# COMPARING KNEE JOINT MECHANICS ACROSS PHASES OF THE MENSTRUAL CYCLE

By

Bethany Kilpatrick

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Committee Membership

Dr. Justus Ortega, Committee Chair

Dr. Whitney Ogle, Committee Member

Dr. Amanda Hahn, Committee Member

Dr. Taylor Bloedon, Committee Member and Graduate Coordinator

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# ABSTRACT

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Bethany Kilpatrick

Anterior cruciate ligament (ACL) injuries are among the most commonly occurring knee injury. Compared to men in the same sports, females are two to eight times at higher risk for ACL injury. Research suggests that hormone fluctuations across the menstrual cycle (MC) play a crucial role in ACL injuries due to their effect on tendons and ligament's mechanical properties. PURPOSE: To examine knee joint laxity during dynamic movements across three-time points of the MC. METHODS: Seven young, healthy females with regular MCs performed three jump-landing tasks (double leg depth jump, single-leg lateral jump, and single-leg forward jump) across the early-follicular, ovulatory, and mid-luteal phases of the MC. Peak frontal and sagittal plane knee joint angles and moments were measured to assess joint stability during dynamic movements in each jump trial were analyzed. The researchers used an analysis of variance (ANOVA) to determine the effects of the menstrual cycle's phases on joint mechanics during each jump task. RESULTS: No significant change occurred in knee joint frontal or sagittal plane angles or moments across MC time points. CONCLUSION: In support of finding from prior studies, the researchers did not observe any changes in knee joint mechanics across the menstrual cycle phases, suggesting that MC hormonal fluctuations do not affect the knee joint's mechanical properties tendons and ligaments enough to cause changes in joint laxity. Future research should examine the specific

relationships between measured hormone levels and knee joint mechanics during dynamic movements across the MC phases to assess these relations more accurately.

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# INTRODUCTION

A common injury among athletes is a knee injury. A knee ligament sprain is one of the most commonly occurring knee injuries, with trauma to the anterior cruciate ligament (ACL) being frequent (Bollen, 2000). In the United States, 150,000 ACL injuries are reported annually, contributing to half-billion dollars in medical costs (Klossner, 2011). With the number of collegiate athletes at an all-time high, women make up 46% of the student-athlete population (Wimmer-Schwarb, 2018). Several studies have shown that female athletes are more likely than men to experience an ACL injury across their athletic careers (Agel et al., 2016; Arendt & Dick, 1995; Kobayashi et al., 2010). More specifically, compared to men in the same sports, female collegiate athletes are two to eight times more likely to experience an ACL injury than male athletes (Arendt & Dick, 1995).

Multiple factors may explain why female athletes have higher ACL injury rates than male athletes, such as sex-related differences in anatomy (Woodland & Francis, 1992a), differences in muscle activation patterns during movement (Zeller et al., 2003), and hormonal fluctuations across menstrual cycles in women (Wojtys et al., 2002). Prior research suggests that hormone fluctuations related to the menstrual cycles may play a specific role in altering tendon and ligament tissue property resulting in decreased joint stability and increased risk of an ACL injury (Liu et al., 1996; Shultz et al., 2006). Decreased knee joint stability, i.e., an increase in knee joint laxity, is a considerable risk factor for knee joint injury due to weakening the knee's structural components. Some research has shown that knee joint laxity changes throughout the menstrual cycle (Park et al., 2009; Shultz et al., 2006), yet other research on the topic has been mixed (Beynnon et al., 2005; Hertel et al., 2006a; Shafiei et al., 2016a). Of the commonly studied phases across the menstrual cycle, early follicular phase, late follicular phase (ovulation), and mid-luteal phase, it remains unclear whether a particular phase across the menstrual cycle is associated with decreased joint stability and increases an ACL injury risk. If any of these particular time points across the menstrual cycle potentially holds the most amount of risk to an athlete's ACL, it is worth investigating to prevent future injury. Thus, the purpose of this study is to examine knee joint instability during dynamic landing movements across three time points across the menstrual cycle.

## Anterior Cruciate Ligament Injuries and Mechanics

Anterior Cruciate Ligament (ACL) injuries are the most commonly reported knee injury, with 150,000 ACL injuries reported annually, contributing to half-billion dollars in medical costs (Klossner, 2011). Complete ACL tears can lead to chronic knee conditions such as knee instability, osteoarthritis, and damage to the knee meniscus (Finsterbush et al., 1990). These injuries are common in many athletic landing and cutting sports, such as basketball, volleyball, and soccer (Agel et al., 2016; Kobayashi et al., 2010). Because there are approximately 216,000 female athletes nationwide, representing 46% of the collegiate athletic population (Wimmer-Schwarb, 2018), and females having a much higher rate of ACL injuries (Arendt & Dick, 1995), identifying factors contributing to ACL injuries among females is imperative.

## The Anterior Cruciate Ligament

The ACL is part of a network of ligaments in the knee's tibiofemoral joint that helps connect the femur and tibia. The ACL comprises type I and type III collagen fibers, making up a thick structural yet elastic matrix of connective tissue (Amiel et al., 1984). Type I collagen fibers are associated with being rigid with a more broad diameter due to the ability to carry a greater mechanical load than type III, which is more elastic (Culav et al., 1999). The ACL originates at the femoral condyle's medial posterior border and attaches at the tibia's anterior medial border (Petersen & Tillmann, 2002). The ACL is strongly associated with knee stability, assisting with the movements of the sagittal plane. Due to the ACL assisting in the sagittal plane, when a rupture occurs to the ACL, it is often in the frontal plane during varus (bow-legged) or valgus (knock-kneed) rotation. ACL ruptures are most common during gameplay and are either contact or non-impact injuries (Kobayashi et al., 2010). Contact ACL injuries occur when there is direct contact to the knee by another person or object, resulting in injury. Non-impact ACL injuries are ruptures of the ACL that occur without contact from another person and are due to the rapid movement in actions such as landing, deceleration, or changing direction (Boden et al., 2000). Non-impact injuries of the ACL are the most common, accounting for an estimated 70% of ACL injuries (Griffin et al., 2000; Kobayashi et al., 2010). Most non-impact ACL injuries in athletes typically occur during gameplay in the frontal plane (Kobayashi et al., 2010).

Injury to elastic tissues such as ligaments and tendons depends directly on the relationship between the forces applied to the tissue and the material's deformation. During regular everyday movements, including running and jumping, ligament tissue behaves like an elastic material; it stretches or deforms when forces are applied but then returns to its standard shape (Kennedy et al., 1976). However, if the force exceeds a certain level (yield point), the tissue breaks down at a microscopic level and behaves like a plastic material where it does not return to its original shape; thus, when there is too much force applied to a ligament and the tissue is stretch beyond the yield point, injury occurs (Kennedy et al., 1976). At a joint level, the relationship between force and deformation is described by joint stiffness, according to Hooke's Law. Hooke's Law, states that the force needed to deform a material is proportional to the deformation's magnitude (Rychlewski, 1984).

Using Hooke's Law, joint stiffness is a biomechanical measurement of the ligaments' deformation concerning the applied moment or load. Torsional joint stiffness is further defined mathematically as, $k_{joint} = \Delta M/\Delta\theta$, M being a change in the joint moment (i.e., torque) and θ being a change in joint angle (Farley et al., 1998). It is essential to consider the knee's torque because this may indicate increased motion at a joint due to a decrease in joint stability. A common technique for directly measuring knee stiffness is by using an arthrometer. An arthrometer is a machine that uses force on the anterior tibia to measure translational displacement within the knee. This type of measurement, referred to as a "drop-drawer test," is considered the gold standard for measuring direct strain on the ACL (Rohman & Macalena, 2016). Although tendons and ligaments do need to have enough stiffness and stability to protect the knee, there is a small range at which healthy knees displaces 5.7 ± 2.3 mm, whereas ACL deficient knees typically move 13.0 ± 3.5 mm (Daniel et al., 1985).

A protective mechanism for the ACL is the muscular co-activation of the quadriceps and hamstrings. Co-activation refers to the simultaneous activation of antagonist muscle groups. Research shows that a balanced co-activation of the hamstrings and quadriceps muscle reduces the ACL strain (Lloyd & Buchanan, 2001). Thus, when the ratio of hamstring to quadriceps muscle activation (Hamstring-Quad Ratio or HQR) is close to 1:1, the risk of ACL injury is reduced. In vivo research has shown that when only the quadriceps activate, there is an anterior pull on the ACL, which activation from the hamstrings mitigates (Draganich & Vahey, 1990). Posterior tension from activation of the hamstrings decreases anterior knee translation movements and reduces frontal plane motion (More et al., 1993), which are risk factors for ACL injury. As well as stabilizing the ACL directly, a balanced HQR also stabilizes the knees, limiting large amounts of varus-valgus frontal plane motion.

## Factors Contributing to ACL injuries in Females

There are several possible determinants of ACL injury among females. Sex-related differences in anatomical and mechanical factors have contributed to more ACL injuries among women (Beutler et al., 2009; Hughes et al., 2008; Zeller et al., 2003). For instance, one anatomical factor is the "quadriceps angle," also known as the "Q-angle," which refers to the angle between the quadriceps muscle and the vertical axis. It is measured by extending a line from the patella to the pelvis's anterior superior iliac spine and then measuring this line's angle relative to a vertical axis going through the patella center. Increased Q-angle inserts more stress on the medial knee joint in the frontal plane and may lead to a greater risk of knee injuries. Prior research has suggested that women may be at a higher risk of knee injury than males because females typically have a wider pelvis and, therefore, greater Q-angle (Woodland & Francis, 1992b).

Moreover, females have more femoral anteversion, ankle pronation, tibial torsion, and rectus femoris activation than males in specific weight-bearing movements, all associated with increased strain on the knee (Zeller et al., 2003). Sex-related differences in muscle activation may also play a role in increased knee injuries among women. Specifically, research has shown that females have greater quadriceps activation and reduced hamstring activation than males (Dos Santos Andrade et al., 2017; Huston & Wojtys, 1996). As previously mentioned, hamstring activation stabilizes the ACL in the posterior direction, minimizing tibial translation (More et al., 1993). These sex-related differences in anatomy and muscle activation likely play a role in females' reduced knee stability.

When comparing female and male basketball players, Rozzi et al. (1999) observed that females had increased knee joint laxity and had a poor sense of knee extension and joint motion, suggesting a deficit in knee proprioception. Research has shown that participants with general joint laxity due to injury in their shoulder joints have shown to have a deficit in proprioception (Allegrucci et al., 1995). It is suggested that since female athletes have inherent joint laxity, the ACL is less taut, therefore not enacting protective proprioceptive mechanisms (Rozzi et al., 1999). If there is a poor sense of internal special awareness of knee movement, protective mechanisms may not be able to enact in time, increasing the risk of injury. Similar studies have also attributed females to increased joint laxity and a delay in hamstring activation to poor knee joint proprioception (Shultz et al., 2004).

In addition to anatomical, mechanical, and proprioceptive differences, physiological differences, such as hormonal fluctuations across the menstrual cycle (MC), may also contribute to higher rates of ACL injuries among females (Liu et al., 1996). Changes in hormone levels at a specific time point across the menstrual cycle possibly affect joint stability of the knee. Such time points would be when estrogen and or progesterone levels are very low across the early follicular or at their peaks during ovulation or mid-luteal phase (Dos Santos Andrade et al., 2017; Park et al., 2009; Shultz et al., 2006; Slauterbeck et al., 2002a; E. M. Wojtys et al., 2002; Yu et al., 1999).

## The Menstrual Cycle

There are two simultaneous cycles occurring across the menstrual cycle (MC), the uterine cycle, and the ovarian cycle. Both cycles coincide, starting at the onset of menses in the uterine cycle. The ovarian MC has two phases, follicular and luteal, with ovulation between the two phases; levels of progesterone and estrogen fluctuate across these phases. For this study, three points of time, or phases, early follicular phase (often referred to as menses), late follicular phase or ovulation, and mid-luteal phase, were analyzed. The first phase of the ovarian cycle is called follicular. The follicular phase, the most variable in length, starts the first day of menstruation and averages about 14 days in females aged 18 to 39 (Grieger & Norman, 2020). At the beginning of the follicular phase or early follicular phase, hormones estrogen and progesterone are at their lowest levels of the MC (Baird & Fraser, 1974). The next sub-phase, the late follicular or, more specifically, pre-ovulation phase, is characterized by a rapid 24-hour peak and a fall in estrogen, with a mild rise in progesterone (Baird & Fraser, 1974). The rise in estrogen through a series of physiological mechanisms also increases luteinizing hormone (LH). Luteinizing hormone's rise triggers the release of an egg from the ovaries after approximately 10 to 12 hours after the LH peak. Ovulation, an event occurring in the late follicular phase, typically occurs around day 14 of the cycle. After ovulation, the corpus luteum, an independent endocrine organ, is formed. The corpus luteum is responsible for releasing the hormones relaxin-2 and progesterone across the last MC phase, the luteal phase. The luteal phase is 14 days in length. Across the mid-luteal phase, estrogen is at its mid-range point, and progesterone rises to its peak. Relaxin-2 serum, a hormone known to give tendons and ligaments elastic properties at childbirth, also peaks in the mid-luteal phase (Loumaye et al., 1984; Nose-Ogura et al., 2017). Towards the end of the luteal phase, the corpus luteum begins to dissolve and undergo luteolysis. This disintegration of the corpus luteum produces a decline in progesterone levels, which, when low enough, the next menstrual cycle begins. The cycle's total length can vary, but the average length is 28 to 35 days (Grieger & Norman, 2020; Mihm et al., 2011a).

## Sex Hormones and Their Proposed Effect on the ACL

Although researchers have mapped hormone fluctuations across the menstrual cycle with great accuracy (Baird & Fraser, 1974), the effects of female sex hormone fluctuations on tendon and ligament matrix properties, specifically joint laxity, are not fully understood and are highly debated. A decrease in joint stiffness brings about less overall stability for the joint, which may bring the ligaments and tendons to their maximal stress point more easily (Butler et al., 2003). In animal studies, the hormones estrogen and relaxin-2 have been shown to inhibit the proliferation of procollagen peptides, thereby degrading collagen matrices (Fischer, 1973; Samuel et al., 1996; Yu et al., 1999). This would suggest that when estrogen and relaxin-2 are at their respective peaks, females have a higher risk of joint injury.

However, just as researchers do not fully understand how each hormone affects tendon and ligament properties, a consensus as to which phases across the MC may pose the highest amount of injury risk due to hormonal fluctuations influencing joint laxity is mixed (Balachandar et al., 2017; Dragoo et al., 2011; Hewett et al., 2007; Shultz et al., 2011; Wojtys et al., 2002). For instance, one study found that 70.3% of athletes tore their ACL in the early follicular phase, when sex hormones are at their lowest across the MC, verified by serum testing at the time of injury (Slauterbeck et al., 2002). However, Wojtys et al. (2002) observed that females are at the highest risk of experiencing a non-impact ACL rupture during ovulation, around the time when estrogen is at its highest. This finding may be related to evidence that knee joint laxity, measured using an arthrometer, increases by 17% during ovulation (Park et al., 2009). These studies suggest that the increase in knee joint laxity and ACL injury may be related to increased estrogen levels during ovulation compared to the early follicular and mid-luteal phases.

Estradiol, one of three estrogen hormones naturally produced in the body, potentially affects the stiffness of connective tissues, such as tendons and ligaments, by inhibiting the rate of fibroblast proliferation and procollagen synthesis (Yu et al., 1999). One study found that oral estrogen-progesterone therapy can decrease type 1 procollagen peptides by up to 30% (Hassager et al., 1991). In animal studies, estrogen administration has shown to reduce collagen and have other degenerative effects on tissues (Fischer, 1973). With increasing estrogen levels and a decrease in type 1 procollagen synthesis, ligaments will weaken (Yu et al., 1999). Thus, increased estrogen levels in the late-follicular phase may reduce ligament strength and stiffness, decreasing joint stability, thus increasing the possible risk of ACL injury.

While many researchers focus their sights on estrogen concentrations in the late-follicular phase (Bell et al., 1990; Lee et al., 2013; Park et al., 2009; Wojtys et al., 2002), relaxin-2 serum, a peptide with the capacity to break down collagen, has its highest concentration in the mid-luteal phase (Loumaye et al., 1984; Nose-Ogura et al., 2017; Samuel et al., 1996). Research has shown that knee joint laxity increases from the early follicular phase to the mid-luteal phase when measuring tibial translation using an arthrometer (Shultz et al., 2011).

In addition to the possible adverse effects of hormones on joint laxity, changes in lower body neuromuscular activation associated with the menstrual cycle may also contribute to knee joint instability and ACL injury. Research suggests that HQR, a known factor influencing knee joint frontal plane stability, is reduced in female athletes' follicular phase (Dos Santos Andrade et al., 2017). However, researchers did not indicate specifically when in the follicular phase, the measurement was collected. In contrast, Dedrick et al. (2008) documented a neuromuscular delay of the semitendinosus hamstring muscle in the luteal phase compared to the follicular. It was speculated that the delay in hamstring firing was possibly due to estrogen altering tissue properties of neural receptors in the ACL (Raunest et al., 1996) and therefore disrupting proprioceptive feedback (Dedrick et al., 2008). Apart from the studies mentioned, minimal research has investigated the potential proprioceptive deficit in female's knee joints or its relation to the phases of the menstrual cycle. Therefore it cannot be concluded that proprioception plays a part in knee stability in frontal plane knee angles.

Delayed motor unit (MU) firing rates may increase the risk of injury among females playing sports due to possibly not activating the leg muscle in time to prevent excessive joint loading and injury. A motor unit is a single motor neuron, and all of the skeletal muscle fibers it innervates (Purves et al., 2001). The MU affects two ways that muscles contract; the recruitment of muscle fibers and the neural firing rate. The MU firing rate is when nerve impulses are sent to the muscle fibers to contract. When the firing rate increases, this produces more muscle force (Purves et al., 2001). Therefore, delays in MU firing may predispose passive tissues such as ligaments to increased stress and strain. Coupled with potential hormone-related increases in knee joint laxity, these neuromuscular activation changes associated with sex hormones throughout the menstrual cycle leave female athletes particularly vulnerable to ligament injury. Although they did not show a difference in leg muscle firing rates across the menstrual cycle, Cesar et al. (2011) found significant differences in varus-valgus knee joint movement between MC phases. As mentioned previously, Dedrick et al. (2008) reported a delay in the semitendinosus hamstring during a time point of the luteal phase in females. However, other studies have noted no difference in neuromuscular firing rates throughout the menstrual cycle (Abt et al., 2007; Hertel et al., 2006; Romero-Moraleda et al., 2019). Moreover, the influence of altered neuromuscular control associated with the menstrual cycle on knee joint stability and injury is still unresolved and not well understood.

Although several studies suggest that hormonal fluctuations within the menstrual cycle influence the prevalence of ACL injuries among females, some studies have shown no correlation between knee laxity or instability across the menstrual cycle (Beynnon et al., 2005; Chaudhari et al., 2007; Hertel et al., 2006a; Shafiei et al., 2016a). Many authors attribute not finding significant results between MC phases possibly due to differences in data collection, variability in hormone levels, as well as potentially small changes in knee laxity did occur but were too minimal to detect (Beynnon et al., 2005; Hertel et al., 2006a; Shafiei et al., 2016). Researchers have also suggested contraceptives that suppress hormonal fluctuations, such as the rise in estrogen or inhibit the creation of the corpus luteum, may act as protective mechanisms against joint instability and injury (Nose-Ogura et al., 2017; Samuelson et al., 2017). With mixed findings on the topic of hormone suppression affecting knee stabilization, no conclusions have been made.

An explanation for mixed findings in prior research may be that some female's knee tendons and ligaments have a greater response to estrogen, progesterone, or relaxin-2, causing an increase in knee laxity than other females who are less responsive to hormonal influence on knee laxity. A hormonal responder would mean that the variability of the magnitudes of hormones from person to person affects knee collagen proliferation. Therefore, individual female knee joint laxity may have a greater response to the magnitude of hormonal fluctuations than others. For instance, Shultz et al. (2004) observed considerable person-to-person variability in knee joint laxity measurements explained by absolute changes in sex hormone levels. Shultz et al. (2004) ascertained that increased knee laxity would either have a positive relationship with all three hormones measured, estrogen, progesterone, and testosterone, a response to a particular sex hormone such as estrogen, or have little relationship between knee laxity and sex hormones. This suggests much variability between individual female's knee tendon and ligament responses to sex hormones.

Moreover, Bell et al. (2014) suggested that female athletes with higher magnitudes of estrogen levels and a mild amount of progesterone around the time of ovulation are the same athletes having ACL injuries. They found that in a group of female athletes with previous ACL injuries, all had more knee laxity and knee frontal plane motion in pre-ovulation than in the early-follicular phase. However, the researchers only compared knee laxity in the early and late follicular phase and not the mid-luteal phase when progesterone and relaxin-2 would be at their highest amounts. Researchers have also found relaxin-2 was in higher quantities in a subgroup of female collegiate athletes who have previously sustained an ACL injury in their athletic career, further adding to the theory of responders versus non-responders (Dragoo et al., 2011). Although the "responders" theory makes sense of why there are mixed results when comparing knee laxity between the MC's time points, it is still unclear which time point across the MC poses the most risk to potential hormonal responders.

Research on the topic of female knee laxity is mixed, with risk associations relating to general anatomical differences between males and women, sex hormone levels, neuromuscular activation timing, and hormonal responders versus non-responders. Although many studies suggest there is no change in knee laxity throughout the MC, there is also much research that reports that knee laxity changes are due to hormonal fluctuations (Beynnon et al., 2005; Park et al., 2009; Shultz et al., 2006; Dedrick et al., 2008). Furthermore, much of the research observing knee laxity changes are done with an arthrometer and not in dynamic movements. Therefore, this study aimed to examine frontal plane knee joint motion and moments in dynamic jump landing across crucial time points of the menstrual cycle. It was hypothesized that frontal plane knee joint motion and moments would change across the menstrual cycle phases. Specifically, that frontal plane knee joint motion and moments will be highest at ovulation when estrogen is at its peak level.

# METHODS

Research on the topic of female knee laxity is mixed, with risk associations relating to general anatomical differences between men and women, sex hormone levels, neuromuscular activation timing, and hormonal responders versus non-responders. Although many studies suggest there is no change in knee laxity throughout the MC, there is also much research that reports that knee laxity changes are due to hormonal fluctuations in passive movements (Beynnon et al., 2005; Park et al., 2009; Shultz et al., 2006). Because ACL injuries are most prevalent during gameplay, in the frontal plane (Kobayashi et al., 2010), it is imperative to attempt to reenact potential movements that the athletes would be doing. No study has researched how hormonal fluctuations may affect knee joint instability across the menstrual cycle while performing non-dominant single-leg landing movements in the frontal and sagittal planes. Therefore, this study aimed to examine frontal plane knee joint motion and moments in dynamic jump landing across crucial time points of the menstrual cycle. The researchers hypothesized that frontal plane knee joint motion and moments would change across the menstrual cycle phases. Specifically, it was hypothesized that frontal plane knee joint motion and moments would be highest during ovulation when estrogen is at its peak level.

## Participants

Twenty-one females were recruited for the study, with seven healthy adult females aged 21.9 ± 1.7 years were retained. Participants had normal menstrual cycles (29 ± 3 days), consistently the same length of time (28 ± 7 days) for at least six months, and were not on any type of hormonal contraceptives for at least six months prior to the study (Mihm et al., 2011a). All participants were free of prior knee injuries and any other lower-body injuries for the six months prior to participation in the study. The researchers excluded participants if they had any neurological disorders. All participants gave their written informed consent before participation in the study. All protocol was reviewed and approved by the Humboldt State University Institutional Review Board before participant recruitment and data collection.

## Experimental Design

The researchers collected jump landing kinematics and kinetics of each participant on three separate occasions; early follicular, late follicular, and mid-luteal phase. Following recruitment and informed consent, participants were instructed to track the timing of two menstrual cycles, starting at the beginning of menstruation, using the menstrual cycle tracking application Clue™. Participants then reported for their first testing session (early follicular phase) within the first five days after the start of their third menstruation, when sex hormones are relatively low (Park et al., 2009). The researchers then gave the participants a series of luteinizing hormone (LH) tests to track when the participants entered their fertile window in the late follicular phase (ovulation) when estrogen is high and progesterone is in mid-range. Once an LH test was positive, within 24 to 48 hours, the participants came in for their second data collection. After their second data collection, the researchers then scheduled participants for their third and final data collection 7 ± 3 days post-ovulation in the mid-luteal phase.

For each time point, the researchers first collected anthropometrics, lower body kinematics (motion), and ground reaction force during the landing phase for three jump landing tasks. For each jump data collection, participants performed three jumping-landing tasks with three trials per task. The three jumping-landing tasks included 1) drop-jump (DJ), 2) single-leg forward jump (SLJ), and 3) single-leg lateral jump (LAJ). Before each experimental jumping task, participants performed several familiarization/practice jumps. If the participant felt fatigued after any particular jump, the researchers gave them time to rest in between jumps. The jump tasks were randomized and counterbalanced across subjects for this study.

## Menstrual Cycle and Late – Follicular Phase Tracking

As mentioned previously, the researchers used the data collected from two menstrual cycles prior to testing to predict the day of ovulation and used luteinizing hormone (LH) testing kits to verify the day of ovulation for our second data collection. To predict the day of onset of menses and the time of ovulation, the researchers used predictions from the phone application Clue $^{TM}$. This phone application uses an algorithm based on previous MC data to predict the day of menses onset and ovulation. The researchers then used the predicted upcoming ovulation date to schedule luteinizing hormone testing.

To assess that participants were tested around the time of ovulation, participants were instructed to take four LH testing kit strips (CVS One Step Ovulation Predictor (sensitivity 20 mIU/ml LH, accuracy 99%); CVS Corporation, Woonsocket, RI), three days before their projected ovulation date, with a fourth LH test taken the day of ovulation, i.e., when LH was present. Participants were instructed to test their first morning urine, thereby avoiding excessive intake of fluids that may dilute the test (Park et al., 2009). The LH testing procedure followed the kit instructions, where participants voided their first morning urine into a cup and placed a testing strip into the urine for 30 seconds before removing the test strip. Then the participants waited three minutes for the test results. A positive LH test indicated LH's presence, indicating that the participant also had high estrogen levels at this time. The researcher contacted the participants every morning to verify a positive or negative test result. Once the participants tested positive for LH, the researcher scheduled the second data collection within 24 to 48 hours after a positive LH test.

If the participants did not test positive for LH on their projected ovulation date, the researcher gave them three extra tests. The researchers excluded participants if they did not test positive for LH after three more days. If the test strip tested positive for LH before their projected date of ovulation, the researchers counted that as their ovulation date. If the participants could not be scheduled within 24-48 hours of a positive LH test, the researchers excluded them from further data collection until their next cycle.

## Anthropometrics

For each data collection trial, before testing, the researchers collected the participant's height (cm), mass (kg), leg length (mm), knee and ankle joint width (mm) anthropometrics for both the left and right sides. The researchers defined leg length as the point from the participant's anterior superior iliac spine to their medial malleolus. Before testing, the researchers instructed participants to fill out a Revised Waterloo Footedness Questionnaire to determine their non-dominant leg (Elias et al., 1998).

## Normalization

For this study, it was important to normalize tasks to create consistency between participants. For the drop-jump, The researchers normalized the drop-box height for each subject, which approximated to 97% of their average maximum counter-movement jump height. Before the familiarization jumps, the researchers determined each participant's maximum counter-movement jump height by having the participant perform three maximal effort counter-movement jump started from a standing position. Using digital motion capture, displacement was measured at the maximal height of the sacral markers' vertical displacement from the standing position.

For the SLJ and the LAJ, the participants landed on their non-dominant leg to possibly amplify any changes that might happen between MC phases. The participants were also asked to jump 90% and 120% of their leg length. These calculated distances were used primarily as a form of normalization between participants but were also challenging yet achievable.

## Jump Landing Tasks

For the drop-jump (DJ), participants started by standing on a normalized height platform. The participants put their hands on their hips, stepped out with their right leg, and dropped down, landing on a force-sensing platform (Model OR6-7, AMTI, Watertown, Maine) evenly between both legs. Upon landing and without stopping movement, the participants performed a maximal effort counter-movement (down/up) vertical jump (Dedrick et al., 2008). Participants perform three recorded DJ trials. If participants did not perform the jump correctly, for example, landing directly on the force platforms, the researchers asked participants to retry the jump.

Participants also performed a single-leg forward jump (SLJ) onto the force platform. Participants held their hands on their hips and stood on their non-dominant leg. Participants then jumped forward to 90% of their leg length, landing on the force platform with their non-dominant leg. The researchers instructed participants to hold their landing foot position for two seconds. Participants repeated the task if they could not hold their landing foot in position for two seconds or placed the opposite foot on the ground for stabilization.

For the lateral jump (LJ), the third jump task, researchers asked participants to jump laterally onto their non-dominant foot at a distance of 120% of their leg length. Participants started the jump trials by standing on their dominant leg with their non-dominant knee and hip flexed to 45 degrees (i.e., stork stand). The participant then jumped laterally 120% of their leg length toward their non-dominant side, landing on the force platform with their non-dominant foot. Participants retried the trial if they could not hold their landing foot in position or placed the opposite foot on the ground for stabilization.

## Measurements

### Leg kinematics

The researchers measured leg kinematics using a nine-camera 3-D digital motion capture system (200 fields/s, Vicon, Centennial, CO, USA). Before data collection, reflective markers (14mm) were placed over the lower body's anatomical landmarks according to a modified Helen Hayes Model with CODA pelvis (A. L. Bell et al., 1990; Davis et al., 1991). Researchers placed markers bilaterally on the posterior-superior iliac spine (PSIS), anterior superior iliac spine (ASIS), lateral femoral epicondyle, lateral malleolus, calcaneus, as well as a cluster of four markers on the upper lateral thigh and lateral lower leg. Raw marker trajectory data was then be filtered using a 6th order zero-lag, low-pass (12 Hz) Butterworth filter (Park et al., 2009).

Using filtered leg marker data, the researchers quantified knee joint motion during the landing phase of each jump; identified as the time between initial contact with the force platform (Force threshold = 10N) and when the center of mass was at its lowest vertical position (Orishimo et al., 2010). Peak knee joint flexion angle ($FLEX_{DEG;°}$) in the sagittal plane and peak knee joint varus-valgus angle ($VV_{DEG}$; °) in the frontal plane were calculated during the landing phase of each jump.

### Leg kinetics

The researchers used force-sensing platforms for each jump's landing phase (Model OR6-7, AMTI, Watertown, Maine) to measure the 3-D ground reaction forces under the landing foot. The researchers collected raw ground reaction force data at a sampling rate of 1500 Hz and then passed through a zero-lag, low pass (50 Hz) (4th Order Butterworth) filter (Park et al., 2009). Using the filtered ground reaction force and leg kinematic data with inverse dynamics equations (Stefanyshyn & Nigg, 1998), the researchers calculated peak sagittal plane extension moment ($EXT_M$; Nm/kg) and peak frontal plane varus-valgus moment ($VV_M$; Nm/kg) for both knee joints during the landing phase of each jump (Hewett et al., 2005). The researchers normalized all joint moments the participant's weight (Nm/kg).

For every trial of each jump condition of a particular session, the researchers averaged absolute peak knee joint kinematics ($FLEX_{DEG}$ and $VV_{DEG}$ angles) and frontal plane joint moments between the right and left sides of the body. The researchers then averaged these values across all three trials of each jump condition.

## Statistical Analysis

The researchers reported participant's means and standard deviations as descriptive statistics. An analysis of variance (ANOVA) was performed on each dependent variable to determine the effects of the menstrual cycle phase (three levels) on 1) leg joint mechanics (peak joint angles and moment). Researchers took absolute peak values for each subject for $VV_{DEG}$, $VV_M$, $FLEX_{DEG}$, and $EXT_M$. Researchers performed four separate one-way-repeated measures analysis of variance (ANOVA) with Bonferroni correction to assess knee joint mechanics throughout the menstrual cycle. Data were analyzed using SPSS version 26.0 (SPSS Inc, Chicago, Illinois). The α- level was set to

0.05 to determine statistical significance. The researchers reported participant's means and standard deviations as descriptive statistics. Before the study, the researchers performed a statistical power test where the results informed us that 10 participants were needed to achieve a power of 0.80 at an alpha level of 0.05.

# RESULTS

Before data collection, all participants reported a healthy menstrual cycle (28 days ± 7). Each participant had two menstrual cycles observed previous to data collection, which averaged 29 ± 3 days. The researchers tested participants across three time points of their cycle, which averaged: follicular 4 ± 1 days, ovulation 15 ± 3 days, and luteal 22 ± 3 days (Table 1).

Table 1. *Participant characteristics*

| Characteristics | n = 7 |
|---|---|
| Age (years) | 21.9 ± 2 |
| Mass (kg) | 62.62 ± 8.90 |
| Height (m) | 1.66 ± 0.04 |
| Leg Length (m) | 0.87 ± 0.03 |
| Vertical Jump Height (cm) | 33.41 ± 3.26 |
| Early Follicular (day tested) | 4 ± 1 |
| Ovulation (day tested) | 15 ± 3 |
| Mid-luteal (day tested) | 22 ± 3 |
| Total cycle length (d) | 29 ± 3 |

Data are represented as mean ± SD

The researchers measured knee joint mechanics in the frontal plane, such as varus-valgus angles and sagittal plane flexion angles, and external moments. The researchers hypothesized there would be a significant phase effect on frontal plane $VV_{DEG}$ and $VV_{M}$ and that $VV_{DEG}$ and $VV_{M}$ would be greatest during ovulation. In contrast to both these hypotheses, the researchers observed no difference in $VV_{DEG}$ and $VV_{M}$ across the three measured phases of the menstrual cycle.

Absolute peak frontal plane angles during landing knee $VV_{DEG}$ angles for SLJ (p=0.204), LAJ (p= 0.166), and DJ (p= 0.739) (Table 2.) were not significantly different between phases. Despite not finding significant differences, $VV_{DEG}$ tended to increase with the testing time points, with the highest $VV_{DEG}$ occurring in the mid-luteal phase of the SLJ and DJ (Table 2). Similarly, $VV_{DEG}$ tended to decrease during ovulation and then increase in the mid-luteal phase for the LAJ. Although the researchers did not determine a significant phase effect on knee joint $FLEX_{DEG}$ for SLJ (p=.564), LAJ (p=.349), and DJ (p=.990), the researchers observed general trends. Specifically, SLJ, $VV_{DEG}$ increased with tested time points, whereas peak knee $FLEX_{DEG}$ tended to decrease. The researchers did not observe this same trend for LAJ or DJ. For LAJ, $FLEX_{DEG}$ lowered slightly during ovulation when $VV_{PK}$ was at its lowest.

Table 2. *Peak joint knee kinematics and kinetics during landing form Single-leg jump (SLJ), Lateral jump (LAJ), and Depth jump (DJ).*

| **Jump** | **Outcome Measures** | | **Early Follicular** | **Late Follicular** | **Mid-luteal Phase** | **Phase Effect (P-value)** |
|---|---|---|---|---|---|---|
| **SLJ** | | | | | | |
| | **Peak knee angle (deg.)** | **Varus /Valgus** | 5.2 ± 2.7 | 5.7 ± 2.6 | 7.6 ± 3.4 | 0.204 |
| | | **Flexion** | 44.2 ± 6.4 | 43.7 ± 6.3 | 41.9 ± 4.8 | 0.564 |
| | **Peak Knee Moment** | **Varus /Valgus** | 0.5 ± 0.2 | 0.6 ± 0.3 | 0.7 ± 0.4 | 0.493 |
| | **(Nm/kg)** | **Extension** | 2.7 ± 0.7 | 2.8 ± 0.5 | 2.5 ± 0.4 | 0.385 |
| **LAJ** | | | | | | |
| | **Peak knee angle (deg.)** | **Varus /Valgus** | 8.0 ± 3.3 | 5.4 ± 2.4 | 8.2 ± 3.0 | 0.166 |
| | | **Flexion** | 42.1 ± 5.9 | 38.6 ± 5.1 | 41.0 ± 4.3 | 0.349 |
| | **Peak Knee Moment (Nm/kg)** | **Varus /Valgus** | 0.5 ± 0.1 | 0.4 ± 0.1 | 0.6 ± 0.2 | 0.131 |
| | | **Extension** | 1.9 ± 0.5 | 1.6 ± 0.4 | 1.6 ± 0.3 | 0.224 |
| **DJ** | | | | | | |
| | **Peak knee angle (deg.)** | **Varus /Valgus** | 7.8 ± 4.2 | 8.8 ± 4.7 | 9.6 ± 4.1 | 0.739 |
| | | **Flexion** | 92.9 ± 12.5 | 92.6 ± 16.1 | 92.7 ± 15.7 | 0.99 |
| | **Peak Knee Moment (Nm/kg)** | **Varus /Valgus** | 0.3 ± 0.2 | 0.3 ± 0.1 | 0.3 ± 0.2 | 0.959 |
| | | **Extension** | 1.7 ± 0.4 | 1.8 ± 0.3 | 1.7 ± 0.3 | 0.822 |

Data are represented as mean ± SD. One way ANOVAs were used at $\alpha = 0.05$.

Due to the researchers analyzing and recording absolute $VV_{DEG}$, the researchers also anecdotally observed what percentage of participants landed in valgus due to large amounts of valgus being a risk factor for ACL injury over the MC course. In the early follicular phase, 100% of participants were landing with their knee in valgus when doing SLJ and LAJ. While performing the DJ at that same time point, only 57.14% landed in valgus. When tested during ovulation, 71.43% of participants performing SLJ landed in valgus, while for LAJ and DJ, 85.71% of participants landed in valgus. SLJ and LAJ both had the greatest amount of participants land in valgus in the follicular phase (85.71% and 71.43%, respectively), while DJ had the most amount of participants land in valgus during ovulation at 85.71%.

Although not statistically significant, $VV_M$ external moments did show slight trends across the time points tested (Table 2.). Specifically, for both SLJ and LAJ, peak $VV_M$ moments were greatest in the mid-luteal phase. For the DJ peak $VV_{M,}$ the moment peaked during the early follicular phase. While also not statistically significant differences across time points, the peak external $EXT_M$ was slightly higher in the follicular phase for the SLJ and LAJ jumps, while DJ had a peak $EXT_M$ during ovulation.

# DISCUSSION

Due to the large discrepancy between males and females with ACL injury rates, it is critical to determine if sex-related physiological mechanisms influence females' joint stability. A large concern being that hormonal fluctuation across different phases of the MC may be responsible for decreased joint stability (Beynnon et al., 2005; Chaudhari et al., 2007; Lee et al., 2013; Park et al., 2009; Shultz, Kirk, et al., 2004; Slauterbeck et al., 2002a; E. m. Wojtys et al., 2002). The purpose of this study was to examine differences in frontal plane jump-landing mechanics across phases of the menstrual cycle among healthy adult females. The researchers hypothesized that peak frontal plane knee motion and moment during jump-landing tasks would be greatest in the late follicular phase compared to other MC times due to high magnitudes of estrogen. The researchers tested participants at three different time points throughout the MC, early follicular phase, late follicular phase (ovulation), and mid-luteal phase. The researchers observed no significant difference in knee frontal plane motion or moment across the menstrual cycle. While knee frontal knee angles and moments of individual participants varied from phase to phase, the researchers did not observe an overall significant phase effect.

Trends the researchers observed for peak $VV_{DEG}$ joint motion occurred at different time points depending on the jump. Although not statistically significant, peak $VV_{DEG}$ primarily occurred during the mid-luteal phase for the SLJ and DJ landings but not LAJ. This would suggest that peak estrogen is not responsible for the majority of frontal plane knee instability in this study.

The LAJ came the closest to statistical significance for $VV_{DEG}$ and $VV_{M}$ for phase by phase differences in frontal plane kinematics and kinetics compared to the other jumps performed (Table 2.). This finding of greater instability in lateral movements compared to sagittal plane movements aligns with previous research (Mornieux et al., 2014; Sinsurin et al., 2013). When landing laterally, different knee flexion and anterior pelvic strategies are being used for balance, compared to a forward jump, creating less overall stability (Taylor et al., 2016; Weltin et al., 2015). It is possible that if hormonal fluctuations throughout the MC do have an effect on joint stability, the LAJ has the largest risk factor.

While prior literature suggests that increased estrogen levels may lead to an increased joint laxity across the phases of the menstrual cycle, our findings agree with those of several other studies showing similar knee joint instability across different time points of the menstrual cycle (Beynnon et al., 2005; Chaudhari et al., 2007; Hertel et al., 2006; Park et al., 2009; Shafiei et al., 2016b). Several factors may explain why the researchers did not observe the difference in frontal plane knee joint motion across the MC phases. Possible explanations for no significance found in results include: changes in the knee instability possibly did happen but were too minute to observe during functional movements, differences in muscle conditioning in physically active versus sedentary individuals, as well as possible non-hormonal responders to hormonal fluctuations. The researchers also had many limitations, such as participant population, no serum hormone testing, and no observations through EMG.

One possible explanation for the observed results may be that knee laxity changes were too small to observe during functional movements tested. Prior studies examining correlations between knee joint laxity and menstrual cycle phases report conflicting results when measuring passive anterior joint manipulation (Beynnon et al., 2005; Eiling et al., 2007; Shultz et al., 2006; Van Lunen et al., 2003). These previous studies used a method of measuring passive tibial translation displacement in the sagittal plane with an arthrometer, a device that applies force to the anterior tibia while measuring the knee's translational displacement. A healthy knee displaces about 5.7 ± 2.3 mm, while ACL deficient knees typically move 13.0 ± 3.5 mm (Daniel et al., 1985). When applying this technique, Shultz et al. (2006) observed a change in anterior knee joint laxity across the MC of 3.19 ± 1.9 mm (Shultz et al., 2006). In comparison to our study, these measurements of knee joint laxity are very controlled. These small joint laxity changes may have been too small to detect with our digital motion capture system.

Although digital motion capture (DMC) is extremely accurate (0.017 ± 0.321 mm) (Massey, 2020) and considered a gold standard in measuring movement in space (Kruk & Reijne, 2018), there are limitations to using DMC. One limitation that may have affected our results is marker balls, which define anatomical landmarks and joint centers in DMC, are placed and taken off each testing session. This may increase data error if the markers are not placed correctly or moved slightly from session to session. Another limitation is that DMC does not measure translational movements within the knee joint, thus not giving us a thorough investigation of movement within the knee joint. The researchers were able to observe the stress of the tissues by using inverse kinematic equations to determine if there was an increase of external torque of the knee as well as observing maximal ROM angles in the frontal plane, which have all shown to be predictors of ACL injury (Hughes et al., 2008). However, if tibial translation or specifically increased strain on the ACL occurred, the researchers would not have been able to measure it directly. Prior research has found significant differences in knee laxity throughout the MC, using a KT – 2000, but no change in knee kinetics or kinematics during running-to-cutting maneuvers (Park et al., 2009). Therefore, it is possible that minimal increases in strain (change in length) within the knee tendons and ligaments during jump landing did occur throughout the MC but were too small to detect during functional movements using digital motion capture.

In this study, the researchers specifically investigated frontal plane joint motion during dynamic movements of jump landing because most ACL injuries among females happen in gameplay when there is a large amount of strain on the knee on the frontal plane (Kobayashi et al., 2010; Podraza & White, 2010). Although our results showed similar joint motion and moments across the menstrual cycle with one prior study that also measured joint motion during depth jump landing and at various time points of the menstrual cycle (Chaudhari et al.,2007), some differences in methodology are worth noting. For example, Chaudhari et al. (2007) do not report the days they tested during the participant's menstrual cycles. Moreover, although they collected serum hormone, an indicator of phase-related hormonal factors that may influence joint laxity, they did not report that data nor report the time points they recorded joint kinematics. The researchers attempted to investigate the depth-jump using more clearly defined time points as measured by hormone test kits based on these methodological limitations. Furthermore, the researchers expanded the investigation to examine joint laxity across multiple types of jumps, including the depth jump, single-leg forward jump, and lateral jumps. Despite expanding the types of jump landing investigated and more clearly define the time points of measurement, the researchers observed no change in joint motion and moment due to the different phases of the menstrual cycle.

Although the researchers did not find significance in our results, the participant's physical activity's diversity is possibly responsible for the variability of measured joint mechanics, specifically the muscle conditioning of the quadriceps and hamstrings. Prior research suggests that physical activity levels influence the balance of quadriceps and hamstrings activation and joint stability during jump landing (Duzgun et al., n.d.; Tourny-Chollet & Leroy, 2002). Because the researchers did not delimit this study based on physical activity or measure our participants' physical activity levels, differences in our subject's physical activity levels may have contributed to greater variability in measured joint motion at each time point. This variability in physical activity possibly influenced our ability to detect joint motion changes during different MC time points.

When observing participant's data, the researchers found that participants stayed consistent in their frontal knee movements, in that they stayed consistently stable (with a smaller frontal plane knee angle ROM) or unstable (larger knee angle ROM). These subject by subject differences in frontal plane motion may be related to differences in hamstrings and quadriceps muscle co-activation. Previous research has shown that when the quadriceps are activated more than the hamstrings during isometric contractions, greater anterior tibial translation occurs, increasing the ACL (Draganich & Vahey, 1990b). The hamstrings are a stabilizing component for the ACL by creating posterior tension for the knee, preventing anterior tibial translation and frontal plane knee motion (More et al., 1993). Prior research suggests that exercise participation is related to a more balanced co-contraction between the HQR and greater joint stability than sedentary individuals (Tourny-Chollet & Leroy, 2002). Previous research showed that training programs targeting HQR on sedentary females increase lower body stability than before the training program when the participants primarily replied on their quadriceps (Duzgun et al., 2017). Previous studies have also demonstrated that training programs based on postural control show that stability improves, compared to control participants who do not participate in this type of training (Imwalle et al., 2009; Myer et al., 2009; Noyes et al., 2005; Paterno et al., 2004). Thus, it is possible that more physically active participants in our study exhibited more consistently stable joint motion in their landings due to more balanced activation between the hamstrings and quadriceps muscles.

Our lack of finding a significant phase effect on joint motion and moment may have been due to variability of the follicular phase between participants and thus variability in hormone levels. The duration of menstrual cycle phases varies among females (Mihm et al., 2011b). The duration of the follicular phase in the MC varies between individuals and even within an individual, and thus the magnitude and timing of hormone fluctuations vary among individuals around the time of ovulation (Shultz, Carcia, et al., 2004). Unfortunately, in this study, the researchers did not directly measure the magnitude of individual hormone levels during the time of data collection, but the researchers did track the onset of the follicular phase and the luteinizing hormone (LH), which is indicative of ovulation. Because the researchers could not confirm estrogen levels, it is plausible that data collected during the projected ovulation date may not have occurred at the time of the expected fluctuation in hormone levels believed to be responsible for joint laxity changes.

With the participants having variable hormone fluctuations, another explanation of why the researchers did not find significance may involve the hypothesis that joint laxity and stability are influenced differently among females. (D. R. Bell et al., 2014; Shultz, Kirk, et al., 2004). Prior research has found compelling yet conflicting results when looking into which hormones during the MC may be responsible at the time of ACL injury. For instance, both Wojtys et al. (2002) and Slauterbeck et al. (2002) recorded what day of the cycle participants were on within 48 hours of an ACL injury and took serum hormone tests. Wojtys et al. found a significant amount of injuries occurred during ovulation, while Slauterbeck et al. (2004) found that a majority of ACL injuries occurred within one to two days of menses onset. A potential reason for this is that Shultz et al. (2004) found that only individual participants' knee laxity was responsive to hormones throughout the MC. They found that increased knee laxity was related to increased estrogen, testosterone, progesterone, all three hormones or had a weak relationship to these hormones. Bell et al. (2014) suggested that female athletes who have a higher magnitude of hormonal peaks during the MC are the athletes that are getting injured. They found that female participants who have already had an ACL injury, while performing drop-jumps increased peak frontal plane external moment during ovulation (D. R. Bell et al., 2014). Together, these results may explain the variability between studies regarding significant differences in joint laxity at different time points of the MC.

# LIMITATIONS

## Participants

A considerable limitation to this study, as previously noted, was the low number of participants in this study. As previously mentioned, the researchers needed 10 participants to achieve statistical significance. Due to strict guidelines for the participants, such as: not having taken birth control within the last six months, no knee injuries within the past year, self-administered LH tests at home, scheduling testing, and factors outside of our control such as COVID-19, the researchers retained seven participants. Although successful studies have been conducted with as few as seven participants (Laeng & Falkenberg, 2007), it is possible the researchers could have observed different results if the researchers had a larger subject pool.

## Menstrual cycle

Another considerable limitation to our study was not confirming hormonal fluctuations through saliva serum hormone testing. This type of testing would have shed light on testing participants' accuracy during their cycle's correct days. This would have also helped us observe a positive correlation between lower body stability and hormone levels. Instead of serum hormone testing, the researchers used a luteinizing hormone (LH) detection four days leading up to the projected ovulation date. The CVS One Step Ovulation Predictor (sensitivity 20 mIU/ml LH, accuracy 99%); CVS Corporation, Woonsocket, RI] kit detects LH's onset, which peaks 24 hours after an estrogen peak during the late follicular phase. Because the researchers did not have serum hormone testing verification of an estrogen peak, there is the possibility that participants were only tested during their fertile window (Gangestad et al., 2016), and not necessarily when they would have the most joint instability. Another potential issues with this testing kit, although 99% accurate, is that it gave ambiguous results at times. For example, when results appear on the test strip, after completing the manufacturer's instructions correctly, one solid line appears as the control, and if a second solid line appears, LH is present. However, it can be challenging to determine a positive test if the test's positive line is somewhat firm but still faint, leading to possible error. The researchers could have implemented a method to verify the correct day of ovulation is a counting back method (Eiling et al., 2007). This method counts 14 days backward from the first day of the next cycle menses, which would be the correct ovulation day. Future studies should verify hormonal peaks through serum hormone testing and the counting back method and LH kits.

## Kinematic Analysis

Although our study focused on functional and dynamic knee movements, the researchers could not measure knee translation. As previously noted, in our study, the researchers used digital motion capture with force sensing platforms to measure frontal plane knee frontal plane motion and torque, which gives us an insight into risk factors of an ACL by indirectly observing strain and stress on the knee joint. Nevertheless, the researchers could not directly measure strain on the ACL or indirectly by measuring tibial translation. Future studies of knee joint laxity during dynamic movements should consider implementing an additional measurement of tibia translation using arthrometrics.

## EMG

Although knee instability is related to muscle activation patterns (Cesar et al., 2011; Dedrick et al., 2008; Huston & Wojtys, 1996b; Pollard et al., 2010), the researchers did not measure surface electromyography (EMG) in this study. The addition of EMG to this study may have provided greater insight into the relation of muscle co-activation to joint laxity. Although EMG could have given us further insight into muscular stabilizing factors, EMG has large inaccuracies due to several methodological factors (Merletti,1999). EMG measurement may be inaccurate due to disruptions in EMG signal from skin movement artifact, subcutaneous adipose signal resistance, and the signal's variability from phase to phase stemming from the EMG electrode in slightly different locations each measurement session. Although there are resources to determine correct placements of the EMG sensor on the muscle, there is still a possibility of signal differences associated with replacing the electrodes between sessions. Although muscle co-activation measurements would have improved our ability to interpret our joint kinematic and kinetic data,  prior studies have shown no difference in muscle firing activation throughout the MC (Abt et al., 2007; Hertel et al., 2006).

# CONCLUSION

This study's results did not find any significant changes in knee frontal plane angles or moment throughout time points across the MC. A potential reason the researchers may not have seen results is that knee laxity changes may have occurred to the knee joint but were too minute to measure due to confounding factors such as muscle conditioning, hormonal variability and equipment used. Future studies should implement a greater number of participants, a more homogenous group of participants with similar muscle conditioning, and directly measure hormonal fluctuations.

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# APPENDIX

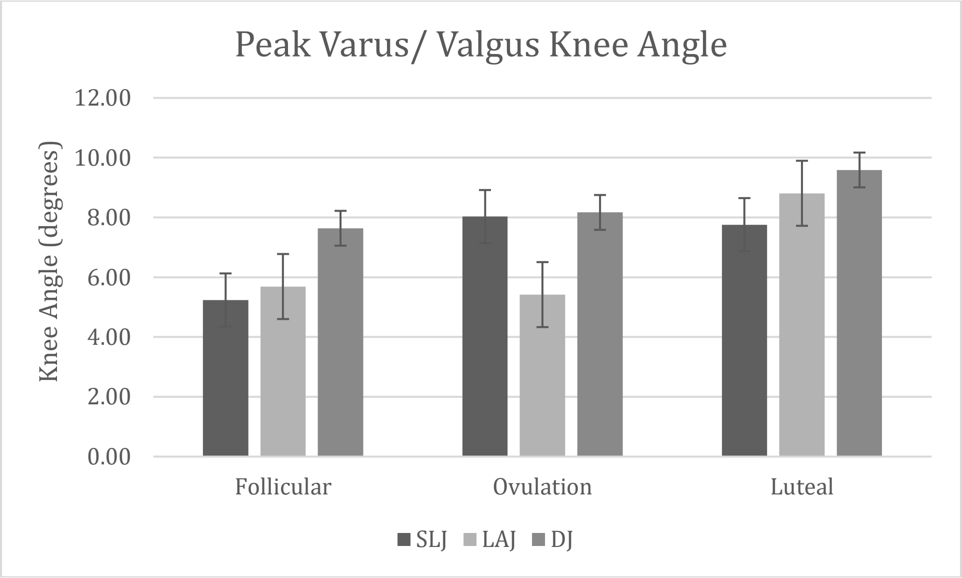

Figure 1. *Average peak frontal plane knee joint kinematics during landing from Single leg jump (SLJ), Lateral jump (LAJ), and Depth jump (DJ).* Error bars represent standard deviation.

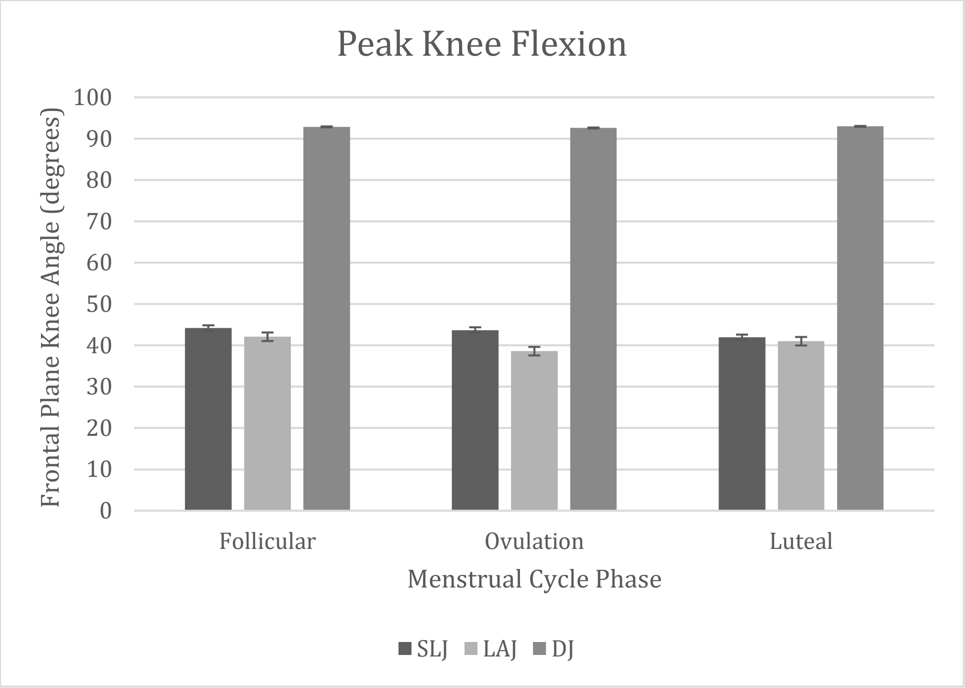

Figure 2. *Average peak sagittal plane knee joint kinematics during landing from Single leg jump (SLJ), Lateral jump (LAJ), and Depth jump (DJ).* Error bars represent standard deviation.

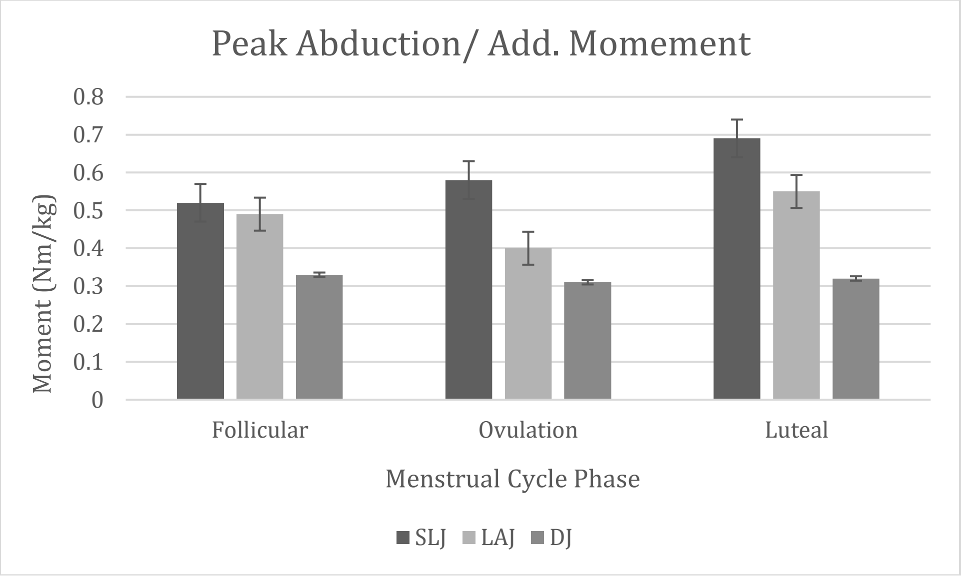

Figure 3. *Average Peak frontal plane knee joint kinetics during landing from Single leg jump (SLJ), Lateral jump (LAJ), and Depth jump (DJ).* Error bars represent standard deviation.

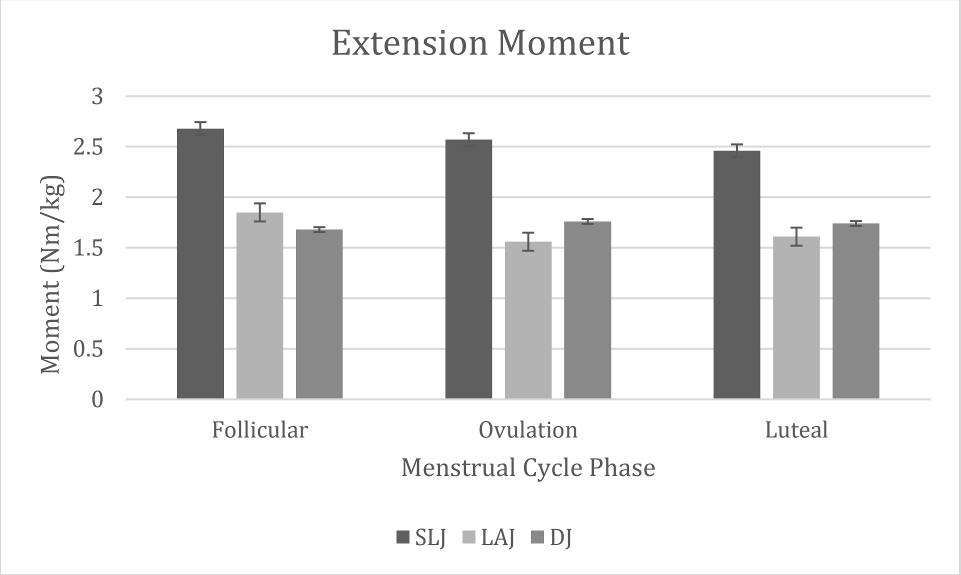

Figure 4. *Average peak sagittal plane knee joint kinetics during landing from Single leg jump (SLJ), Lateral jump (LAJ), and Depth jump (DJ).* Error bars represent standard deviation.