. A total of 17 studies of varying quality met the inclusion criteria for this systematic review and were evaluated. Strong evidence from this review indicates that female athletes have a significantly greater risk of ACL injury in the pre-ovulatory phase during dynamic sporting activity[25,38,41,43,45]. Strong evidence from this review indicates a sub-group of female athletes with significantly greater anterior knee laxity in the pre-ovulatory phase, in whom significantly greater knee valgus and knee external rotation moments represent possible mechanisms behind greater ACL injury risk [27,31,32].

### Quality assessment and validity

Seven studies scored full points for participant inclusion by providing a suitable explanation of how participants were screened and how alternate causes of pain were excluded (Tab. II). The majority of studies were consistent in their inclusion criteria for participants; however, a number of studies did not state that they excluded patients with previous knee surgery, or exclude patients with an irregular menstrual cycle history or suspected/possible pregnancy in the last year. These omissions may reflect an assumption that these individuals were excluded following recruitment questionnaires, or that pregnant participants would all also experience irregular menses. Nevertheless ensuring these criteria form part of routine inclusion provides clarity and avoids confounding pathology.

Methodological quality of biomechanical studies was assessed using a modified Down and Black scale with varied results. Many studies failed to recruit participants that were representative of the entire population and to blind the outcome measurer however these issues may be less important for biomechanical variables than ACL injury risk. Ten studies investigated the effects of menstrual cycle on ACL injury risk[25,37-45]. All studies describe a prospective screening for previous ACL injury, however, all athletes were retrospectively interviewed and tested following ACL injury. Menstrual cycle variability exists in adolescent female athletes, influenced by activity levels, changes in weight, diet, and stress[46]. While retrospective questioning has been found a reliable method[47], it is possible that inconsistencies in cycle patterns may be identified between prospective and retrospective design methods and may present a threat to internal validity and reliability of findings. We recommend that researchers consider prospective evaluation to better differentiate cause and effect between ACL laxity and ACL injury during pre-ovulatory phase of the menstrual cycle.

### Possible mechanisms

The anterior cruciate ligament is the primary stabiliser of the knee acting synergistically to limit anterior translation of the tibia on the femur and to limit tibial internal rotation. The significance of greater knee valgus and greater anterior knee laxity as mechanisms behind ACL injury during functional activity is well documented in the literature[4,6-8,10,17,18]. One hypothesis suggests that increased dynamic valgus increases frontal plane valgus collapse and ACL injury in females[4,8,19]. A number of cadaveric, *in vivo*, and computer modeling studies report that greater knee valgus can increase ACL strain sufficiently to rupture the ligament[48-50]. However, strong evidence from our review indicates that the menstrual cycle has no direct effect on levels of knee valgus in females during functional movements[27,31,32,35]. Importantly of note, strong evidence from our review indicates a subgroup of female participants with significantly greater anterior knee laxity in the pre-ovulatory phase who experienced significantly greater knee valgus and knee external rotation during cutting and drop jump movements[27,31,32]. Consequently, this may explain the significantly greater risk of ACL injury in female participants in the pre-ovulatory phase during functional activity.

While some studies included in this systematic review investigated the effects of the menstrual cycle on ACL laxity, laxity cannot predict joint behavior during dynamic and functional activities as tests are typically performed without the compressive joint forces required to properly engage the conforming condylar surfaces, which play an important role in joint stabilisation[18]. While ACL laxity lies outside the scope of this review, it is considered as part of our discussion. Limited evidence from Belanger's recent systematic review suggests that increased ACL laxity in the pre-ovulatory phase represents an important mechanism behind greater ACL injury risk[24]. A number of studies has identified the presence of estrogen receptors within human ACLs, which, when activated, produce a decrease in fibroblast proliferation and subsequent decrease in type 1 collagen synthesis[20,21,51,52]. Studies have reported this reduces overall ACL strength, integrity, and its ability to withstand higher loads[20,21,52,53]. Put together, an increase in estrogen and ACL laxity in the pre-ovulatory phase may lead to greater knee valgus and knee external rotation resulting in greater ACL strain and injury risk in a subgroup of female participants.

Neuromuscular control is an important factor in lower limb biomechanics and imbalances or deficits are often implicated knee pathology[6,10,18,54]. Supporting this, previous studies have identified delayed gluteus medius onset timing as a risk factor for ACL injury in female athletes. Our review indicates that changes in neuromuscular control between phases of the menstrual cycle may contribute to ACL injury during functional activity. Limited evidence from one study indicates that oestrogen may increase quadriceps contractile strength and slow relaxation during the ovulatory phase[27]. One hypothesis suggests that fluctuations in serum oestrogen concentrations during the menstrual cycle affect muscle function and may represent a mechanism of ACL injury in females[23,24]. A second hypothesis suggests the menstrual cycle has a significant influence on knee joint kinesthesia and ACL injury risk in females[35]. Considering the limited and conflicting evidence, and the different variables measured, further high-quality evidence is required to identify potential changes in neuromuscular control during the menstrual cycle and how this may contribute to ACL injury risk.

### *Limitations and future research*

High quality prospective evaluation of two themes identified by this systematic review should be a priority for future research. During the pre-ovulatory phase of menstrual cycle, 1) are females with greater ACL laxity at increased risk of ACL injury, and 2) are females with greater ACL laxity have greater lower-limb valgus, tibial external rotation, or other abnormal biomechanics. High quality prospective studies will help strengthen our understanding of the influence of hormonal mechanisms on ACL injury risk.

High quality prospective evaluation of the effects of the menstrual cycle on possible proximal and distal biomechanical mechanisms should also be covered. Specifically, the influence of pelvis width and Q-angles, femoral notch widths, hamstring-quadriceps muscle, muscle onset and activity, and ankle biomechanics on ACL injury should be sought.

Negative findings from studies using movements such as two-leg drop landing, horizontal jump, and vertical jump may be a result of these less functional movements. Furthermore, variability of movements between studies makes direct comparisons difficult to make. The literature suggests that investigations of the ACL should focus on dynamic movements such as rapid deceleration and change of direction[10]. High quality prospective evaluation of biomechanics between phases of menstrual cycle using specific higher-risk movements will improve validity and allow direct comparisons to be made.

Retrospective studies in this review are required to make assumptions based on responses from questionnaires regarding length and timing of participants' cycles. The majority of studies in this review separate the menstrual cycle into three main phases: follicular, ovulatory, and luteal. While retrospective questioning has been found a reliable method[47], accurate differentiation between these three phases of the menstrual cycle requires prospective serum analysis before injury and an understanding of the length and timing of a participant's cycle[55]. Ovulation is a specific event in the menstrual cycle and future studies should either use serum analysis to identify the specific menstrual phase or group subjects in preovulatory/postovulatory phases.

## Conclusion

In summary, our systematic review provides strong evidence that females are at greatest risk of ACL injury during the pre-ovulatory phase of the menstrual cycle. The greater ACL injury risk in the pre-ovulatory phase occurs through a combination of greater ACL laxity, greater lower-extremity valgus, and greater tibial external rotation. High quality prospective evaluation should be a priority for future research given the devastating impact of ACL injury on physical and psychological disability.

## Conflicts of interest

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