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Effects of Hormonal Changes Throughout the Menstrual Cycle on Joint Laxity in Females

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EFFECTS OF HORMONAL CHANGES THROUGHOUT THE MENSTRUAL CYCLE ON JOINT LAXITY IN FEMALES

by

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ABSTRACT

EFFECTS OF HORMONAL CHANGES THROUGHOUT THE MENSTRUAL CYCLE ON JOINT LAXITY IN FEMALES

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The objective of this study was to examine the levels of estrogens, progesterone, LH, FSH, estradiol and testosterone and their relation to ACL laxity throughout the menstrual cycle. Twelve females ($\bar{x}_{age} = 24.25 \pm 4.94$) presented with a dominant right leg free of injury. They were mild to moderately active and had a 12-month history of normal menstrual cycles (28-35 days) that were not due to the use of hormonal therapy. Subjects were tested at the onset of menses, the first day of ovulation (days 8-17) and day 23 at the mid-luteal phase. At each session, 14 ml of blood was drawn from the antecubital vein in the forearm. All blood samples were analyzed via radioimmunoassay to determine the concentration of each hormone. Immediately following the blood collection, subjects were tested for ACL laxity using the MEDmetric KT-2000 in conjunction with simultaneous radiographs. A Pearson Product Moment Correlation Coefficient revealed that there were no statistically significant relationships between laxity measurements, of the KT-2000 or radiographic comparisons, and elevated concentrations of any of the hormones. LH was observed in the follicular phase, at the onset of menses, to have a negative correlation with only the radiographic reading ($r = -.628$, $p = .029$). A 2-way repeated measures ANOVA was used to determine if there were any statistically

significant changes in the KT-2000 or radiographic comparisons over time. Values obtained by reading the KT-2000 graphs from the X-Y plotter showed no statistically significant changes throughout the menstrual cycle ($F_{2,22} = .709$, $p = .503$). There were no significant changes in laxity over the duration of the menstrual cycle as determined by radiographic comparisons ($F_{2,22} = 1.095$, $p = .352$). We concluded that there was no relationship between concentrations of estrogens, progesterone, LH, FSH, estradiol or testosterone and ACL laxity. Both the KT-2000 and radiographic measurements have high intra-method reliabilities, but inter-method reliability is low.

I would like to dedicate this paper

to my parents, Jack and Bernita.

Whose love and guidance

have been the one constant

throughout my entire educational career

and life.

And to Pam, for showing me

unconditional love and support.

Always and Forever.

I love you.

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CHAPTER I

Introduction

Estrogens, progesterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol and testosterone are hormones that are normally present during the menstrual cycle. These hormones are produced at varying levels throughout the cycle, with the amounts being dependent upon the corresponding phases.[1-8] In the past, it has been postulated that these changes in hormonal levels may be related to the occurrence of peripheral joint laxity.[1,2,7,9] It has also been demonstrated that increasing joint laxity compounds the risk of injury to the involved ligaments.[9-13]

At the knee joint, the anterior cruciate ligament (ACL) has been given significant attention. This ligament is injured at an alarming rate in the athletic population, especially among females.[7, 9-15] Few studies have examined hormonal effects throughout the menstrual cycle in relation to knee joint laxity.[1,2,8] Replication of various aspects of the current studies, with appropriate modifications, will help to examine the reliability and validity of the proposed techniques and methods, as well as to examine the roles of other various hormones effecting knee joint laxity.

Statement of the Problem

The purpose of this study was to examine the levels of estrogens, progesterone, LH, FSH, estradiol and testosterone and their relation to ACL laxity throughout the menstrual cycle.

Research Hypothesis

There was to be a statistically significant increase in the amount of anterior tibial translation corresponding with elevated levels of estrogens, progesterone and estradiol at the mid-luteal phase therefore inferring an increase in ACL laxity and a higher potential for injury.

Null Hypothesis

There was to be no statistically significant change in the amount of ACL laxity as estrogens, progesterone, LH, FSH, estradiol and testosterone levels fluctuate throughout the menstrual cycle.

Independent Variables

1) Menstrual cycle phase with the following levels: Follicular, Ovulatory, Luteal.
2) Method of tibial displacement with the following levels: KT-2000 (Figure 1), Radiographic Comparison (Figure 2).

Dependent Variables

1) Hormonal levels of estrogens, progesterone, LH, FSH, estradiol and testosterone.
2) Anterior tibial translation measured in millimeters at 133 N. (30 lbs.) of force.

Figure 1: KT-1000, KT-2000 (Adapted from MEDmetric Corp Buyers' Guide).[16]

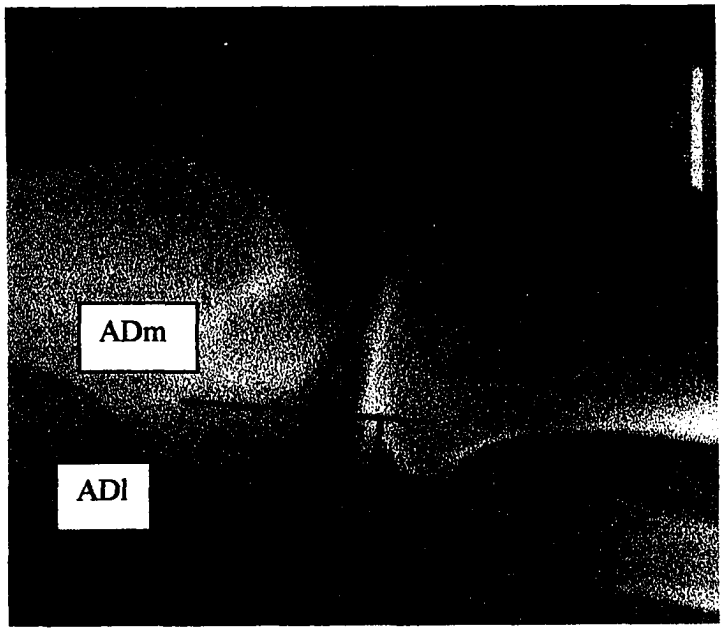


Figure 2: Radiographic assessment. Lateral radiograph measuring anterior displacement medially (ADm) and anterior displacement laterally (ADl) with applied stress.

Significance of the Study

There has been relatively little information published on ACL laxity and its relation to hormone levels.[1,2,8] No literature could be found that discussed knee laxity regarding varying levels of LH, FSH, estradiol or testosterone. The intent of this study was to support or refute former findings regarding estrogens and progesterone and provide further information on the relationship of LH, FSH, estradiol and testosterone to ACL laxity.

The objectives of this proposed study were to: 1) measure the levels of estrogens, progesterone, LH, FSH, estradiol, and testosterone in the blood on a specified day from each phase of the menstrual cycle, 2) measure the amount of laxity in the ACL on the same days hormonal levels were evaluated, 3) compare the two findings, 4) discuss possible relationships between individual and/or collective hormonal releases and ACL laxity to injury and 5) compare the results between the KT-2000 assessment and the radiograph assessment to determine the accuracy and effectiveness of both methods.

Delimitations

1) Subjects consisted of 12 college-aged, females not participating in varsity athletics.
2) Subjects presented with the right knee as dominant and free of any past or current pathological conditions, including, but not limited to ACL deficiency, meniscal injury, tendinitis, chondromalacia and ligamentous sprains.

3) There was no use of hormonal therapy or supplementation (ie. birth control) for 3 months prior to the first testing session or during the study.
4) There was minimal risk of subjects becoming pregnant during the course of the study.
5) Subjects had experienced eumenorrhea for the year immediately prior to the start of the study (10 or more menstrual cycles in a 12 month time period).
6) The average length each subject's menstrual cycle was in a range of 28-35 days, which is considered medically normal.
7) The length of each subject's dominant tibia was not less than 34 cm. (Smaller lengths would require a smaller arthrometer).
8) Each subject's quadriceps angle (Q-angle) was less than 15°.

Limitations

1) There was no control for the release of estrogens, progesterone, LH, FSH, estradiol or testosterone for each individual. Hormonal levels will always vary per individual, per hormone and per menstrual cycle.
2) The size of ACL (ie. width and thickness) and the tensile strength of the ligament could not be compensated for within the scope of this study.
3) Pre-existing notch stenosis could have resulted in deterioration of the ligament from excessive amounts of friction.
4) Muscular tension of the hamstrings may have affected the amount of anterior displacement if the subjects were not completely relaxed.

Assumptions

1) The KT-2000 remained calibrated throughout all testing sessions.
2) Blood sampling instruments and storage units were in proper working condition.
3) Subjects promptly and accurately reported the first day of their menstrual cycle and the first day of ovulation.
4) Ovulation kits were used correctly by each subject.
5) Radioimmunoassay procedures were conducted correctly.
6) The correct statistical analysis formulas were chosen and used properly.

Operational Definitions

1) KT-2000 laxity measurements: The KT-2000 knee arthrometer measures the amount of ACL laxity by anteriorly displacing the tibia at known loads. An X-Y plotting graph is printed to determine the exact amount of displacement, in millimeters, at a specific applied force, in Newtons or pounds. For the purposes of this study, the load observed was 133 N or 30 lbs.
2) Radiographic laxity measurements: Two lateral radiographs were taken of the knee. The first was in a resting position with the knee in 20-30° of flexion. The second was taken while a constant force of 133 N was applied anteriorly using the KT-2000. Anterior displacement of the medial (ADm) and lateral (ADl) compartments of the knee were compared relative to tangent lines drawn parallel to the posterior tibial cortex. Displacements of the 2 compartments were averaged for both films individually ([ADm+ADl]/2).

The number obtained by the first film was subtracted from the number obtained by the second film to provide the amount of anterior tibial displacement in millimeters.[17]

3) Normal menstrual cycle: Twenty-eight to 35 days in length.
4) Follicular phase: Days 1 to 9, approximately, of the menstrual cycle.
5) Ovulatory phase (Menstrual phase): Days 10 to 14, approximately, of the menstrual cycle.
6) Luteal phase: Approximately day 15 through the end of the menstrual cycle.
7) Hormonal levels: The concentration of each hormone in the blood from samples taken at the antecubital vein in the forearm. This study used the method of radioimmunoassay to measure serum concentrations of estrogens (pg/ml), progesterone (ng/ml), FSH (mlU/ml), LH (mlU/ml), estradiol (pg/ml) and testosterone (ng/ml).

CHAPTER II

REVIEW OF LITERATURE

To fully understand the presentation of the effects that estrogen, progesterone, FSH, LH, estradiol and testosterone can have on ACL laxity, it is best to have a good understanding of the structures and concepts involved. The following review of literature addresses several general topics: 1) anatomy of the ACL, 2) etiology, 3) epidemiology, 4) assessment of ACL laxity, 5) the menstrual cycle, 6) hormonal functions and characteristics and 7) the relationship of ACL laxity to the menstrual cycle.

Anatomy

The knee is composed of three joints, which include the patellofemoral joint, the superior tibiofibular joint and the tibiofemoral joint. The patellofemoral joint acts in a modified sagittal plane within the trochlear groove of the femur.[18] The patella enhances the efficiency of knee extension from 0 to 30°, since it ensures that the quadriceps tendon remains away from the axis of movement.[19] The articulation of the tibia and fibular head is a synovial joint in which a gliding motion occurs when there is activity at the foot.[19] The tibiofemoral joint is the primary joint involved in the etiology of ACL injuries. The primary actions that occur at the tibiofemoral joint are knee flexion and extension. There are also moderate amounts of internal and external rotation of the tibia on the femur ranging from approximately 10-20° in either of the two directions, and is therefore classified as a modified hinge, or ginglymus joint.[18] It is held together, almost

entirely, by four ligaments: 1) medial collateral ligament (MCL), 2) lateral collateral ligament (LCL), 3) posterior cruciate ligament (PCL) and 4) ACL. The MCL lies slightly posterior on the medial aspect of the knee and is composed of a superficial and a deep layer. The superficial layer has a strong, broad, triangular shaped band that begins distal to the adductor tubercle of the femur and runs to the medial surface of the tibia approximately 6 cm below the joint line. It is separated from the capsule by a bursa. The deep layer is simply a thickening of the joint capsule and blends together with the medial meniscus. The MCL is taut throughout the range of motion (ROM) of the knee, with the anterior fibers tighter in flexion, the posterior fibers tighter in midrange and the entire ligament being taut in full extension. The LCL is more of a round cord, approximately one half to 1 cm in width, lying under the biceps femoris tendon. It also lies slightly posterior, but on the lateral side, and is separated from the lateral meniscus and joint capsule by a small fat pad.[18,20]

The PCL courses anteriorly, superiorly and medially from the posterior aspect of the tibial crest to the lateral aspect of the medial femoral condyle. The ACL attaches to the anteromedial surface of the tibial crest and runs superiorly, posteriorly and laterally to insert on the posteromedial surface of the lateral femoral condyle. The knee joint is surrounded by an extensive synovium that communicates with the bursae and pouches of the knee. Although the synovial membrane covers the entire knee, its arrangement is such that the cruciate ligaments are extrasynovial, because they are pinched within the articular folds.

Even though the cruciate ligaments are considered extrasynovial, they are conversely considered as being intracapsular[19] (Figure 3).

The ACL can be described in two manners regarding its separation into bundles. The first separates the structure into two bundles of fibers. The anteromedial bundle is taut in flexion and extension, but serves its main function when the knee is flexed. The posterolateral fibers are tight in extension.[19] There is also the suggestion of a third intermediate bundle.[20,21] The bundles are interwoven with and twist around one another. The presence of the third bundle would increase the amount of torsion placed on the ACL. It could cause an increased amount of friction and decreased amount of blood supply due to a "wringing out" effect.[20] Norwood and Cross[21] observed a separation of the ACL into three bundles. They found the anteromedial bundle to be the longest and that it aids in resisting anteromedial and anterolateral rotary instability. The intermediate bundle has its greatest effects on straight anterior instability. The posterolateral bundle is the shortest of the three and it prevents posterolateral rotary instability. Each of the three bundles aid in the prevention of hyperextension of the knee. The ACL has, as a single unit, the least amount of stress placed upon it between 30-60° of flexion. While the tibia is internally rotated the ACL becomes taut again, as opposed to the release of pressure produced by externally rotating the tibia on the femur.[19] The midsection of the ACL is mainly viscoelastic, which provides for resistance to deformation at low and moderate applied forces. The ends of the ligament are more neural in nature and receive more strain during higher loads.[22]

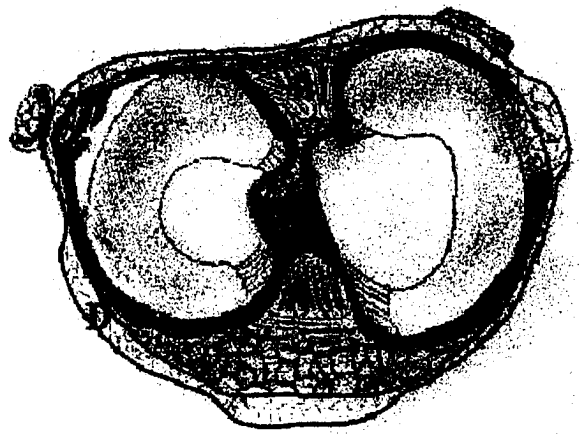


Figure 3: A) ACL B) PCL C) Synovium D) Joint Capsule (Adapted from Netter 1994).[23]

The anterior cruciate ligament's vascularization primarily derives from the middle genicular artery and the surrounding synovium. After it separates from the popliteal artery, the middle genicular artery moves along the dorsal aspect of the ACL to provide arterial branches to the ligament itself. These branches form a vast network that is interwoven to run transversely across the ACL and form an anastomosis with longitudinally oriented intraligamentous vessels. These transverse branches are connected to the intraligamentous vessels at the proximal and distal ends of the ACL, with the proximal insertions being slightly larger. The middle genicular artery also supplies the femoral epiphysis and proximal tibial epiphysis and then bifurcates to the right and left to supply their respective tibial condyles. The inferior medial and lateral genicular arteries also supply blood to the ACL. They branch from the posterior surface of the popliteal artery and course anteriorly. The inferior medial genicular artery passes inferior to the medial tibial condyle and deep to the MCL. The inferior lateral genicular artery passes proximal to the fibular head and deep to the LCL. It also brings a supply of blood to the periphery of the lateral meniscus. Both arteries come to an anastomosis at the infrapatellar fat pad on the posterior surface of the patellar ligament. Terminal branches of the inferior genicular arteries supply portions of the ACL directly.[24]

Kennedy et al[25] described two groups of afferent nerves that supply the knee: 1) a posterior group of the posterior articular and obturator nerves and 2) an anterior group of branches from the femoral, common peroneal and saphenous nerves. The posterior articular nerve is the major contributor, supplying

everything from the posterior capsule to the infrapatellar fat pad, including the menisci and cruciate ligaments. The other nerves each supply various aspects of the joint capsule, synovium, collateral ligaments, surrounding muscles, bursae and fat pads.

The ACL has a mechanoreceptor system that is able to respond to the tension of the ligament that is generated by the motion of the joint. Schutte et al[26] identified three types of mechanoreceptors and free-nerve endings, including: 1) two types of Ruffini end-organs, 2) Pacinian corpuscles and 3) free nerve-endings. Several of the mechanoreceptors have been found to be similar to Golgi tendon organs.[23,25-27] The ACL has an extensive intraligamentous neural network. Few receptors, however, have been reported in the deep fibrosis substance of ligaments or menisci.[25] Neural fibers enter the ligament by an unmyelinated axon through connective tissue and terminate within various receptors.[26,27] The mechanoreceptors function as transducers, in that they are able to convert physical stimulus of tension into a specific neural signal. They provide information on kinesthesia, or position, motion and acceleration, but not pain. Most mechanoreceptors have been found to be closer to the insertion to the tibia and the synovial coverings.[25,26] Conversely, Schultz et al[27] reported finding receptors at both ACL insertions, with more being present at the femoral attachment. The discovery of neural concentrations at the ACL bony attachments, along with EMG readings, has led Solomonow et al[22] to speculate that during high applied forces, the ACL signals the hamstrings to contribute in the prevention of anterior tibial displacement.

The Ruffini endings are slow adapting mechanoreceptors with a low threshold (high sensitivity) that respond to tensile changes within the ligament. Their function is to signal when the joint is near its terminal ends of flexion and extension. There is also a Ruffini mechanoreceptor that is similar to the pilo-Ruffini complex associated with hair, whose function is not certain. Rapidly adapting Pacinian corpuscles are activated by joint movement in any degree of movement, as opposed to only the terminal ranges. They also have a low threshold and seem to be sensitive to the speed of movement. The sensory input to the ACL is provided by specialized receptors and free nerve-endings in the ligament, which only make up 1% of the area of the ACL. Free nerve-endings were identified within the collagenous fibers and are responsible for pain.[26] Since a relatively small number have been identified, this could be why the ACL is almost insensitive to pain with isolated tears.

Etiology

The primary purpose of the ACL is to prevent anterior tibial translation of the tibia on the femur. It also prevents hyperextension, acts as rotary stabilizer and provides for the "screw home" mechanism in which the tibia slightly externally rotates as the knee achieves terminal extension.[19,20,28] Injuries to the ACL can be the result of various mechanisms, such as an anterior blow to the knee causing hyperextension on a weight bearing leg, noncontact hyperextension, noncontact deceleration, hyperflexion and noncontact deceleration with tibial internal rotation or femoral external rotation on a fixed tibia.[19,20] The majority of

noncontact ACL injuries involve a history of landing from a jump and planting the foot while cutting or pivoting.[10] Contact and noncontact ACL injuries generally involve foot fixation resulting in a closed chain type of activity.

There are several other structures that aid the ACL in its preventive tasks. The hamstrings help prevent hyperextension of the knee, while the quadriceps muscle group aids in resisting hyperflexion. Resistance against internal rotation is assisted by the biceps femoris muscle and via the combined effort from the tensor fasciae latae and the iliotibial band. The menisci, the medial meniscus in particular, aid in the reduction of anterior translation of the tibia.

There are many possible etiological factors to consider when discussing injuries to the ACL. These are generally classified as intrinsic, extrinsic and partially controllable. Intrinsic factors, which are not controllable, include hormonal changes, malalignments, structural content of the ACL and other structures in the knee. Extrinsic factors, which may be controllable, are skill and coaching levels, experience, equipment and physical conditioning. Partially controllable factors involve a combination of intrinsic and extrinsic measures. They include neuromuscular actions and acquired skills.

Different authors and researchers focus on a wide variety of etiological aspects. Gray et al,[9] after conducting a survey of 76 female basketball related injuries, postulated that position being played, prior laxity of the ligament, weak quadriceps mechanism and hormonal fluctuations could all predispose an individual to an ACL injury. Saunders[11] also focused intrinsically on the effect of estrogens on the cellular matrix of the ACL, the effect of the menstrual cycle and

the width of the femoral intercondylar notch. Estrogens have been found to inhibit the proliferation of human ACL fibroblasts and Type I procollagen synthesis. Narrow femoral intercondylar notches may be disruptive, because the smaller space may cause an increased amount of friction on the ACL causing excessive microtears within its outer fibers. Other intrinsic factors include alignment, hyperextension, physiologic rotary instability, ACL size, notch width and shape, hormonal levels and inherited skill and coordination. Lower extremity malalignments and conditions that may contribute to ACL related injuries are excessive foot pronation, genu recurvatum, external tibial torsion effecting rotary alignment, a quadriceps angle (Q-angle) in excess of 15° and poor dynamic stabilization assistance for the quadriceps and hamstrings.[10,29,30] Rosene and Fogarty[31] examined differences in anterior tibial translation among the categories of sports, sex and leg dominance. They found females with healthy knees to have an overall greater amount of anterior tibial translation then their male counterparts and that there was a significant interaction between sex and forces applied through a KT-1000 ($F_{1.1,64.93} = 8.08$, $P < .05$). Bonci[29] reported that most etiological factors, when studied, provide inconclusive and conflicting results. It is difficult to isolate one specific factor in multiple subjects because all other factors, intrinsically and extrinsically, must be accounted for and equal for each subject. Decoster et al[30] found, contrary to common belief, that hypermobility did not play a role in the predisposition of knee injuries. This contradiction to the norm substantiates Bonci's[29] statement of conflicting and inconclusive results.

Female athletes of the past have had lower skill levels, less experience and a lower faculty of coaching compared to their male counterparts. These differences may, in part, account for the greater risk of injury to the female ACL. In recent years, however, this gap regarding these extrinsic factors has narrowed. Ireland[12] examined conditions that may be a partially controllable. Strength, conditioning, footwear, and motivation are all extrinsically controlled, while proprioception, neuromuscular recruitment and firing patterns and acquired skills are only partially controllable. In Ireland's[12] review, a position of no return was described, which relates to positioning of the body from the back through the foot. The back is forward flexed and rotated to the opposite side. The hips are adducted and internally rotated while the knee is near or in full extension with a valgus force. The tibia is externally rotated and a valgus pressure is placed on the ankle when the medial aspect of the foot is in contact with the ground. This position places every joint and body part, between the back and the ground, in a manner that maximal tension on the ACL is reached and surpassed causing certain injury.

Epidemiology

Sports where most injuries to the ACL are seen within female populations are basketball, soccer, volleyball and cheerleading, respectively.[9-13] Gray et al[7] found that 73% (n=55) of 76 basketball related injuries in women involved the knee and 35% (n=19) of these were ruptures to the ACL. Ireland[12] has taken into consideration gender and level of play and reported on the incidents of knee

injuries, ACL injuries, injuries requiring surgery and season ending injuries. Overall, women experience a greater number of injuries to the knee, and more specifically, to the ACL, when compared to men. They also seem to have more general injuries, as well as injuries requiring surgery and season ending injuries.[9-13, 32] Gwinn et al[15] evaluated the relative risk of injuries to the ACL in female and male midshipmen at the United States Naval Academy. In an overview of varsity basketball, soccer, and rugby, coed soccer, basketball, softball and volleyball and military training, women had a relative risk injury of 2.44 compared to men. The relative risk in women to men was 1.40 in coed sports and 9.74 during military training. Ireland's[12] studies from 1985-1993 found that females playing basketball received almost 3 times as many injuries to the ACL than their male counterparts and 2.3 times as many injuries in soccer. Between 1988 and 1990 in 29 schools from the Atlantic Coast, Big Ten and Pacific Ten basketball conferences, women received over 6 times as many ACL injuries as men. In considering 14 colleges in New Jersey and a random sampling of high schools, the ratios for female to male were significantly higher regarding knee injuries (Table 1). It also seems to be that females lose fewer days to knee injuries, but more days to overall injuries. At the 1988 Olympic trials, women had over three times as many combined knee injuries, surgeries and ACL reconstructions, with only 2/3 of the number of participants.[12]

The National Collegiate Athletic Association (NCAA) noted an overall increase in female participation of 9% from 1989 to 1992. This increase varied for each activity. The number of soccer programs increased from 308 to 455

Table 1: Ratio of Female to Male Knee Injuries at New Jersey Colleges and High Schools (Adapted from Ireland 1999).

LEVEL/INJURY	Knee	ACL	Surgery	Season-ending	ACL & season-ending
NJ colleges	2.21:1	6.23:1	7.61:1	5.07:1	n/a
High school	2.1:1	n/a	n/a	n/a	3.52:1

(48%) between 1990 and 1995. The NCAA Injury Surveillance System (NCAA-ISS) has shown a higher rate of ACL injuries for female basketball and soccer athletes than in males. Non-contact mechanisms are also the primary cause of injuries to the ACL in females. The most common histories were straight knee landing from a jump, cutting, pivoting and sudden deceleration with a one-step stop causing hyperextension. Untrained females have increased chance (3.7:1) of injury during activity than trained females.[12] It has been noted that proper neuromuscular training appears to decrease the risk of injury in basketball, soccer and volleyball. Following training modifications, there was an 89% decrease in the rate of injury to the ACL in basketball at two Division I schools in Kansas. Adjustments involved improving techniques with accelerated and rounded turns off the inside leg, flexed knee landings when jumping and 3-step stops with flexed knees.[10,12] In addition to the aforementioned training modification, eccentric hamstring strengthening has also aided in preventing ACL injuries. This allows for a more controlled deceleration and a better counteraction to the anterior force of the quadriceps on the tibia.[10]

Karageanes et al[8] found there to be a significantly greater amount of ACL laxity in the left knee than the right knee ($p < .05$), without regard to leg dominance, in the follicular (.47 mm) ovulatory (.81 mm) and luteal phases (.47 mm). They also noted that age ($p > .404$) and sports played ($.164 < p < .631$) had insignificant roles in the amount of ACL laxity.

Knee Laxity Assessment

Precision and accuracy are a necessity in research when determining the amount of laxity presented in a ligament. There are several manual ligamentous tests that are good for on-field and clinical evaluations, as well as several means to mechanically manipulate the ACL to determine objective measurements. The most common manual tests for anterior laxity are the anterior drawer test, Lachman's test and the pivot shift test.

The drawer test (Figure 4) is conducted with the patient lying supine while the knee flexed to 90° and the hip to 45°. The examiner sits on the patient's foot while it is in neutral rotation, to stabilize it on the examination table. The examiner's hands are placed behind the gastrocnemious muscle belly and around the tibia just below the knee joint to ensure that the hamstrings are relaxed.[18,19,28] A force is applied in an anterior direction to the tibia with counter pressure applied by the thumbs to the femoral condyles and the amount of displacement is compared bilaterally. Anderson and Lipscomb[33] found the anterior drawer test to be effective in 20% of acute and 60% of chronic ACL injuries preoperatively (n=50).

The Lachman test (Figure 5) also measures one-plane anterior laxity, but is considered to be more reliable than the anterior drawer test because other stabilizing structures of the knee are less taut at 30° of knee flexion, which allows for more anterior tibial translation when damage has occurred to the ACL. The patient lies supine with the knee flexed to 30°. The examiner places the stabilizing hand around the lower thigh just above the patella while the other hand

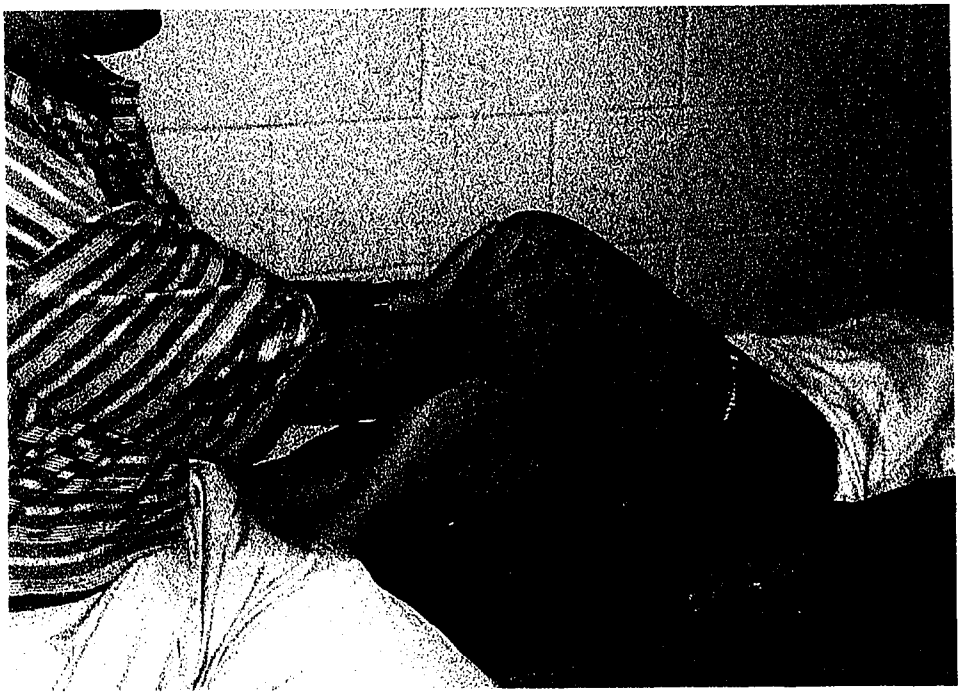


Figure 4: Anterior drawer test for ACL instability.

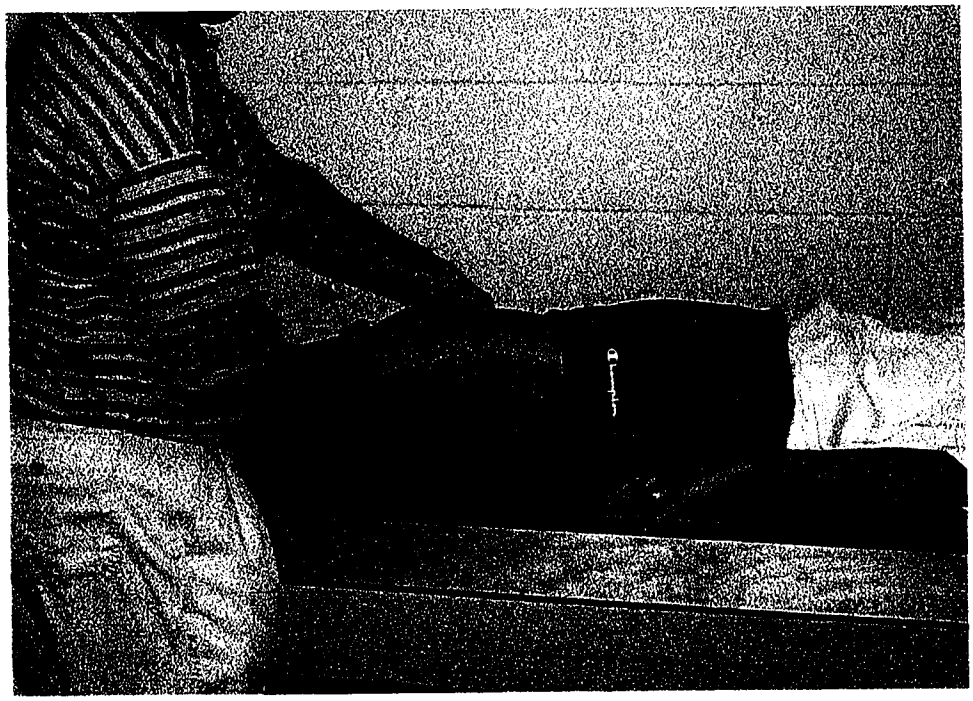


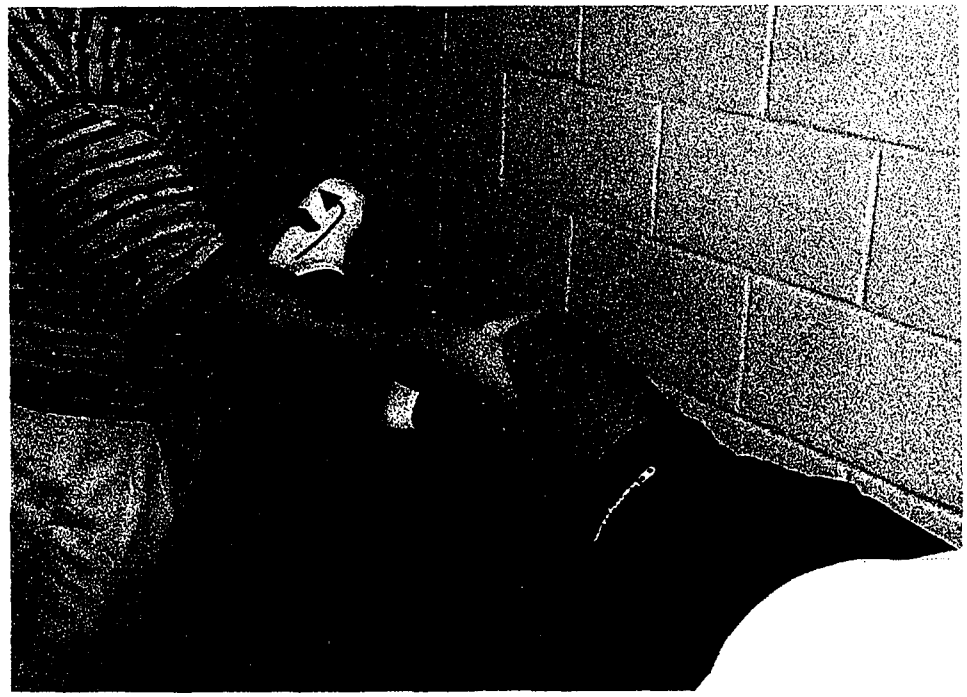
Figure 5: Lachman test for instability of the ACL.

grasps the proximal aspect of the tibia. An anterior force is applied to the tibia and again the amount of displacement is compared bilaterally.[18,19,28] Anderson and Lipscomb[33] found the Lachman to be effective in 91% of acute and 100% of chronic ACL injuries preoperatively (n=50).

The pivot shift test (Figure 6), also known as the lateral pivot shift test, assesses anterolateral instability. The patient is supine with the examiner gripping the heel. The other hand is placed on the lateral aspect of the tibia with the thumb behind the fibular head. The knee is passively brought into slight flexion with the tibia being internally rotated. At the same time the examiner pushes the proximal tibia anteriorly while lifting the head of the fibula with the thumb. In a positive test the lateral tibial plateau will sublux anteriorly from under the femoral condyle. The tibia will conversely reduce when the examiner applies valgus stress with the distal hand in a more flexed position, which is the second indication of a positive test.[18-20] There are modifications of all three manual tests that have been developed to compensate for incompatible size ratios of examiner to patient and poor patient positioning.

Although clinical assessments are the most common form of identifying ACL deficiencies, subtle changes that may not be detectable by manual tests must be noted when considering intrinsic fluctuations in laxity. There are several mechanical devices that are capable of this task while also providing for more objective results. This is not only helpful when conducting research, but also aids in monitoring rehabilitation progress after surgical intervention. Some of these include the Stryker (Stryker Corp., Kalamazoo, MI), KT-1000 and KT-2000

A)



B)

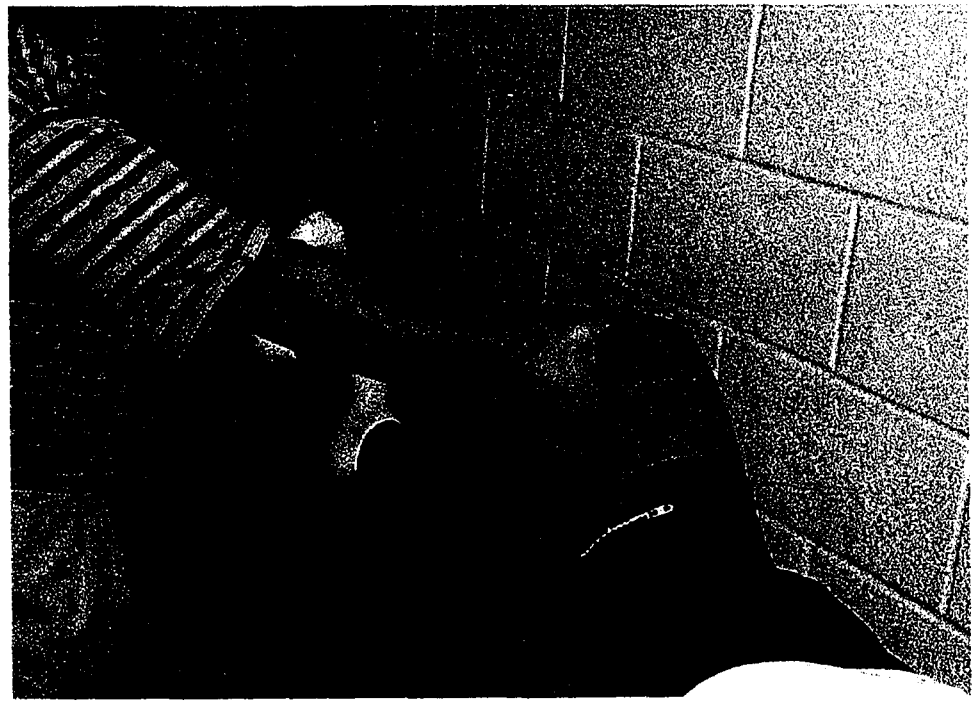


Figure 6: Pivot shift test A) tibia subluxes in slight flexion and B) reduces as valgus stress is applied and the knee reaches 20-40° of flexion.

(MEDmetric, San Diego, CA), Genucom Knee Analysis System (FARO Medical Technologies Inc. Montreal, Canada) and the Knee Signature System (KSS, Acufex, Boston, MA). All of these instruments can measure both anterior and posterior translation of the tibia on the femur, in millimeters, with repeatable loads applied through a spring gauge at varying degrees of flexion at the knee and hip. It is up to the clinician to decide which one best suits the needs at hand, based on clinician experience, device reliability and portable verses stationary devices.

The Stryker unit consists of a bar that is applied to the anterior aspect of the tibia with elastic straps and rods that maintain a distance of 4 cm between the leg and the machine. A piston at the proximal end contains a plunger, which is attached to the patella. This portion is at a right angle to the long axis of the leg. It is preloaded against the patella so the amount of pressure remains the same. Along the shaft of the device, which is in the line with the tibial tuberosity, are "stick/slip pointers" to measure the millimeters of displacement, which remain in position after maximal force is applied in anterior and posterior directions. This device measures pure anterior and posterior motions and does not compensate for the difference in anterior translation of the lateral verses the medial tibial plateaus. Movement of the lateral tibial plateau is significantly increased, in comparison to the medial tibial plateau, in the absence of an intact ACL. This causes the medial portion to almost act as a fixed axis of rotation causing a value that is less than the true reading. Single patient, bilateral variations of 2 mm or more can be observed when considering one examiner over time (22.2%), multiple examiners (21.5%),

between positioning at 30° and 90° (41.5 % and 35% respectively) and between 10 and 20 lbs. or 44.5 N and 89 N (14% showed greater motion at 44.5 N).[34] The Stryker unit has been shown to incorrectly diagnose ACL tears and present false positives (+) in 10% of normal knees and correctly identify 72% of knees with isolated ACL tears.[33]

The KT-1000 and KT-2000 (Figure 1) knee arthrometers are applied to the anterior aspect of the tibia, while the patient is supine, so they are parallel to the line of the tibial tuberosity. Two straps are applied at the proximal and distal ends of the tibia at points to allow for alignment with the tibiofemoral joint line as indicated on the devices and so that the patellar sensor remains in a position over the patella. A thigh support is placed under the distal femur, to cause the resulting position of the knee to be at 20-30° of flexion. Tibial rotation is at a neutral position and controlled for with a foot support and a third strap being placed around the thighs. Pressure is applied to the patellar sensor pad and maintained at a constant level throughout the use of the device. Force is given in either an anterior or a posterior direction with applied forces equaling 15, 20 and 30 lbs. or 67, 89 and 133 N, respectively. This is indicated by audible tones at each level. The KT-2000 also produces tones when these forces are produced anteriorly, but because it also contains an X-Y plotter (Figure 7), displacement can be measured at any amount of force since the readings are recorded on the Patient Evaluation Form (Figure 8). This added characteristic also increases the amount of accuracy and reliability because it allows for a more exact comparison of the millimeters of

Figure 7: X-Y plotter attachment for the KT-2000 (Adapted from MEDmetric Buyers' Guide 1995).[16]

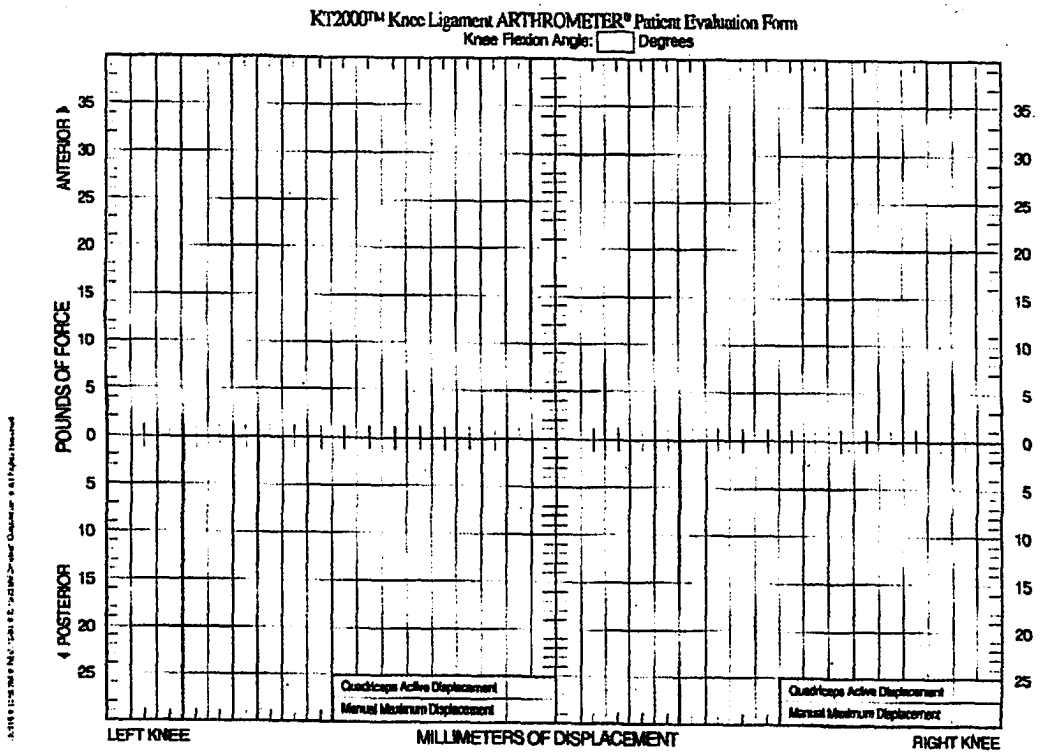


Figure 8: KT-2000 knee ligament arthrometer patient evaluation form (MEDmetric Corporation).

displacement to the pounds of force applied. Reliability of both devices has been found to be dependent on experience in using the device, knee positioning, relaxation of the hamstrings and proper application. It is also recommended that repeated measures be taken during each use to confirm intratester reliability.[35,36] Tibial rotation will adversely affect readings produced by this arthrometer. Fiebert et al[37] found significant a difference ($p < .0001$) to exist between internal rotation (3.415 ± 1.827 mm) and both a neutral position (4.607 ± 2.124 mm) and external rotation (4.548 ± 2.269 mm) of the tibia. They also found intratester reliability of high ($r = .90$) for tester 1 and good ($r = .86$) for tester 2. Intertester reliability between the two was poor ($r = .64$). Intertester reliability was reduced with less amounts of experience in using these machines. The KT-1000 has been found to be reliable in that it allows for the production of consistent measures (mean error = $.13 \pm .12$ mm).[35] Intratester reliability ranges average from .87 to .97, with reliability between males and females, regardless of experience level, ranging from .77 to .88. Examiner experience reliability levels range from .12 to .78, with measurement errors between 1 mm and 2.2 mm, indicating poor intertester reliability.[36] The KT-1000 has been found to correctly identify all normal ACLs and 76% of those with an isolated tear.[33] The KT-2000 has demonstrated small estimates of absolute reliability (95% confidence intervals), indicating little variability (high reliability) among multiple testing sessions.[38] The correlation coefficient between the KT-2000 readings and the direct transducer readings has been reported as $.97 \pm .44$ mm.[39]

The Genucom Knee Analysis System combines a computer, a biomechanical digitizer, an electrogoniometer, a dynamometer and an inkjet printer. It allows for testing analysis of total knee stability, including: anterior-posterior, valgus-varus, pivot shift and recurvatum.[40] No literature could be found on the set up or application of this device, although the manufacturers do offer a testing certification program. Several studies reported on its overall effectiveness, including Anderson and Lipscomb[33] who found that for anterior displacement it misdiagnosed 10% of normal knees as having ACL tears and correctly diagnosed 72% of those with isolated tears to have the same specificity and sensitivity, respectively, as the Stryker unit, but only found an average laxity of 2 mm, as opposed to the KT-1000 and Stryker unit, which produced 4.4 mm and 4.6 mm of displacement, respectively, all at 20 lbs of force. Conversely, Highgenboten et al,[40] found the Genucom (93.45 N) to produce laxity values significantly higher than the Stryker (89 N) and KT-1000 (89 N). Steiner et al[41] also found the Genucom to produce higher displacement values at 89 and 133 N of force than the Stryker, KT-1000 and Acufex and also noted that the Genucom did not allow for reproducible measurements.

The Acufex KSS can be used in conjunction with a Hall effect transducer, which is implanted arthroscopically within the knee itself. An electrogoniometer provides a constant measure of flexion/extension and internal/external rotation of the knee. Anterior-posterior loads are used and monitored by a load sensor that is located on a T-bar attached to the distal aspect of the tibia. Information from a Hall effect transducer, electrogoniometer, anterior-posterior load and extension-

flexion load sensors can be used in compliment to transfer the physical results to a data acquisition board (Tecmar, Cleveland, OH) to be converted to a digital frequency of 20 Hz.[42] Steiner et al[41] found the Acufex could be used to identify 80% to 90% of normal and ACL deficient knees and could give reproducible measures.

Portable knee ligament arthrometers can also be used in conjunction with plain radiograph assessment. Staubli and Jakob[17] used the KT-1000 with lateral radiographs taken before and after the force is applied. The radiographic equipment was set at a film-to-tube length of 120 cm. To measure the anterior position of the tibia with respect to the femur, a line is drawn on the posterior tibial cortex (PTC) at the midshaft level. The most posterior aspects of the medial tibial plateau and medial femoral condyle are identified at the subchondral bone level. A tangent line is drawn to the most posterior aspect of the tibial plateau and parallel to the PTC. A second parallel line to the PTC is drawn tangent to the posterior aspect of the femoral condyle. The distance between the two tangent lines is the anterior displacement of the medial compartment (ADm). This measurement defines the anterior position of the medial tibial plateau with regard to the media femoral condyle. Anterior displacement is also measured in the lateral compartment (ADl) using the most posterior aspects of the lateral tibial plateau and lateral femoral condyle. The sum of the ADm and ADl is then divided by two. The arithmetic mean of measurements of both compartments represents the anterior knee motion by describing the anterior position of the tibia in regard to the femur at the midpoint of the knee. This is done on two

radiographs. Then the amount of displacement for a film with no applied force is subtracted from the amount of displacement for a film with the known load being applied to obtain a reading for the actual millimeters of displacement (Figure 2). This technique is only possible if the knee arthrometer being used is portable. It should not be used to monitor rehabilitation progress due to the amount of radiation exposure, but is an excellent means of tracking laxity over time.

The Menstrual Cycle

Follicular Phase

The menstrual cycle is traditionally described by 3 stages: the follicular phase, ovulation and the luteal phase.[3,4] The follicular phase is divided into the initial follicular growth stage, the mid-follicular stage, and the preovulatory stage. The follicular phase as a whole allows for proper follicular development, which in humans is usually considered to be one surviving mature follicle. This entire process generally takes 10-14 days.[3] Initial follicular growth (Days 2-6) appears to be independent of stimulation. The mechanism for determining which follicles, or how many will develop, during any one cycle is not known. In most cases, growth is limited and quickly eliminated. This rapid follicular death is referred to as atresia. The pattern is disrupted when the menstrual cycle begins and a group of follicles begin to grow in response to hormonal changes. Folliculogenesis begins in the latter days of the luteal phase of the prior cycle. The corpus luteum is in regression and secretes smaller amounts of steroids and induces a rise in FSH levels.[4] Diminished steroidogenesis permits a rise in the gonadotropins, FSH and

LH. Initially, only FSH is found in the fluid of smaller follicles. It is simpler to think of FSH as the gonadotropin responsible for growth, while LH stimulates steroidogenesis. FSH receptors are limited to the granulosa cells of the ovary where FSH may contribute to steroidogenesis by stimulation of the aromatizing system within the cells. However, growth and steroidogenesis depend upon the cooperation of FSH, LH and estradiol. In terms of steroidogenesis, the role of FSH is to increase the activity and number of LH receptors. During this stage, there is almost no detectable change in plasma levels of gonadal hormones. A rise in LH occurs, but its only function at this point is to maintain smaller amounts of steroidogenesis.[3] The eventual targets for LH are thecal, stromal, luteal and granulosa cells.[4] Initial follicular growth ends when an increase in plasma estrogen first becomes detectable 7-8 days before the preovulatory surge of LH that signifies ovulation.[3]

In the mid-follicular stage (Days 7-10), estradiol, and FSH itself, enhance the number of FSH receptors. This increase in the number of gonadotropin receptors is the result of continued follicular maturation and increased estradiol levels. This continued growth is the result of an increased use of gonadotropins. This is not because more FSH is being produced, rather there is an increased ability to respond. There are now more FSH receptors available, because the granulosa cells they are attached to are in more abundance.[4] Estradiol increases may stimulate granulosa cell proliferation by increasing the ovarian uptake of FSH. This increased action of estradiol and FSH also allows them to act together to prepare follicles to react to LH surges during ovulation and luteinization. This

sequence has 5 steps that are important: 1) initial follicular growth, 2) FSH stimulation into the mid-follicular stage, 3) estradiol production, increasing the sensitivity to FSH by enhancing FSH action, 4) FSH induction of LH receptors that is enhanced by estradiol and 5) thecal cell differentiation causing even greater amount of estradiol to be produced.[3]

Estradiol interacts with both FSH and LH. As estradiol levels increase LH is initially suppressed, but as estradiol levels continue to rise FSH becomes inhibited. *Estradiol levels still rise, but now the secretion of LH is promoted* (Figure 9). These hormonal fluctuations are essentially feedback mechanisms. FSH reacts negatively to estrogens, yet estradiol increases the number of FSH receptors. LH has a negative response to low levels of estrogen, but as estrogens increase, so do the levels of LH. This positive interaction is seen during the follicular and ovulatory phases. LH is suppressed during the luteal phase by progesterone. The positive feedback is dependent on the concentration of estradiol and the duration of time for which its levels are elevated. The negative feedback mechanism consists of 2 components: 1) a system within the hypothalamus involving the tonic center and 2) a modulating system controlling the response of the pituitary to GnRH.[3,4] Rapid increases in estradiol inhibit the pituitary's response to GnRH, but moderate elevation of estradiol enhances its response (Figure 10).[3]

In the preovulatory phase (Days 10-14), or the late follicular phase, estrogens rise moderately and then spike quickly just before ovulation. At the same time, there is a decrease in FSH, but LH also rises slowly at first and quickly

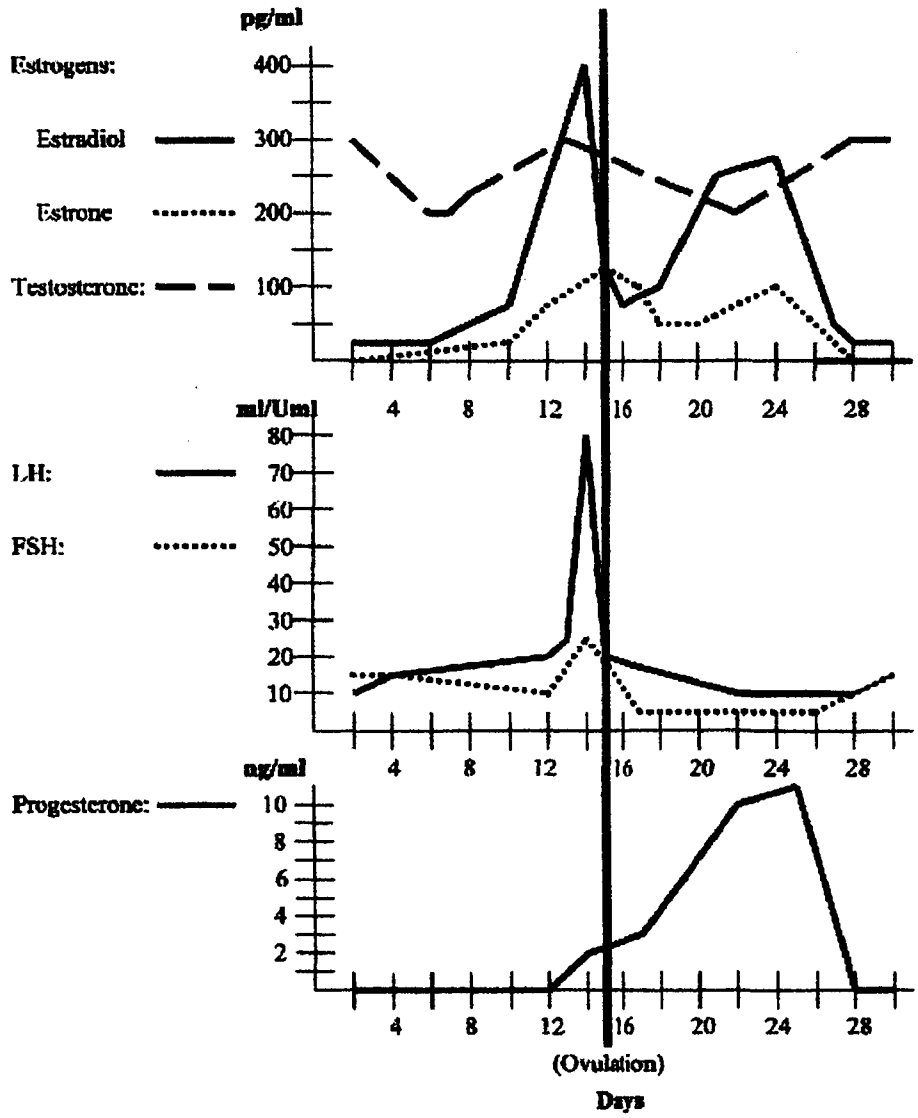


Figure 9: Hormonal concentrations throughout the menstrual cycle (Adapted from Speroff et al 1978, Yen and Jaffe 1986).[3,4]

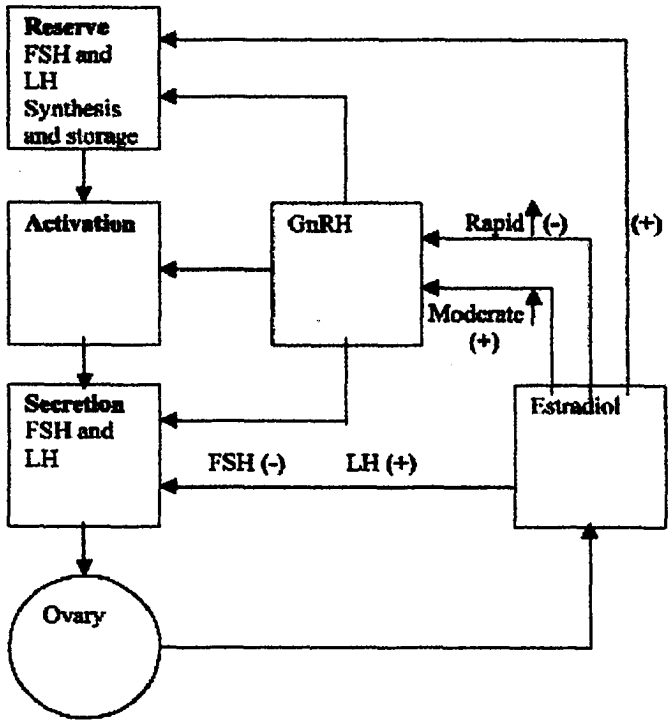


Figure 10: Hormonal effects and reactions of FSH, LH and estradiol (Adapted from Speroff et al 1978).[3]

peaks at midcycle, immediately after the estrogens peak (Figure 9). The follicle grows rapidly now as FSH levels are still diminished. This indicates that as the follicle matures, it becomes increasingly sensitive to FSH. The follicle is able to protect itself from premature atresia by producing its own hormones of highly localized estradiol concentrations, which increases the follicles sensitivity to FSH, encouraging follicular binding to FSH. The FSH concentration remains high in the follicles where estradiol still has concentrations of 5000 pg/ml, but the circulating levels of FSH still decrease due to the overall negative feedback from estradiol. This is the reason that a mass of atresia is seen as estrogen levels rise. Luteinizing hormone is not detectable in follicular fluid until luteinization begins in the granulosa cells 24-36 hours before peak LH levels. Luteinization is the process in which the granulosa-thecal cell complex is transformed into luteal cells. These luteal cells have an increased number of LH receptors and the capacity for progesterone biosynthesis.[4] This increase in the number of LH receptors is a response caused by FSH and estradiol. The overall result is a high concentration of progesterone in preovulatory follicles. While follicular growth is occurring, stromal cells are organized into a thecal layer where LH will bind to promote steroidogenesis. The stromal cells are the primary source of estradiol before ovulation. Most estradiol at midcycle is produced by the follicle that is going to ovulate. Mass atresia will occur when all of the other follicles fail to reach full maturity. This is accompanied by a decrease in the number of receptors for FSH, LH and estradiol. At this time, thecal cells return as a component of stromal tissue, but keep the ability to react to LH for steroid production. Principal

stromal products are androgens rather than estrogens, namely androstenedione and testosterone. This redistribution into the stromal tissue during the late follicular phase is accompanied by a rise in androgen levels in the peripheral plasma, specifically the 20% increase of testosterone levels. The production of androgen enhances the process of atresia and stimulates the libido. Androgens within the ovary regulate the number follicles that reach ovulation by accelerating granulosa death and follicle atresia.[3]

Ovulation

The rise in estradiol in the late follicular phase is most likely the reason for the surge of the gonadotropic hormone LH. The hypothalamus responds by releasing GnRH. Androgens are thought to prevent this when at elevated levels by suppressing the function of the cyclic center that produces GnRH. There are two requirements for an LH surge at midcycle: 1) a minimal estradiol concentration of 200 pg/ml and 2) an exposure of the cyclic center to estradiol for approximately 50 hours.[3] Luteinizing hormone levels will surge prior to the withdrawal of estrogens. At this time, there is a simultaneous incline in the FSH due to the release of GnRH (Figure 10). Follicle-stimulating hormone may be required for the production of a normal corpus luteum, because FSH induces LH receptors, which in turn allow LH to begin the process of ovulation and luteinization in the corpus luteum.[3,4] At midcycle there are high levels of estradiol and slightly elevated levels of progesterone that are responsible for the surge of LH. The hypothalamus and pituitary gland must both respond to GnRH for the release of FSH and LH. There is also a small increase in progesterone

levels during the ovulatory period that is probably due to a small rise in LH and luteinization. Progesterone then has a positive feedback in that it will enhance the release of gonadotropins. LH specifically, however, seems to be inhibited at the hypothalamus by the combination of progesterone and estrogens. The elevated levels of LH last about 24 hours and decrease during the luteal phase (Figure 9). Release of gonadotropins is not consistent. It is an episodic release by the pituitary, mostly of LH. There is no known mechanism for what then shuts down the surge of LH, but within hours there is a decrease in plasma estrogens. This is thought to be secondary to the luteinization of the follicle. This change is said to be associated with a shift of estrogens secreting thecal dominance to a dominance of progesterone secreting granulosa cells. The rapid decline in LH may represent a lack of its content in the pituitary gland, which demonstrates a negative feedback upon the hypothalamus. The LH surge, however, does not guarantee ovulation. This is dependant on the maturity level of the follicle itself. These two events, however, do coincide since the gonadotropins are regulated at this point by estradiol. After 24 hours from the time LH has peaked, the follicle, which is most likely single, will rupture. Degenerative changes in the collagen of the follicular wall occur, due to passive stretching prior to ovulation, allowing the ovum to escape. The increased blood flow that goes along with vascularization of the granulosa layer in response to LH causes an increase in the clearance of blood constituents from the capillary walls and passage into the antrum. Elasticity reaches minimal levels so there is no change in pressure when ovulation occurs.

The follicular wall will rupture, because of the increase in the volume of the antrum.[3]

Luteal Phase

Once the follicle has ruptured and the ovum is released, the granulosa cells will increase in size for three days after ovulation and develop characteristics of lutein. This is the process of luteinization and the formation of the corpus luteum. Capillaries penetrate the granulosa layer and fill the central cavity with blood. Peak vascularization is reached 8-9 days after ovulation and is accompanied by peak levels of estradiol and progesterone. The corpus luteum is able to synthesize all three classes of sex steroids: androgens, estrogens and progestins. A plasma level of at least 3 ng/ml is a reliable determinant of ovulation. Ten to 12 days following ovulation, the corpus luteum begins to regress. This is seen through a gradual decrease of blood in the capillaries followed by a decrease in the amount of progesterone that is secreted. Large follicles begin to appear in the ovary again. Their presence is most likely because of the surge in FSH at midcycle, but there is no growth at this time since levels are still diminished. Fourteen days is the normal time frame between the midcycle surge of LH and menses, but varies among individuals. LH and FSH fall to their lowest levels during the luteal phase when estradiol and progesterone are at maximal levels, due to the negative feedback mechanisms of these steroids when they are combined (Figure 9). A normal corpus luteum requires the constant presence of minimal LH to function properly, confirming that steroidogenesis is impossible without LH. The corpus

luteum abruptly declines 9-11 days after ovulation, and is termed luteolysis, but the reason for this occurrence is not known. The 3-4 days before the onset of menses a decline in estrogen and progesterone will accompany the sudden demise of the corpus luteum causing another increase in FSH and LH concentrations.[4] This degeneration is unavoidable unless the individual becomes pregnant. A short luteal phase has been considered the possible cause for infertility. The short luteal phase has certain characteristics: low secretion of progesterone, early regression of the corpus luteum, with menses occurring 6-9 days after peak LH levels and increased circulating aldosterone.[3]

Speroff et al[3] identified five characteristics that are key points to a normal menstrual cycle. These include 1) the beginning of the cycle is initiated by a rise in FSH which occurs in response to the decline in estradiol in the preceding luteal phase, 2) estradiol maintains follicular sensitivity to FSH by inducing FSH receptors, 3) estradiol enhances follicular response to LH by working synergistically with FSH to induce LH receptors, 4) ovulation is triggered by the rapid rise in estradiol and LH at midcycle and 5) regression of the corpus luteum may depend on its own production of estradiol and a local luteolytic effect.

There are significant differences regarding hormonal releases and concentrations during a pregnancy. Instead of tracking a general sequence of changes over a 4-5 week menstrual cycle, a hormonal fluctuation pattern is now monitored throughout the duration of the approximate 9 months of pregnancy. At the onset of menstruation, most of the endometrium of the uterus moves away from the uterine wall and is expelled. If this were to happen after an ovum had

implanted itself the pregnancy would be terminated. This is prevented by the secretion of human chorionic gonadotropin (HCG). This increased secretion of HCG begins 8-9 days after ovulation and continues until it peaks between 10-12 weeks. At weeks 16-20, the level of secretion decreases and maintains a level that is slightly elevated from the period before the initial increase. It remains at this concentration throughout the duration of the rest of the pregnancy (Figure 11).

HCG is similar to LH in structure. It prevents the normal usage of the corpus luteum at the end of the cycle. It also causes increased secretion of estrogens and progesterone. This increase in estrogens and progesterone prevents menstruation and causes the endometrium to continue to grow and store nutrients. HCG also exerts an interstitial cell-stimulating effect on the testes resulting in the production of testosterone in males up until the point of childbirth. This small production of testosterone is what causes the growth on male sex organs and not female sex organs. It also causes the testes to descend into the scrotum near the end of the pregnancy.

During pregnancy, the placenta secretes estrogens and progesterone. Daily production of placental estrogens increases by 30 times the normal amount by the end of pregnancy. Most of the estrogens released in this manner is estriol. During pregnancy, estrogens cause 1) enlargement of the uterus, 2) enlargement of the breasts and growth of the ductal structure and 3) enlargement of the external genitalia. Estrogens also relax the pelvic ligaments so the sacroiliac joints and symphysis pubis become more elastic in nature. The rate of production for progesterone production increases 10 times above normal by the end of

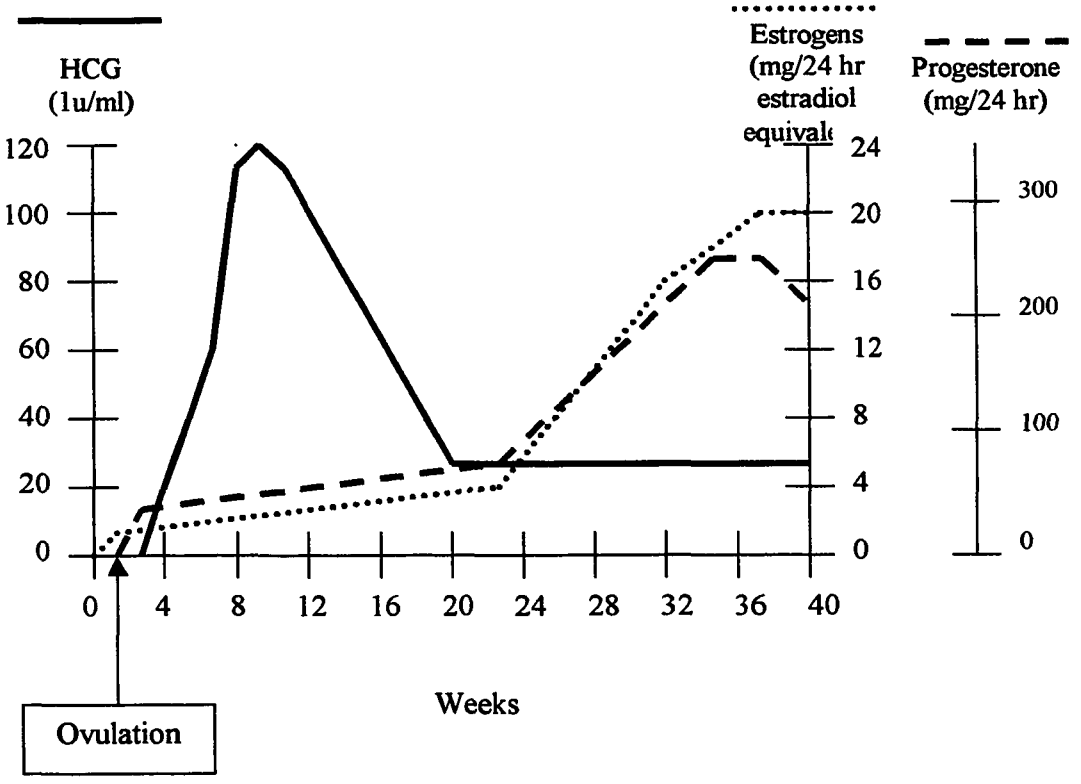


Figure 11: Hormonal concentrations throughout a pregnancy. (Adapted from Guyton and Hall, 1996).[43]

pregnancy. During pregnancy, progesterone 1) causes decidual cells to develop which provide nutrition to the embryo, 2) decreases the contractility of the uterus to prevent contractions from causing a spontaneous abortion, 3) increases the secretion from the fallopian tubes to provide appropriate nutrition and 4) helps estrogens to prepare the breasts for lactation. LH and FSH are almost completely suppressed by the increased levels of estrogens and progesterone produced by the placenta. This prevents the preovulatory surge of LH throughout the pregnancy that would occur during a normal menstrual cycle.[43]

Hormonal Characteristics

Estrogens

Estrogens are composed of a group of sex steroids secreted by the ovaries, adrenal cortex and fetoplacental unit in women. They are responsible for the development of several female sex characteristics and for normal menstruation. During the menstrual cycle, estrogens allow the female genital tract to become more suitable for fertilization, implantation and nutrition of the early embryo.[44] It has a negative effect on FSH in that when estrogens' levels are elevated, FSH production becomes diminished. The average concentration in the blood stream during each phase of a normal menstrual cycle are: 1) 1-10 days range from 60-400 pg/ml, 2) 11-20 days range from 120-440 pg/ml and 3) 21-30 days range from 150-350 pg/ml.[3,4,43,44]

Progesterone

Progesterone is essential for the female reproductive system to function properly. It is produced at various times and locations. During the second half of the menstrual cycle it is produced in the ovaries, as opposed to the placenta during pregnancy. Smaller amounts also come from the adrenal glands. After ovulation, an increase in progesterone levels has been noted to cause a thickening of the uterine lining in order to prepare for the implantation of a fertilized egg. If this does not occur then progesterone and estrogens levels decrease and the uterine lining is shed.[44] The combined effect of progesterone and estrogens is the inhibition of FSH and LH production. The standard range for progesterone is 0.15-80 ng/ml with ranges of 0.2 – 0.9 during the follicular phase and 3-35 ng/ml during the luteal phase.[3,4,43,44]

Luteinizing Hormone

Luteinizing hormone is produced by the pituitary gland in response to FSH and estradiol in various amounts throughout the menstrual cycle. In females, LH acts with FSH to stimulate the follicle growing within the ovary to secrete estrogens. High levels of estrogens in turn cause a surge in LH to stimulate ovulation. LH then induces the ruptured follicle to development into the corpus luteum. The corpus luteum then secretes estrogens and progesterone.[44] The standard range for LH is 2.5-200 mlU/ml with levels normally between 2-20 mlU/ml, and elevating to 30-200 mlU/ml at midcycle.[3,4,43,44]

Follicle-Stimulating Hormone

Follicle-stimulating hormone is a gonadotropin that stimulates the growth and development of the follicles in the ovary. It is secreted by the anterior pituitary gland. The hypothalamus, however, controls the release of FSH by regulating the amount of FSH-releasing factor that is produced. FSH, along with estradiol increases the availability of FSH receptors and LH concentrations prior to ovulation. Increased amounts of FSH are released at the end of the luteal phase of the menstrual cycle, which causes an immature follicle to develop into a mature grafian follicle that in turn produces estrogen at higher levels before ovulation begins. This surge of estrogens will suppress FSH until the mid-luteal phase when follicles begin to develop for the following cycle.[44] The concentration for FSH varies from 2.5-200 mlU/ml. Average levels throughout the menstrual cycle include: 1) the follicular phase from 2.5-15 mlU/ml, 2) the midcycle from 12-50 mlU/ml, 3) the luteal phase from 2.5-15 mlU/ml.[3,4,43,44]

Estradiol

The menstrual cycle is mainly dependent upon critical adjustments of estradiol at specific points in time. It is the most potent naturally occurring estrogen in the human body. Its key roles include: 1) declining in levels during the luteal phase to allow for elevated FSH concentration in the following cycle, 2) maintaining follicular sensitivity to FSH by inducing FSH receptors, 3) enhancing follicular response to LH by working synergistically with FSH to induce LH receptors, 4) increased levels at midcycle to induce ovulation and 5) regression of the corpus luteum through its own production of estradiol.[3] The standard

concentration ranges from 10-3000 pg/ml. In ovulating females, this ranges from 40-500 pg/ml in the follicular phase, 200-600 pg/ml at midcycle and 120-350 pg/ml in the luteal phase. [3,4,43,44]

Testosterone

Testosterone is the most important of the male sex hormones, but it is still present in the female endocrine system. It is responsible for stimulating bone and muscle growth and sexual development. It is an androgen produced in small amounts by the ovaries. If overproduction of testosterone occurs in a female, masculinization symptoms, which include amenorrhea, voice deepening, and excessive growth of body hair, will occur.[44] During the redistribution of the stromal tissue in the late follicular phase, a rise in testosterone levels in the peripheral plasma occurs. The production of the androgen enhances the process of follicular atresia and stimulates the libido. Androgens within the ovary regulate the number follicles that reach ovulation by accelerating granulosa death and follicle atresia.[3] The average concentrations for testosterone during the follicular and luteal phases are 0.2 – 0.8 ng/ml. The range may be higher at midcycle. [3,4,43,44]

Phlebotomy

Phlebotomy is the act of drawing or removing blood from the circulatory system through a cut (incision) or puncture in order to obtain a sample for analysis and diagnosis. It can also be done as part of a patient's treatment for certain blood disorders.[45] The entire process requires little equipment: a vacuum-

tube adapter and needle with protective device, alcohol and povidine-iodine swabs, tourniquet, gloves, gauze pads and an adhesive bandage.[46] Blood is usually drawn from a vein on the back of the hand or at the anterior aspect of the elbow. Other blood tests require samples from an artery. The skin over the injection site is wiped with an antiseptic and an elastic band is tied around the arm proximal to the site. The band acts as a tourniquet to slow the blood flow to the arm and increase the visibility of the veins. The patient makes a fist in order to again increase the visibility of the veins and allow the phlebotomist to select an appropriate site. The needle is inserted into the chosen vein and the elastic band is removed. Once the appropriate amount of blood is drawn the needle is removed.[45] The patient should always be in a seated position when conducting the procedure.[46]

When testing requires a smaller amount of blood, the technician can use a finger stick instead. A small needle, or lance, is used to break the skin of the surface of the fingertip. The finger can be squeezed to obtain a slightly larger sample.[45]

Patients are usually instructed to cease the use of medication or avoid eating for a designated length of time prior to the sample being draw. Exercise and physical activity may also affect the results of blood tests. After the blood has been drawn, pressure is applied to the site to stop the bleeding and a bandage is applied. Side effects may include dizziness or nausea, which is why it is important to be seated during the procedure. To avoid this occurrence, patients are instructed to rest for a short period of time following the procedure and to

drink plenty of fluids and eat regularly for the following 24 hours to replenish the blood volume. A small bruise and mild soreness are also possible for several days. Infection is a risk as with any invasive procedure, but can be minimized through the use of sterile equipment and proper cleansing of the sample site.

Ligamentous Laxity and Menstrual Cycle Associations

Many studies in the past have suggested the further investigation of the possible relevance of hormonal fluctuations to peripheral ligamentous laxity and more specifically ACL injuries.[1,2,8,47] Given that the elevated levels of relaxin, produced with pregnancy and labor, allow for increased ligamentous laxity of the pelvis to permit a large enough birth canal for a newborn child,[8,43,47] leads to the hypothesis that there may be other hormones capable of the same laxity inducing characteristics in peripheral locations. Serum relaxin levels have been found to have no peripheral effects on laxity during pregnancy. There have been demonstrated statistically significant changes in knee joint laxity over time ($p = .0001$). There have also been statistically significant elevations of relaxin levels over time ($p < .0001$). However, there has been no statistically significant correlation reported between joint laxity and relaxin.[47] Since there is no correlation between these two characteristics, there may be a correlation involving one or more other hormonal variations, which could explain the changes that have been observed in knee laxity.

Wojtys et al[1] conducted a survey reporting on the occurrence of ACL injuries in correspondence with the phases of the menstrual cycle. They found

more injuries were reported as happening in the ovulatory phase, from days 10 to 14, than were expected and less injuries than expected occurred during the follicular phase, days 1-9. This is about the same time that estrogens, LH, FSH and estradiol reach peak levels and progesterone and testosterone levels begin to rise slightly. According to McShane et al,[48] this finding was not statistically significant, but Wojyts[49]still observed that there was at least a trend that had occurred to suggest further investigation.

Heitz et al[2] reported the menstrual cycle as being divided into three phases: 1) menstrual phase (days 1-5), 2) follicular phase (days 6-13) and 3) luteal phase (day 15 to end of cycle). The menstrual phase is the approximate five days of menses caused by a reduction in estrogens and progesterone. The follicular phase is responsible for the developing follicle so it can secrete increased amounts of estrogens, which are the dominant ovarian hormones throughout this phase. The luteal phase is the time from ovulation to the next menses. LH is secreted after ovulation to stimulate the developing corpus luteum, which in turn secretes increased amounts of estrogens, progesterone and relaxin. The greatest amount of ACL laxity was noted, and had a significant difference ($F_{1,6} = 13.41$, $p = .006$), during the luteal phase, between days 20 and 23, with an increased amount of progesterone. A significant difference ($F_{1,6} = 3.56$, $p = .048$) was also observed with peak levels of estrogens during days 10 through 13 of the follicular phase.[2]

Karageanes et al[8] tracked ACL laxity during the menstrual cycle for both knees in adolescent female athletes. Their findings suggested that there is no specific "time of the month" when females are more likely to sustain an injury to

the ACL due to changes in laxity. The findings over the course of all 3 phases showed insignificant differences in ACL laxity for the right ($p = .7977$) and left knees ($p = .9$).

Estrogens and progesterone receptors have been reported as being present in normal connective tissue.[50] In rat studies, estrogens have been shown to decrease the total collagen content in tendon and fascia and the amount of collagen synthesis. For this reason, there is the assumption that estrogens may affect the collagen production of fibroblasts and the tensile strength of the ACL.[50] Guyton and Hall[43] report that, in women, relaxin serves to soften the cervix at the time of delivery. They also stated that relaxin has been shown to play a role in relaxing the ligament of the symphysis pubis in estrous rats and guinea pigs, but has a poor effect in women. In human females, the relaxing effects are more likely to be from the release of estrogens.[43] A study, using the ACL of rabbits, found collagen levels to be significantly decreased when local estradiol levels were elevated ($p < .001$). Collagen synthesis decreased by more than 40%. Fibroblast proliferation was also reduced as estradiol increased ($p = .023$). Collagen, produced by fibroblasts, provides for the majority of the ACL's load-bearing function. Changes in the metabolism of the fibroblasts can have an effect on the quantity, type and stability of collagen in the ACL. These changes may be caused by local stimuli, growth factors, mechanical strain or hormones.[50] Lui et al[50] also reported, that while there are several studies that have investigated the effects estrogens may have on collagen metabolism in various tissues, ligaments themselves have not been the source of investigation. Comprehensive or quick

fluctuations in serum estrogens may result in structural or compositional changes in the ACL, causing decreased ligamentous strength and stability, which would predispose an individual to injury. The ability to perform motor skills may also be affected by the fluctuation of hormones.[50-53] Clapp et al [51] found that sustained exercise produces increased levels of estradiol. This increase is intensity dependant and most likely reflects decreased hepatic clearance caused by a fall in splanchnic blood flow.

According to survey, female athletes have reported feeling a decrease in overall physical performance during the preovulatory stage of the follicular phase. This is when estrogens, progesterone and LH levels are elevated.[1] Conversely, Lebrun[52] found that there was no effect produced by the follicular or luteal phases on performance levels, but did suggest a slight influence on aerobic capacity. Wojyts et al[1] could not categorized these differences for those individuals using birth control, because the number of respondents involved in the study using birth control was too little to extrapolate any significant information. In a review of literature, Lebrun[53] found that estrogens and progesterone have direct and indirect effects on athletic performance. The review noted that estrogens, particularly when used with oral contraceptives, have detrimental effects on the cardiovascular system, such as alterations in plasma fibrinolytic activity and platelet aggregation with a corresponding increase in thrombosis. Estrogens also cause retention of sodium and chloride, which leads to subsequent edema and weight gain. Metabolically, estradiol affects lipid availability and utilization. Metabolism is centered toward the use of free fatty acids due to the increased lipid

synthesis and enhanced lipolysis in muscle and adipose tissue. Progesterone in the luteal phase is at increased levels and increases the core body temperature and basal metabolic rate. Other effects that were noted by progesterone include a shift in substrate metabolism to more dependence on fat, lower respiratory exchange ratios, lower blood lactate levels at submaximal exercise and higher circulating free fatty acids.[53] Moller-Nielson and Hammar[14] found that women soccer were more prone to traumatic injuries in the premenstrual and menstrual stages as compared to the rest of the cycle ($p < .05$), especially for those women who experienced premenstrual symptoms, such as irritability/irascibility, swelling/discomfort in the breasts and swelling/congestion in the abdomen. They also found that women soccer players using oral contraceptives had a lower rate of traumatic injuries ($p < .05$) compared to those not using them. A review, by Hewett,[54] stated that oral contraceptives significantly affect endurance activities by decreasing VO_2 max. The article also suggested that hormone stabilization through the use of oral contraceptives could possibly aid in maintaining dynamic stability in a female's knee. The study found that female athletes using oral contraceptives had lower impact forces and decreased valgus and varus forces at the knee, increased hamstring to quadriceps strength ratios, increased unilateral stability and decreased knee laxity on average in comparison to those not using oral contraceptives. Dennison et al[55] reported no significant correlation between the use of birth control and ligamentous laxity ($p > .05$).

When compiling the facts together, it is difficult to disregard the possible relationship of hormones and ACL injuries. Six details are important to consider

when making such an extrapolation: 1) the majority of ACL injuries in females appear to occur at a time when hormones are peaking, near peak levels or just beginning to increase in concentration, 2) estrogens and progesterone receptors have been located within the structural composition of the ACL, 3) the greatest amount of ACL laxity corresponds with the peak levels of estrogens and progesterone, 4) hormonal concentrations are interdependent upon one another and a variation of one hormonal concentration will cause a chain reaction of variations among others, 5) females have greater fluctuations in hormonal concentrations than males and 6) females have shown to receive the vast majority of ACL injuries, even though there are fewer female participants overall.

These differences in peripheral laxity, athletic performance and reports of the occurrence of ACL injury provide reasonable evidence to conduct this investigation and future studies. Due to the interaction and interdependence of one hormone to another, all aspects of the menstrual cycle should be evaluated. It is possible that estrogens, progesterone, FSH, LH, estradiol and testosterone may have direct or indirect effects, individually or in combination with one another, on ACL laxity.

CHAPTER III

METHODOLOGY

Subjects

Subjects consisted of twelve college-aged ($\bar{x}_{age} = 24.25 \pm 4.94$ years, $\bar{x}_{height} = 166.16 \pm 5.75$ cm) female volunteers who replied to advertisements through Health, Physical Education and Recreation classes at Old Dominion University. Criterion for approval of subject participation was limited by several factors. Qualifying conditions included: 1) the right leg to be dominant with no pathologies of that knee, 2) minimal risk of injury during the testing period 3) no use of hormonal therapy or supplementation for the prior 3 months, 4) minimal risk of becoming pregnant during the course of the study, 5) a history of eumenorrhea for the year immediately prior to the beginning of testing, 6) the average length of the individuals' menstrual cycles were between 28 to 35 days, 7) a dominant tibia could not be less than 34 cm and 8) a Q-angle less than 15°. All participants had an apparently healthy right, dominant knee to be assessed during the testing sessions. If the dominant knee had an injury that may have caused a change in structural composition, such as the formation of scar tissue after a ligamentous sprain, tendinosis or ACL deficiency, the potential participant was excused from the study. Any subjects receiving an injury to the knee being tested were removed from the study. Subjects were asked to refrain from exercise during the 24 hours prior to each testing session. Subjects were removed from the study if any hormonal supplementation, or therapy, was taken during the testing period. This included, but was not limited to, birth control and was abstained

from for at least three months prior to the start of testing. If a subject was to have become pregnant before or between testing sessions she would have been removed from the study due to the fact that pregnancy would have affected the normal changes in hormonal levels for that individual. All of the subjects were eumenorrheac for one year prior to the beginning of testing. Each of them had a history of 10 or more menstrual cycles within a time frame of one year prior to data collection. All subjects had histories of normal menstrual cycles (28-35 days) prior to and during the testing to ensure that hormonal levels were relatively normal and consistent from subject to subject. The length of the each subject's dominant tibia was 34 cm or longer due to the fact that shorter legs would require a smaller knee arthrometer. The Q-angle (Figure 12) was measured at the time of subjects' consideration for the study. This was defined as the angle between the rectus femoris muscle and the patellar tendon. This was done while the potential subject was standing with the knee and hip fully extended and in neutral rotation. The angle was determined by drawing a line from the anterior superior iliac spine (ASIS) through the midpoint of the patella and from the tibial tubercle, again, through the tibial tubercle. The angle formed was measured using a goniometer.[19]

Once subjects were chosen, according to the responses made on the questionnaire (Appendix A), the protocols were explained. After a verbal agreement to participate in the testing, all subjects were required to sign a consent form (Appendix B), which explained all testing protocols again and provided a written list of all requirements and limitations placed on each individual

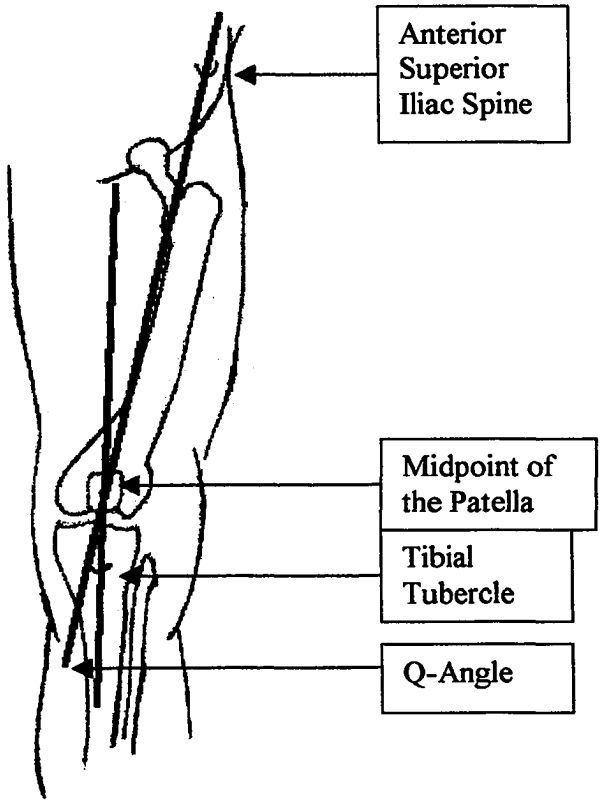


Figure 12: Q-angle measurement using the ASIS, midpoint of the patella and tibial tubercle (Adapted from Magee 1992).[19]

throughout the testing period. The Old Dominion University Human Subjects Review Board approved the consent form.

Instrumentation

All blood samples were drawn at Old Dominion University by one of three testers, who were all trained in phlebotomy. Fourteen milliliters (14 ml) of blood was obtained from the antecubital vein of the forearm in two 7 ml K_3EDTA tubes on three separate occasions, for a total of 42 ml per subject for the entire study. Whole blood was taken from one 7 ml tube to measure hemoglobin and hematocrit. Samples were placed in a centrifuge at 1500 RPM for 10 minutes to extract the plasma. The extracted plasma was then immediately frozen and stored at -60°C until it was ready to be analyzed. Upon completion of all testing sessions for every subject, samples were analyzed via radioimmunoassay at Old Dominion University Human in the Life Science Building to determine hormonal concentrations of estrogens (pg/ml), progesterone (ng/ml), LH (mlU/ml), FSH (mlU/ml), estradiol (pg/ml) and testosterone (ng/ml).

ACL laxity was assessed using the KT-2000 knee arthrometer (MEDmetric, San Diego, CA) (Figures 1,7 and 8) and plain radiographic films (Figure 2). The KT-2000 was used to displace the tibia anteriorly to allow for a measurement to be taken to establish objective readings for laxity at 30 lbs. or 133 N of force. One of two examiners, who had established good intratester reliabilities, took measures for the KT-2000. The initial examiner, who tested the first two subjects, became pregnant and could not test the remaining subjects due

to the radiation exposure. Each subject was tested completely by only one of the testers to avoid differences that may occur regarding intertester reliability. Although intertester reliability was examined and proved to be within a satisfactory range it would have had little effect on the outcome of the sessions since the subjects were monitored over time and not compared to one another. Data was initially analyzed using only the ten subjects tested by the second examiner, who was the primary tester. There was no change in significance levels with the addition of the two subject tested by the first examiner. The plain radiographs were taken at Sentara Norfolk General Hospital (Norfolk, VA) by trained radiology technicians. This method of laxity measurement permitted a more precise and accurate reading by comparing two films taken, respectively, before and after the force was applied. Both methods were measured in millimeters.

An over-the-counter (OTC) Answer Quick and Simple One Step at home ovulation test kit (Gray's Pharmacy, Norfolk, VA) was issued to the subjects so they could monitor themselves for a surge in the LH concentration, which signifies the onset of ovulation. Each subject began monitoring for this occurrence on day 9 of the cycle. This was later changed to day five due to the loss of several subjects. Each kit contained five days worth of testing materials. Each day's supply included one thin plastic strip with a thumb grip, urine collection pad and result window.

Experimental Design

After the experimental group was chosen based on the criterion of the delimitations, all subjects were given 1-3 familiarization periods with the KT-2000 prior to the start of any testing to become acclimated to the application of the device to allow for more accurate readings. Test subjects were then required to track their own menstrual cycle and contact the primary examiner on the first day of their menstrual cycle, which was the first day of the onset of menses. The initial testing session was done within 36 hours of the onset of menses.

Testing began by taking a blood sample (14 ml). All blood samples were drawn at Old Dominion University by one of three examiners, who were all trained in phlebotomy. The site of injection was properly cleaned prior to venipuncture. Fourteen milliliters (14 ml) were obtained from the antecubital vein of the forearm in K_3EDTA tubes. Aliquots of well-mixed whole blood were taken to determine hemoglobin and hematocrit. Samples were placed in a centrifuge machine at 1500 RPM for 10 minutes to extract the plasma. The extracted plasma was immediately frozen and stored at –60°C until it was ready to be analyzed. The sites of injection were properly cleaned and bandaged upon completion of the procedure.

Once the process of venipuncture was complete, a combination measurement of the KT-2000 knee arthrometer and plain radiographs was taken at Sentara Norfolk General Hospital to assess ACL laxity. Patients were placed on the examination table in a supine position to obtain a cross-table, lateral view radiograph of the knee. Using each subject's right, dominant leg, the KT-2000

was applied to the anterior aspect of the tibia, so that it was parallel to the line of the tibial tuberosity as specified by the manufacturer. Velcro straps were applied at the proximal and distal ends of the tibia at points that allowed for alignment with the tibiofemoral joint line as indicated on the device and so the patellar sensor remained in a position over the patella. A third strap was placed around both thighs to maintain a position of neutral hip rotation. The thigh support was placed under the distal femur, causing the resulting position of the knee to be at 20 to 30° of flexion. The thigh support was also placed so that it was only under the right leg to allow the placement of the radiographic film cartridge between the subjects' knees. Tibial rotation was in a neutral position and controlled for with a foot support to reduce external rotation and a towel to block internal rotation. Once in position, the first radiographic picture was taken with no force being exerted upon the knee. Pressure was applied to the patellar sensor pad and maintained at a constant level throughout the use of the device. Force was exerted in an anterior direction with the applied force equaling 133 N. This was indicated by the presence of an audible tone produced by the unit and a point on the X-Y plotter that was attached to the device.

When the 133 N. of force was applied, the radiologists were instructed to take a second film of the patient's knee. To prevent radiation exposure to the examiners' hands, the pressure for the patellar sensor pad was applied to the top of the handle rather than the pad itself. The two films were compared, according to Staubli and Jakob's[17] protocol, to determine the amount of tibial displacement

in millimeters. Each film taken was a lateral radiograph in the anterior position with the radiographic equipment set at a film-to-tube distance of 120 cm.

To measure the anterior position of the tibia with respect to the femur, a line was drawn on the PTC at the midshaft level. The most posterior aspects of the medial tibial plateau and medial femoral condyle were identified at the subchondral bone level. A tangent line was drawn to the most posterior aspect of the tibial plateau and parallel to the PTC. A second parallel line to the PTC was drawn tangent to the posterior aspect of the femoral condyle. The distance between the two tangent lines was considered the anterior displacement of the medial compartment (ADm). This measurement defined the anterior position of the medial tibial plateau with regard to the medial femoral condyle. Anterior displacement was also measured in the lateral compartment (ADl) using the most posterior aspects of the lateral tibial plateau and lateral femoral condyle. Then the sum of the ADm and ADl was divided by two. The arithmetic mean of the measurements for both compartments represented the anterior knee motion by describing the anterior position of the tibia in regard to the femur at the midpoint of the knee (Figure 2). The amount of displacement was determined by subtracting the amount of tibial translation according to the radiograph with no exerted force from the radiograph taken during the application of 133 N.

After the first testing session, the subjects were given an OTC Answer Quick and Simple One-Step at home ovulation test kit (99% accurate). Its purpose was to detect the LH surge in the urine, which normally occurs 24-36 hours before ovulation. Each subject followed the instructions indicated on the

ovulation kit box. The individual was instructed to hold the test stick by the "thumb grip" and place the "urine collection pad" in the urine stream for 10 seconds. The stick was then laid down on a flat surface with the "result window" facing up. A positive test was represented by the appearance of the test line being as dark or darker than the reference line inside the "result window." If the test line was lighter than the reference line, then the LH surge had not occurred and the subject continued to monitor each day until it did. Each kit contained 5 days worth of testing materials. Subjects were instructed to begin monitoring for the first day of ovulation on day 9 of the menstrual cycle. When the subject tested positive (+), the examiners were again notified and tests for hormonal concentrations and ACL laxity were conducted within 36 hours from the time the test was taken using the same procedures as performed on day 1. Subjects were later instructed to begin monitoring themselves for ovulation on day 5 rather than day 9 due to the loss of several subjects who did not test positive using the ovulation kits early in the study. It was believed that several of these subjects did not test positive because they ovulated before the first use of the kits. Regardless of the day in which ovulation falls, a third series of tests was completed on day 23 of the cycle to obtain a reading from the mid-luteal phase.

After all of the laxity measurements were obtained and all blood samples drawn and frozen, the samples were analyzed at Old Dominion University in the Life Science Building to measure levels of estrogens, progesterone, LH, FSH, estradiol and testosterone. The procedure used was commercial radioimmunoassay (ICN Pharmaceuticals, Inc., Costa Mesa, CA). Each kit

contained standards of known concentrations, allowing for the construction of standard curves from which the hormonal concentrations of our unknown samples were drawn. For estrogens, this began by adding .1 ml of plasma and 6 ml of a ethyl acetate: hexane mixture (3:2 ratio) being shaken for 60 seconds. Then 5 ml were withdrawn from the organic phase (top phase) and allowed to evaporate. The sample residue was reconstituted with 2.5 ml of diluent buffer and incubated at room temperature for 30 minutes. Then .5 ml of extracted serum, .1 ml of anti-total estrogen and .1 ml of ^{125}I 17β estradiol being placed in a tube and incubated at room temperature for 90 minutes. Then .1 ml of second antibody was added, incubated for 1 hr. at room temperature and placed in the centrifuge for 15 minutes at 1000 x g. After the tubes were aspirated, they were counted in a gamma counter calibrated for ^{125}I. Standard curves were then plotted on semi-log paper based on known concentration provided with the kit. The total amount of estrogens was determined according to the value that was inversely proportional to the amount of radioactivity.

Evaluating progesterone consisted of 100 µl of the extracted serum and 1 ml of progesterone being placed in a tube and incubated for 2 hours at 37° C. After the tubes were aspirated, they were counted in a gamma counter calibrated for ^{125}I. Standard curves were then plotted on semi-log paper based on known concentration provided with the kit. The amount of progesterone was determined graphically from the curve obtained from the results of the progesterone standards.

Analyzing LH consisted of 100 µl of the extracted serum and 200 µl assay buffer being incubated at 37° C for 45 minutes. The tubes were then aspirated and washed and 300 µl of ^{125}I anti-LH is added. The tubes were incubated again at 37° C for 45 minutes. After the tubes were aspirated and washed a second time, they were counted in a gamma counter calibrated for ^{125}I, which constructed a standard curve to determine LH concentrations.

Determining FSH levels consisted of 100 µl of the extracted serum and 200 µl of assay dilution being incubated 45 minutes at 37° C. The tubes were then aspirated and washed and 300 µl of ^{125}I anti-FSH was added. The tubes were incubated again at 37° C for 45 minutes. After the tubes were aspirated and washed a second time, they were counted in a gamma counter calibrated for ^{125}I, which constructed a standard curve to determine FSH concentrations.

For estradiol, 100 µl of the extracted serum and 1 ml of ^{125}I estradiol were placed in a tube and incubated for 90 minutes at 37° C. Then 500 ml of a second antibody/PEG mixture was added and placed in the centrifuge for 15 minutes at 1000 x g. After the tubes were aspirated, they were counted in a gamma counter calibrated for ^{125}I. Standard curves were then plotted on semi-log paper based on known concentration provided with the kit. The total amount of estradiol was the value that was inversely proportional to the amount of radioactivity.

For testosterone, 25 µl of the extracted serum and 1 ml of ^{125}I testosterone were placed in a tube and incubated at 37° C for 2 hours. After the tubes were aspirated, they were counted in a gamma counter calibrated for ^{125}I. Standard

curves were then plotted on semi-log paper based on known concentration provided with the kit. The total amount of testosterone was inversely proportional to the amount of radioactivity.

Hematocrit and hemoglobin levels were measured to assess plasma volume. Due to changes in fluid retention throughout the menstrual cycle, it was important to estimate plasma volume changes in order to compensate for an accurate reading of relative hormonal levels. Hematocrit levels were simply observed after removal from the centrifuge by comparing the ratio of red blood cells to total blood cells and plasma in order to obtain a percentage of RBCs in the blood. Determining hemoglobin (Hb) required the preparation of Drabkin's reagent. Sodium bicarbonate (1 g), potassium cyanide (.05 g) and potassium ferricyanide (.29 g) were combined in a brown bottle with distilled water so the total contents was equal to 1000ml, forming a clear, yellowish solution. Using the Drabkin's reagent, stock cyanmethemoglobin and Hb with a spectrophotometer allowed for a reading to be taken to determine the mean absorbance of each sample. Hematocrit and hemoglobin were evaluated after each testing sessions using whole blood.

Statistics

Statistical analysis was conducted using SPSS Windows 9 to discuss the significance of the findings. A Pearson Product Moment Correlation Coefficient was used to measure the relationship of ACL laxity to hormonal levels throughout the three phases of the menstrual cycle. A Repeated Measures ANOVA was used

as a post hoc test to determine the relationship for the KT-2000 and radiographic measures of laxity. All ANOVA values were based on the Spericity Assumed values from the within-subjects effects. An α level of .05 was the criterion for statistical significance. An Intraclass Correlation Coefficient (2,1) was used to evaluate intratester reliability for the KT-2000 and radiographic readings.

CHAPTER IV

RESULTS

Subject Attributes

All subjects fell within categories that provided relatively similar demographics (Table 2). Subjects were between the ages of 18 and 34 years old. Height ranged from 156.21 to 176.53 cm and Q-angles were between 5 and 15°. Tibial length had a minimum of 34.5 cm and a maximum of 43 cm. All subjects' weight means for all three sessions ranged from 53.52 to 91.63 kg and showed no significant differences over time ($p = .3614$). Data for individual subjects are located in Appendix C.

The first testing session was conducted between 16 and 34 hours of the onset of menses. The amount of time from the onset of menses to the positive ovulation tests ranged from 182 to 390 hours with the second testing sessions occurring between 9.75 and 34 hours of the positive ovulation tests. Mean times and standard deviations are summarized in Table 3.

The change in percent volume for hematocrit and hemoglobin of each subject was between –18.86 and 7.7% ($p = .012$) for the time period between the onset of menses to ovulation. The change in percent volume between the onset of menses and the mid-luteal phase ranged from –32.29 to 4.98% ($p = .091$). Mean changes in percent volume and standard deviations are included in Table 3. Data for individual subjects are located in Appendix D.

Table 2: Subject Demographics

Attributes	Mean	Standard Deviation
Age (years)	24.25	± 4.94
Height (cm)	166.16	± 5.75
Q-Angle (degrees)	9.75	± 3.57
Tibial Length (cm)	37.92	± 2.84
Weight Menses (kg)	64.86	± 11.14
Weight Ovulation (kg)	65.09	± 11.03
Weight Mid-luteal (kg)	65.03	± 11.10

Table 3: Time Intervals for Testing Sessions and Percent Volume Changes in Hematocrit and Hemoglobin.

Time / %ΔPV	Mean	Standards Deviation
Menses – Test 1 (hours)	23.44	± 7.54
Menses – Ovulation (hours)	270.27	± 64.63
Ovulation – Test 2 (hours)	20.08	± 8.83
Menses – Test 3 (hours)	519.98	± 24.19
Ovulation – Test 3 (hours)	249.50	± 59.28
%ΔPV at Ovulation (Test 2)	-5.46	± 7.70
%ΔPV at Mid-luteal (Test 3)	-4.83	± 10.29

Hormone Concentrations

Descriptive statistics for total estrogens, progesterone, LH, FSH, estradiol and testosterone are summarized in Table 4. A Pearson Product Moment Correlation Coefficient revealed that there were no statistically significant relationships between laxity measurements, of the KT-2000 or radiographic comparisons, and elevated concentrations of hormones. LH was observed in the follicular phase, at the onset of menses, to have a negative correlation of $r = -.628$ ($p = .029$) to the radiographic readings. This was not observed in the coinciding reading from the KT-2000. All other values ranged from significance levels of $p = .076$ to .953 (Table 5). Individual subjects' raw data for hematocrit, hemoglobin, hormonal concentrations and laxity readings at menses, ovulation and the mid-luteal stage are summarized in Appendix E. All hormonal values were obtained from standard curves (Appendix F) based on known standard concentrations provided with each assay kit in order to maintain consistency regarding the half-life of each assay kit.

Laxity Readings

An ICC (2,1) was used to determine the intratester reliability for the KT-2000. The primary tester proved to be reliable over time with an ICC of .9211 ($F_{12,12} = 12.6677$, $p = .0001$). Raw data and descriptive statistics are located in Appendix G. The second tester proved to be reliable in a prior study. Radiographs were read a second time to obtain values to assess intratester

Table 4: Hormonal Descriptive Statistics for the Mean and Standard Deviation at Each Phase.

Phase	Hormone	Mean	Standard Deviation
Follicular	[E] (pg/ml)	71.0	+ 61.0
(Menstration)	[P] (ng/ml)	0.5	± 0.3
	[LH] (mlU/ml)	5.5	± 3.8
	[FSH] (mlU/ml)	9.8	± 2.5
	[E2] (pg/ml)	55.5	± 15.4
	[T] (ng/ml)	0.3	± 0.1
Ovulation	[E] (pg/ml)	200.2	± 161.5
	[P] (ng/ml)	2.2	± 2.4
	[LH] (mlU/ml)	17.3	± 18.9
	[FSH] (mlU/ml)	12.7	± 5.5
	[E2] (pg/ml)	116.5	± 64.4
	[T] (ng/ml)	0.4	± 0.2
Mid-Luteal	[E] (pg/ml)	215.4	± 255.8
(Day 23)	[P] (ng/ml)	15.9	± 8.1
	[LH] (mlU/ml)	4.9	± 2.3
	[FSH] (mlU/ml)	6.2	± 2.0
	[E2] (pg/ml)	111.5	± 40.8
	[T] (ng/ml)	0.3	± 0.1

Table 5: Hormone and Laxity Correlations.

Phase	Hormone	Statistic	KT-2000	Radiograph
Follicular (Menstration)	[E]	r	-0.239	0.057
		p	0.455	0.859
	[P]	r	0.100	0.022
		p	0.756	0.946
	[LH]	r	-0.216	*-0.628
		p	0.501	0.029
	[FSH]	r	-0.404	-0.262
		p	0.193	0.410
	[E2]	r	-0.419	-0.530
		p	0.175	0.076
	[T]	r	-0.044	0.223
		p	0.893	0.486
Ovulation	[E]	r	-0.200	0.192
		p	0.532	0.549
	[P]	r	-0.316	-0.479
		p	0.317	0.115
	[LH]	r	-0.208	-0.419
		p	0.516	0.175
	[FSH]	r	-0.026	-0.269
		p	0.937	0.398
	[E2]	r	-0.113	-0.340
		p	0.726	0.280
	[T]	r	-0.259	0.233
		p	0.416	0.467
Mid-Luteal (Day 23)	[E]	r	0.159	0.335
		p	0.621	0.287
	[P]	r	-0.174	0.157
		p	0.588	0.626
	[LH]	r	0.173	0.044
		p	0.591	0.893
	[FSH]	r	-0.384	-0.394
		p	0.217	0.206
	[E2]	r	0.114	0.019
		p	0.724	0.953
	[T]	r	0.112	0.366
		p	0.730	0.242

* Indicates significance of $p < .05$

reliability for the radiographic comparisons. An ICC (2,1) showed a reliability of .8079 ($F_{34,34} = 5.2057$, $p < .0000$). Raw data appears in Appendix H.

A repeated measures ANOVA was used to determine if there were any statistically significant differences in the KT-2000 or radiographic comparisons over time. There were, however, minute decreases throughout the cycle, as seen in Figure 13. Contrary to the arthrometric readings, radiographic comparisons, although not statistically significant, revealed a slight increase in laxity over time (Figure 13). There was a main effect for testing methodology ($p < .0001$, $F_{1,11} = 74.667$) with an observed power of 100%. There was no main effect over time ($F_{2,22} = .470$, $p = .631$). The observed power was 5%. There was no interaction testing methodology over time regardless of menstrual phase ($F_{2,22} = 1.201$, $p = .320$) with an observed power of 22% (Appendix I). A Pearson Product Moment Correlation Coefficient was used to measure the relationship of the KT-2000 and radiographs over time. There were no statistically significant relationships between the KT-2000 and radiographic interpretation at menstruation ($r = .461$ and $p = .131$), ovulation ($r = .02$ and $p = .951$) or the mid-luteal phase ($r = .236$ and $p = .461$).

Several radiographic readings showed that rotation had occurred when force was applied with the KT-2000. A Repeated Measures ANOVA showed no main effect between lateral and medial displacements of the tibia ($F_{1,11} = .005$, $p = .948$). There was no main effect over time ($F_{2,22} = .471$, $p = .631$). There was also no interaction while comparing lateral versus medial displacements over time

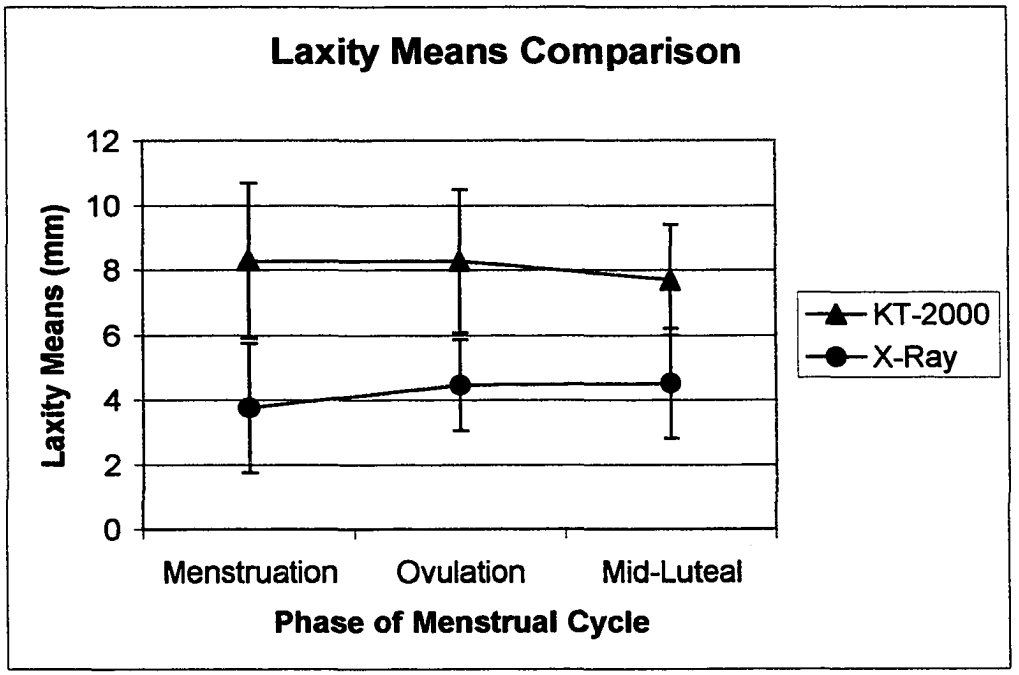


Figure 13: Comparisons of laxity measuring methodology at each phase of the menstrual cycle.

regardless of menstrual phase ($F_{2,22}$ = .1.407, p = .266), (Appendix J). Raw data are contained in Appendix K.

CHAPTER V

DISCUSSION

The purpose of this study was to examine the levels of estrogens, progesterone, LH, FSH, estradiol and testosterone and their relation to ACL laxity throughout the menstrual cycle. Total estrogens and progesterone have both been previously evaluated in regard to ACL laxity.[2] ACL laxity has also been tracked throughout the menstrual cycle in adolescent females.[8] We could not find any literature regarding knee laxity and a possible relationship with fluctuating concentrations of LH, FSH, estradiol or testosterone. Several authors have recommended the further study of these potential associations.[1,2,8,9,47,50,52] The intent of this study was to support or refute former findings regarding estrogens and progesterone and to provide further information on the relationship of LH, FSH, estradiol and testosterone to ACL laxity.

Hormone Concentrations and Laxity Readings

The research hypothesis stated that there was to be a statistically significant increase in anterior tibial translation corresponding with elevated concentrations of estrogens, progesterone and estradiol at the mid-luteal phase therefore inferring an increase in ACL laxity and a higher potential for injury. Contrary to this, the null hypothesis appears to be true according to the collected data. There were no statistically significant changes in the amount of anterior tibial translation as estrogens, progesterone, LH, FSH, estradiol and testosterone levels fluctuated throughout the menstrual cycle.

A retrospective survey (n = 28) by Wojyts et al [1] reported that 13% of ACL injuries occurred between days 1 and 9, 29% occurred from days 10 to 14. The remaining 58% of ACL injuries were received between day 15 and the end of the menstrual cycle. These results were later found to be statistically insignificant[48], but do seem to at least show a trend occurring that contradicts what would be expected to occur in a randomized dispersion 31, 18 and 50%.[49]. Heitz et al[2] found a significant difference ($F_{1,6} = 3.56$, $p = 0.048$) in ACL laxity occurring in conjunction with the approximate time of ovulation and pre-ovulation (days 10 – 13). This increase in ACL laxity coincided with increased estrogens in comparison to baseline levels of estrogens at the onset of menses to days 10 through 13. They also noted a significant difference ($F_{1,6} = 13.41$, $p = 0.006$) in laxity occurring from days 20 to 23 of the mid-luteal stage. This is approximately the same time when estrogens and progesterone peak in comparison to baseline levels at the onset of menses. Karageanes et al[8] found no significant differences in ACL laxity for the left ($p = .9$) or right knee ($p = .7977$) during the course of the menstrual cycle in adolescent female athletes. They did not monitor hormonal fluctuations due to complications regarding obtaining parental consent. To estimate the time of ovulation the examiners counted 14 days back from the onset of menses. Three days were added prior to this time to include an estimated preovulatory phase. To minimize error in this methodology all subjects selected had menstrual cycles lasting 26 to 30 days and menses lasting 4 to 7 days. Heitz et al[2] conducted their second series of tests from days 10 through 13, the approximate time of ovulation. Our study found subjects to begin

ovulating between days 8 and 17. To avoid the possibility of missing ovulation, subjects were asked to use an Answer Quick and Simple At-Home Ovulation Kit beginning on day 5 on their menstrual cycle. A familiarization period was also done with each subject to allow for more accurate readings at the time of testing. This practice session allowed the subjects to become acquainted with the protocol for administering a KT-2000 test before data was collected in order to decrease the amount of apprehension they may have.

Hormone concentrations within this study followed what are considered normal fluctuation patterns for women with regular menstrual cycles (Table 4, Appendix E).[1-5] It is assumed that hormone concentrations for days of the menstrual cycle that were not observed also fell within normal ranges and fluctuations.

The data collected in this study supports the findings of Karageanes et al[8], but refutes Heitz et al[2] in that there were no statistically significant changes found in the amount of anterior tibial translation, as estrogens and progesterone levels rose in the mid-luteal stage. While no statistical differences were observed over time for the use of the KT-2000 or radiographic comparisons, their means show minute changes that contradict one another. The KT-2000's means, although they are not significant, decreased over time. The means observed by the radiographs showed a slight increase in ACL laxity over time, however these were not significant either (Figure 13). This could be the result of tibial rotation or soft tissue mimicking straight anterior tibial translation.

Past research has examined ACL laxity through the use of the KT-1000[8] or KT-2000 by itself.[2] Our methodology used the KT-2000 along with simultaneous radiographs for more objective results. The addition of the X-Y plotter allows the KT-2000 an increase in reproducibility as opposed to the KT-1000. Myrer et al[38] found within tester correlation ranging from .92 to .95 for the KT-2000 and Highenboten et al[40] found a test-retest correlation of .87 for the KT-1000. One reason for the increase may be due to the fact that the examiner does not have to rely completely on the arthrometer's internal tibiofemoral anterior/posterior internal measuring instrument for a laxity reading. More attention may be given to the force being applied and the direction of the tibial translation since the data for the laxity measure can be read after the test from the graph produced by the X-Y plotter. This study added another precision in that a radiograph was taken as the predetermined 133 N of force was applied. This radiograph was compared to another film taken immediately prior while no force was exerted. Using tibial and femoral landmarks, according to the procedure by Staubli and Jakob,[17] allowed for more accuracy regarding total tibial translation rather than permitting possible tibial rotation or soft tissue interference to give an exaggerated amount of laxity.

There was no relationship between the KT-2000 and radiographic values. The X-Y plotter provided laxity readings from all 36 testing sessions that were greater than those obtained by reading the radiographs, with the exception of only one. Staubli and Jakob[17] found no significant correlation between the use of the KT-1000 and radiographic measures for determining the amount of laxity in ACL

intact knees. Their study (n = 16) reported a mean radiographic measure of 4.9 ± 1.6 mm and a mean of 6.2 ± 2.5 mm for the use of the KT-1000. In order to match their study to ours, all laxity measurements were averaged together to obtain an overall mean (n = 36). The present study found a greater difference between the two means (radiographs: 4.23 ± 1.68 mm, KT-2000: 8.08 ± 2.08 mm) with less of a standard deviation in the use of a KT-2000 in comparison to a KT-1000. An independent t-test found there to be reasonable agreement between the data provided the radiographic measures of both studies ($t = -2.83$, $p > .05$). A comparison of the data obtained by the KT-1000 and KT-2000, respectively, found no significant agreement between the studies ($t = 1.21$, $p < .05$). While interpreting the radiographs in our study, it was noted that there was an occasional degree of tibial rotation that occurred when force was applied using the KT-2000. This rotational error is difficult to compensate for manually since a millimeter is such a small unit of measurement. This may also partially explain the fact that the means from the KT-2000 were larger than those seen in the radiographs. It is possible that the arthrometer and X-Y plotter could have been displaying the anterior displacement of one compartment while the other remained in a position of less translation. In cases of internal rotation of the tibia, anterior tibial translation will be decreased in comparison to positions of neutral and external rotation.[37] Another explanation may be the amount of soft tissue between the upper strap of the arthrometer and the tibia. The Velcro strap may be secured tightly below the knee, but with certain individuals larger amounts of soft tissue will compress between the strap and the tibia more when an anterior force is

applied imitating tibial translation. Regardless of the proper application of the Velcro thigh strap and foot support or examiner experience there is still a large amount of room for human error, since the arthrometer measures laxity externally[8] and the measurement taken is to the nearest .25 of a millimeter. This information justifies the importance for the development of a device that has the ability to measure tibial rotation or control for it when manually applying forces to observe ACL laxity. MEDmetric states in their Buyers' Guide[16] that a KT-3000 is being studied that will measure coupled motions of rotation and anterior and posterior displacements.

Several authors, while not all describing the KT-2000 or radiographic measures, all agree that there are a variety of valid manual and arthrometric methods for measuring ACL laxity.[33,40,41] All have varying degrees of reliability and accuracy. While some are more comparable to others, all of these authors are in agreement that each is most reliable within itself. When monitoring laxity over time it is important to use the same testing method and that examiner experience is the factor that most affects reliability. This also appears to be true for the two methodologies used in our study.

Karageanes et al[8] used the KT-1000 to monitor adolescent females (n = 26: 52 knees). The means for the right knee were 4.98 mm in the follicular phase, 5.24 mm in the ovulatory phase and 5.09 mm in the luteal phase. Means for the left knee were 4.51 mm in the follicular phase, 4.43 mm in the ovulatory phase and 4.62 mm in the luteal phase. Standard deviations were not reported, but the right knee presented with significantly more laxity ($p < .05$). While the right to

left comparison itself is not relevant to our study, it is still noted that there were no significant differences in laxity for either knee throughout the menstrual cycle. Heitz et al[2] used the KT-2000 to record ACL laxity in females (n = 7) using readings obtained at menstruation as a baseline (5.6 $\pm$ 1.34 mm). Laxity readings were collected on days 10 through 13 (6.4 + 1.64 mm), a time that ovulation generally occurs, and days 20 through 23 (7.0 $\pm$ 1.66) of the luteal phase. While our study found higher laxity readings using the KT-2000, we also observed higher standard deviations. It is also noted that their readings from the X-Y plotter obtained later in methodology are closer to our overall mean of 8.08 $\pm$ 2.08. The smaller standard deviations may be attributed to the fact that a smaller random sample was used by Heitz et al[2] and that each subject was measured four times for both ovulation and the luteal phase providing multiple readings from the same ACL and the ability to compensate for outliers. Tester experience may also provide a reason for the smaller standard deviation. While both examiners in our study using the KT-2000 proved to have sufficient intratester reliabilities, our primary tester had a limited period of one month to become proficient in its application and execution.

Conclusions

This study showed that there were no significant differences in laxity measurements over time. Both methodologies revealed mean differences of less than 1 mm throughout the menstrual cycle. Therefore no relation between hormonal concentration and ACL laxity could be determined.

This study supports the fact that both the KT-2000 and radiographic measurements are reliable over time if measured within themselves. It is important to note that when monitoring ACL laxity there are different complications with each device. The radiographic comparisons deliver an amount of radiation exposure that limits its repetitive use. The KT-2000 has a large opportunity for human error regardless of tester experience and it is difficult to compensate for soft tissue displacement in larger individuals and for the minute changes in rotation that may occur. These are situations that would appear to only present complications in a research setting. Clinically, these should not cause difficulties in comparing a potentially injured ACL to that of the contralateral side.

Further research is warranted on this topic, with this study producing results that are in accord with Karageanes et al[8] showing no significant changes in ACL laxity but contradict Heitz et al,[2] who found a potential relationship between increased laxity and elevated concentrations of estrogens and progesterone. This is the first known study to examine the possible relationship of LH, FSH, estradiol and testosterone to ligament laxity. The results show there is no relationship involving any of theses hormones.

Recommendations

The following are recommendations proposed for further research to both improve and expand upon this study. A larger sample group would be a better representation of the total female population. Our search for subjects was greatly

restricted by two features. First the use of birth control was an exclusionary criteria due to the fact that it not only alters hormonal levels in comparison to what may be considered normal for an individual but it completely inhibits ovulation. This use of hormonal therapy calls for further investigation as well to determine the effects of such medications as birth control. Second was that testing sessions occurring at the onset of the menstrual cycle and at ovulation were difficult to schedule with the subjects given such short notification.

More testing sessions per subject would provide more accurate results as to what changes are actually occurring over time. In the case of this study, radiation exposure from the radiographs became the limiting factor. It is also advisable to track women over time to note when ACL or other ligamentous injuries are occurring within the menstrual cycle. The examination of other ligamentous structures could also be a source of research in regards to the possible associations with the menstrual cycle. It would be prudent to evaluate those ligaments containing specific hormone receptors.

Monitoring for ovulation should occur at an earlier time frame than what most ovulation kits suggest and what this study initially implemented. The loss of subjects due to the fact that ovulation had occurred before they began to monitor themselves was another factor limiting the progress of the study. This was compensated for midway through the study by changing the day to begin monitoring for ovulation from day 9 to day 5.

The final recommendation regards the development of a better and more valid arthrometric device. While studies have shown the KT-2000 to be reliable

over time, there are still many chances for human error with its use. This includes alignment and positioning of the feet, hips and thigh support and placement of the KT-2000 itself. It would also be beneficial to develop a device that could detect more minute changes in displacement. Clinically and practically, a millimeter may be a sufficient measurement and the KT-2000 has proven to be effective in that setting. But scientifically, noting more precise changes may be advised. The more objective the device, the more reliable the data. Further research is warranted to determine what degree of laxity difference will affect an individual's proneness to injury. Compensation for tibial rotation is also advisable due to the fact that readings taken from the X-Y plotter for the KT-2000 more closely resembled displacements of lateral or medial compartments while the opposite side showed smaller values or even a decrease in laxity.

APPENDIX A

INCLUSIONARY CRITERION QUESTIONNAIRE

Name (for records only):_____

Last 4 digits of SSN# (for identification purposes):_____

Phone Number:_____

Age:_____ Height:_____

Weight:_____ Dominant Leg:..... R L

Have you ever injured your dominant knee before?..... Y N

If yes, explain:_____

_____

How many times do you exercise in a week?_____

Are you currently taking any form of hormonal therapy or

supplement (ie. birth control)?..... Y N

Have you taken any form of hormonal therapy or supplementation

within the past 3 months?..... Y N

Are you currently pregnant?..... Y N

What is your current method of birth control?_____

What is the average length of your menstrual cycle (in days)?_____

Have you had at least 10 menstrual cycles with in the past year?.....Y N

Below Line For Examiners Only

Has the subject read and signed the consent form?..... Y N

Length of tibia:_____ Q-angle:_____

Does the applicant fit the criterion for inclusion in the study?..... **Y** **N**

APPENDIX B

INFORMED CONSENT DOCUMENT

Old Dominion University Darden College of Education
Department of Exercise Science, Physical Education and Recreation
Masters Thesis for John C. Roberts

Title of Research: Effects of hormonal changes throughout the menstrual cycle on joint laxity in females

Primary Investigator: John C. Roberts, ATC

Assisting Investigators: Bonnie L. VanLunen, PhD, ATC
Elizabeth A. Dowling, PhD
J. David Branch, PhD

Description of Research:

It is well known that hormonal levels are in constant fluctuation throughout the menstrual cycle. Several hormones have been given special attention, in that they are said to play a role in increasing the amount of laxity in the pelvis during pregnancy in order to allow passage for a newborn baby. It has been suggested that some of these same hormones, in addition to others, may also have the same effect on other ligaments throughout the body. The anterior cruciate ligament (ACL) of the knee is considered in this study. This is important information for women participating in high intensity levels of activity and sports, because as the amount of laxity in a ligament increases, so does its chance for injury. This study will monitor the levels of estrogens, progesterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol and testosterone and their relation to the laxity of the ACL throughout the menstrual cycle. The purpose is to determine whether individual hormones play a role in increasing ACL laxity.

You are agreeing to participate as a subject in this study. If selected to participate, you will be participating in a study observing hormonal fluctuations of

Subject's Initials_____

estrogens, progesterone, LH, FSH, estradiol and testosterone throughout the menstrual cycle as they relate to the laxity of the ACL in the knee. Prior to being selected you will complete a screening questionnaire to determine your eligibility to participate. If selected, you will undergo testing for ACL laxity and hormonal levels. The blood collection will be done at in the HPER Human Performance Laboratory at Old Dominion University. Ten milliliters (10 ml) of blood will be drawn from your arm by one of three trained phlebotomists at each session, so the total amount of blood withdrawn throughout the entire testing will be 30 ml. The testing for ACL laxity assessment will be conducted at the Norfolk Sentara Medical Hospital Radiology Lab. You will be provided with the appropriate directions, once you have decided to participate. The ACL laxity is measured using a knee arthrometer (KT-2000) that will apply 30 lbs. or 133 N of force to your lower leg. This instrument will translate your tibia (lower leg) forward on you femur (thigh bone). A plain film radiograph (x-ray) will be taken while the device is strapped to your leg before and after the force is applied.

This procedure will be done 3 times throughout one menstrual cycle. The first session is the first day of your menstrual cycle. You will be required to contact the primary investigator immediately so that you can be tested within the next 24 hours. The second session will be the first day of ovulation. You will monitor yourself for this occurrence through the use of an at-home-ovulation kit that will be issued to you. Each kit has testing materials to last for 5 days. You will begin testing yourself on day 9 of your menstrual cycle. It is again your responsibility to contact the primary investigator immediately so that you can be tested within 24-36 hours of a positive test. The third session will occur on day 23 of your menstrual cycle and will follow the same protocol as other testing sessions.

Subject's Initials_____

Exclusionary Criteria:

You must be college-aged (18-35 years of age) and not currently participating in any form of competitive athletics. To the best of your knowledge you cannot present with knee pathologies of your dominant leg, and that being your right. You cannot currently be using hormonal therapy or supplementation (ie. birth control) and have minimal risk of becoming pregnant during the course of the study. You must have had 3 consecutive normal menstrual cycles immediately prior to the start of the study that are not due to hormonal therapy or supplementation and at least 10 within the past year. The length of your tibia cannot be less than 34 cm., because that would require a smaller arthrometric device. You cannot have a quadriceps angle (Q-angle) greater than 15°.

Risks and Benefits

When taking radiographs there is a slightly increased amount of radiation exposure. This total amount of background exposure is considered to be equal to living in Norfolk for 9 months. The precise risk from such exposure is not known, but is thought to be small. There is the possibility of bruising and/or soreness due to the collection of blood. There is the possibility of infection. Collection of samples, by trained phlebotomists, will be done under aseptic conditions to minimize these risks.

The benefit to you is that you will each know your own respective amount of laxity within your knees. This serves as a general predictor as to the chance of injury to your anterior cruciate ligament.

Cost and Payments:

Your efforts in this study are voluntary. Any information obtained during the course of this testing will provided to you upon your request based on your willingness to comply and complete the entire study.

Subject's Initials_____

Confidentiality:

Any information obtained from this study, including questionnaires, medical history and laboratory findings, will be kept confidential. Only the primary investigator and the assistant investigators will have access to your files, in which you will be assigned a random identification number. The data derived in this study could be used in reports, presentations and publications, but you will not be referred to by more than your identification number. However, your records may be subpoenaed by court order or may be inspected by federal regulatory authorities.

Withdrawal Privilege:

You are free to refuse to participate in this study or to withdraw at any time. Should you choose to withdraw, your decision will not adversely affect your relationship with this institution or cause a loss of benefits to which you may be otherwise entitled. If you decide to withdraw, you agree to undergo all trial evaluations necessary for your safety and well being as determined by the investigators. The investigators reserve the right to withdraw your participation at any time throughout this investigation if they observe any contraindication to your participation.

Compensation for Illness or Injury:

In the event of illness or injury resulting from the research protocol, no monetary compensation will be made, but immediate emergency medical treatment, including first aid by the investigators, will be made available to you. If any injury should result from your participation in this study, Old Dominion University does not provide insurance coverage, free medical care or any other compensation. If you suffer injury as a result of your participation in this study, contact the primary investigator, John C. Roberts, ATC, (xxx-xxxx), who will review the matter with you.

Subject's Initials_____

Voluntary Consent:

I certify that I have read the preceding sections of this document, or it has been read to me; that I understand the contents; and that any questions I have pertaining to the research have been, or will be answered by John C. Roberts, ATC (xxx-xxxx). If I have any concerns I can express them to Patricia Pleban, Chairperson of the Old Dominion University Institutional Review Board (xxx-xxxx). A copy of this informed consent form has been given to me. My signature below indicates that I have freely agreed to participate in this investigation.

_____

Subject's Printed Name

_____ _____

Subject's Signature Date

_____ _____

Witness's Signature Date

Subject's Initials_____

Investigator's Statement:

I certify that I have explained, to the subject whose signature appears on the previous page, the nature and purpose and the potential benefits and possible risks associated with participation in this study. I have answered any questions that have been raised by the subject and have encouraged her to ask additional questions at any time during the course of this study.

John C. Roberts, ATC

_____ _____

Investigator's Signature Date

Subject's Initials_____

APPENDIX C

Table 6: Raw Data for Individual Subjects' Demographics.

Attributes	Age (yrs)	Height (cm)	Q-Angle (degrees)	Tibial Length (cm)	Mass at Menses (kg)	Mass at Ovulation (kg)	Mass at Day 23 (kg)
Subject 1	23	156.21	10	34.5	91.40	91.40	91.63
Subject 2	34	160.02	5	38.5	61.69	61.24	61.69
Subject 3	20	167.64	9	41.0	81.87	81.65	81.87
Subject 4	26	170.18	13	34.5	70.31	70.76	69.63
Subject 5	22	170.18	5	37.5	62.60	63.50	63.50
Subject 6	21	162.56	11	40.0	53.52	54.43	54.43
Subject 7	18	167.64	15	40.0	57.15	57.49	56.25
Subject 8	22	172.72	11	43.0	60.56	60.33	61.24
Subject 9	23	176.53	10	39.5	61.24	62.26	62.14
Subject 10	27	165.10	15	35.5	58.97	59.42	59.42
Subject 11	22	162.56	5	35.0	56.70	56.02	56.25
Subject 12	33	162.56	8	36.0	62.37	62.60	62.26
Average	24.25	166.16	9.75	37.9	64.86	65.09	65.03
SD	4.94	5.75	3.57	2.8	11.14	11.03	11.10

APPENDIX D

Table 7: Raw Data for Individual Subjects' Time Intervals for Testing Sessions and Percent Volume Changes in Hematocrit and Hemoglobin.

Time/%ΔPV	Menses – Test (hrs)	Menses – Ovulation (hrs)	Ovulation – Test (hrs)	Menses – Day 23 (hrs)	Ovulation – Day 23 (hrs)	%ΔPV – Ovulation	%ΔPV – Day 23
Subject 1	19.50	225.50	10.50	523.25	297.25	-1.63	4.98
Subject 2	16.00	390.00	9.75	544.50	154.50	-2.91	-1.62
Subject 3	20.00	201.00	15.00	523.00	322.00	0.64	2.39
Subject 4	17.00	221.00	34.00	519.00	298.00	-6.73	-2.52
Subject 5	16.50	182.00	26.00	518.00	336.00	-18.86	-4.89
Subject 6	26.25	216.00	16.00	506.00	290.00	-16.70	-14.81
Subject 7	35.00	329.75	29.25	541.00	211.25	-4.56	2.83
Subject 8	17.00	353.00	34.00	531.00	178.00	0.64	-8.07
Subject 9	35.50	296.00	13.50	538.25	242.25	-3.08	-6.33
Subject 10	24.50	262.00	21.50	451.50	189.50	1.41	-0.22
Subject 11	20.00	293.00	19.50	525.25	230.25	3.71	2.56
Subject 12	34.00	274.00	12.00	519.00	245.00	-13.64	-32.29
Average	23.44	270.27	20.08	519.98	249.50	-5.46	-4.83
SD	7.54	64.63	8.83	24.19	59.28	7.70	10.29

APPENDIX E

Table 8a: Individual Subjects' Raw Data for Hematocrit and Hemoglobin.

	Hct (%) Menses	Hct-CV Menses	Hb Menses	Hb-CV Menses	Hct (%) Ovulation	Hct-CV Ovulation	Hb Ovulation	Hb-CV Ovulation	Hct (%) Day 23	Hct-CV Day 23	Hb Day 23	Hb-CV Day 23
Subject 1	38.50	0.00	13.61	0.18	39.5	1.07	13.61	0.36	37.1	1.12	13.26	0.55
Subject 2	38.10	1.30	13.78	1.44	39.9	0.71	13.99	2.13	39.1	0.36	13.19	1.30
Subject 3	38.80	0.73	12.45	0.79	34.7	1.02	13.22	0.74	39.6	1.61	12.00	5.77
Subject 4	39.10	0.36	12.45	0.39	41.6	0.34	12.80	0.19	40.3	2.81	12.52	0.78
Subject 5	36.60	0.00	11.51	0.21	40.8	1.21	13.29	0.00	36.7	2.50	12.14	2.05
Subject 6	37.30	3.03	12.84	0.57	43.5	1.30	13.89	1.06	42.8	0.99	13.75	0.89
Subject 7	36.00	0.98	11.05	0.22	36.6	0.58	11.47	1.95	35.6	0.40	10.81	1.36
Subject 8	39.80	0.18	12.56	0.97	38.9	0.18	12.66	2.35	40.9	0.69	13.40	0.91
Subject 9	36.20	0.00	11.65	0.00	38.0	1.12	11.68	1.26	39.0	0.36	11.89	1.24
Subject 10	32.10	0.00	11.79	1.04	35.9	3.94	11.26	2.20	37.7	0.19	10.67	0.00
Subject 11	37.30	0.38	11.16	0.44	36.2	0.78	10.95	0.45	38.7	1.28	10.67	1.38
Subject 12	21.60	1.64	9.24	0.80	32.0	0.44	9.27	1.32	32.5	0.65	11.75	0.00
Average	35.95		12.01		38.1		12.34		38.3		12.17	
SD	4.94		1.24		3.2		1.43		2.7		1.09	

Table 8b: Individual Subjects' Raw Data for Hormonal Concentrations at Menses.

Menses	E (pg/ml)	E-CV	P (ng/ml)	P-CV	LH (mIU/ml)	LH-CV	FSH (mIU/ml)	FSH-CV	E2 (pg/ml)	E2-CV	T (ng/ml)	T-CV
Subject 1	210	37.0	**0.2**	0.1	5.0	2.0	10.0	1.7	51	5.0	0.5	0.1
Subject 2	110	0.9	**0.2**	0.3	17.0	30.0	11.0	4.5	82	2.2	0.4	0.2
Subject 3	73	4.3	0.6	1.4	4.7	2.3	6.7	5.9	62	4.9	0.6	8.0
Subject 4	**10**	0.7	0.2	0.4	4.4	13.0	8.5	6.5	50	1.6	**0.2**	1.0
Subject 5	26	2.5	0.5	1.2	3.7	0.5	9.1	0.0	46	0.0	**0.2**	0.5
Subject 6	**10**	1.4	**0.2**	1.5	4.1	4.8	8.6	3.6	31	1.4	**0.2**	0.8
Subject 7	29	2.8	0.4	0.9	2.6	2.4	6.7	4.1	31	3.3	**0.2**	0.9
Subject 8	100	43.0	0.5	2.7	5.4	4.5	11.0	6.6	65	0.0	**0.2**	0.7
Subject 9	105	0.3	0.3	0.9	6.4	1.0	12.0	2.9	73	0.7	0.3	0.4
Subject 10	29	1.7	1.1	3.3	4.4	0.9	7.5	1.8	60	0.3	0.4	0.9
Subject 11	25	1.8	0.6	3.7	3.2	3.7	15.0	3.6	50	3.2	**0.2**	1.2
Subject 12	125	20.0	0.6	0.4	5.4	2.9	12.0	4.5	65	2.0	**0.2**	1.1
Average	71		0.5		5.5		9.8		56		0.3	
SD	61		0.3		3.8		2.5		15		0.1	

† Bold values represent those above or below the respective obtained standard curve.

Table 8c: Individual Subjects' Raw Data for Hormonal Concentrations at Ovulation.

Ovulation	E (pg/ml)	E-CV	P (ng/ml)	P-CV	LH (mIU/ml)	LH-CV	FSH (mIU/ml)	FSH-CV	E2 (pg/ml)	E2-CV	T (ng/ml)	T-CV
Subject 1	420	0.6	1.1	1.0	29.0	2.0	21.0	6.5	210	6.7	0.9	1.8
Subject 2	160	3.5	6.8	2.9	14.0	4.8	12.0	2.7	145	3.5	0.3	1.0
Subject 3	75	9.7	0.4	1.8	6.1	3.0	7.2	6.5	80	0.2	0.6	0.2
Subject 4	95	0.7	**0.2**	0.4	4.7	6.3	6.3	1.0	120	3.0	**0.2**	0.7
Subject 5	50	0.6	2.0	1.5	20.0	13.0	16.0	2.8	165	2.4	**0.2**	1.4
Subject 6	125	1.7	0.3	2.1	4.2	1.7	8.1	4.2	62	3.9	**0.2**	0.2
Subject 7	135	3.4	6.8	1.0	6.7	0.1	5.9	6.7	45	2.5	**0.2**	1.2
Subject 8	510	68.0	1.5	2.9	19.0	7.3	15.0	19.0	87	1.9	0.6	1.2
Subject 9	340	21.0	1.2	0.2	72.0	1.5	21.0	72.0	250	0.9	0.4	3.4
Subject 10	85	2.6	4.3	3.6	6.3	2.3	8.5	6.3	68	3.1	0.5	2.3
Subject 11	360	5.4	0.2	3.3	8.5	6.9	17.0	8.5	61	0.5	**0.2**	1.9
Subject 12	47	1.3	1.7	0.9	17.0	3.9	14.0	17.0	105	4.4	**0.2**	1.6
Average	200		2.2		17.3		12.7		117		0.4	
SD	162		2.4		18.9		5.5		64		0.2	

† Bold values represent those above or below the respective obtained standard curve.

Table 8d: Individual Subjects' Raw Data for Hormonal Concentrations at Day 23.

Day 23	E (pg/ml)	E-CV	P (ng/ml)	P-CV	LH (mIU/ml)	LH-CV	FSH (mIU/ml)	FSH-CV	E2 (pg/ml)	E2-CV	T (ng/ml)	T-CV
Subject 1	245	2.8	4.5	0.3	7.0	8.1	6.0	16.0	140	4.5	0.4	0.3
Subject 2	145	1.5	2.1	3.5	8.8	0.8	11.0	3.9	105	1.9	0.3	3.1
Subject 3	170	3.2	18.0	1.2	3.8	0.9	4.3	6.2	160	2.4	0.6	0.1
Subject 4	100	1.8	12.0	2.6	2.3	10.0	5.4	5.7	98	2.6	**0.2**	1.6
Subject 5	170	26.0	12.0	0.2	6.4	5.6	4.2	0.9	75	2.3	**0.2**	0.2
Subject 6	250	0.7	9.8	2.6	4.9	2.2	7.4	0.5	68	0.0	**0.2**	5.5
Subject 7	56	1.7	22.0	1.0	2.0	22.0	5.1	2.0	75	0.0	**0.2**	1.5
Subject 8	**1000**	52.0	19.0	4.9	7.2	1.1	5.3	2.3	200	6.3	0.4	1.3
Subject 9	190	0.8	16.0	0.2	2.5	7.7	5.4	5.4	135	2.7	0.2	5.6
Subject 10	68	4.6	30.0	0.3	4.1	5.9	5.6	5.2	69	6.1	**0.2**	4.7
Subject 11	135	4.4	23.0	1.8	6.9	8.2	8.7	1.6	115	3.6	0.3	0.6
Subject 12	56	0.0	22.0	2.1	2.3	1.7	6.0	5.9	98	2.6	**0.2**	0.7
Average	215		15.9		4.9		6.2		112		0.3	
SD	256		8.1		2.3		2.0		41		0.1	

† Bold values represent those above or below the respective obtained standard curve.

Table 8e: Individual Subjects' Raw Data for Laxity Readings

	X-Ray (mm) Menses	KT (mm) Menses	X-Ray (mm) Ovulation	KT (mm) Ovulation	X-Ray (mm) Day 23	KT (mm) Day 23
Subject 1	7.375	10.000	6.000	8.500	5.625	10.000
Subject 2	0.125	7.000	2.625	5.000	2.000	4.750
Subject 3	5.000	7.000	5.000	8.250	6.250	7.500
Subject 4	4.875	8.750	6.625	8.000	3.375	6.500
Subject 5	6.250	12.750	2.250	13.250	4.625	10.500
Subject 6	3.125	11.250	5.125	11.000	6.625	8.500
Subject 7	3.750	6.500	4.500	9.250	1.875	7.500
Subject 8	2.250	8.250	5.125	7.500	5.875	7.750
Subject 9	2.000	5.750	2.625	6.500	3.125	8.000
Subject 10	3.250	10.750	3.750	6.250	6.000	5.500
Subject 11	4.125	6.000	5.000	8.750	3.250	9.000
Subject 12	2.875	5.500	4.750	7.000	5.378	6.750
Average	3.750	8.292	4.448	8.271	4.500	7.688
SD	1.959	2.409	1.373	2.209	1.696	1.693

APPENDIX F

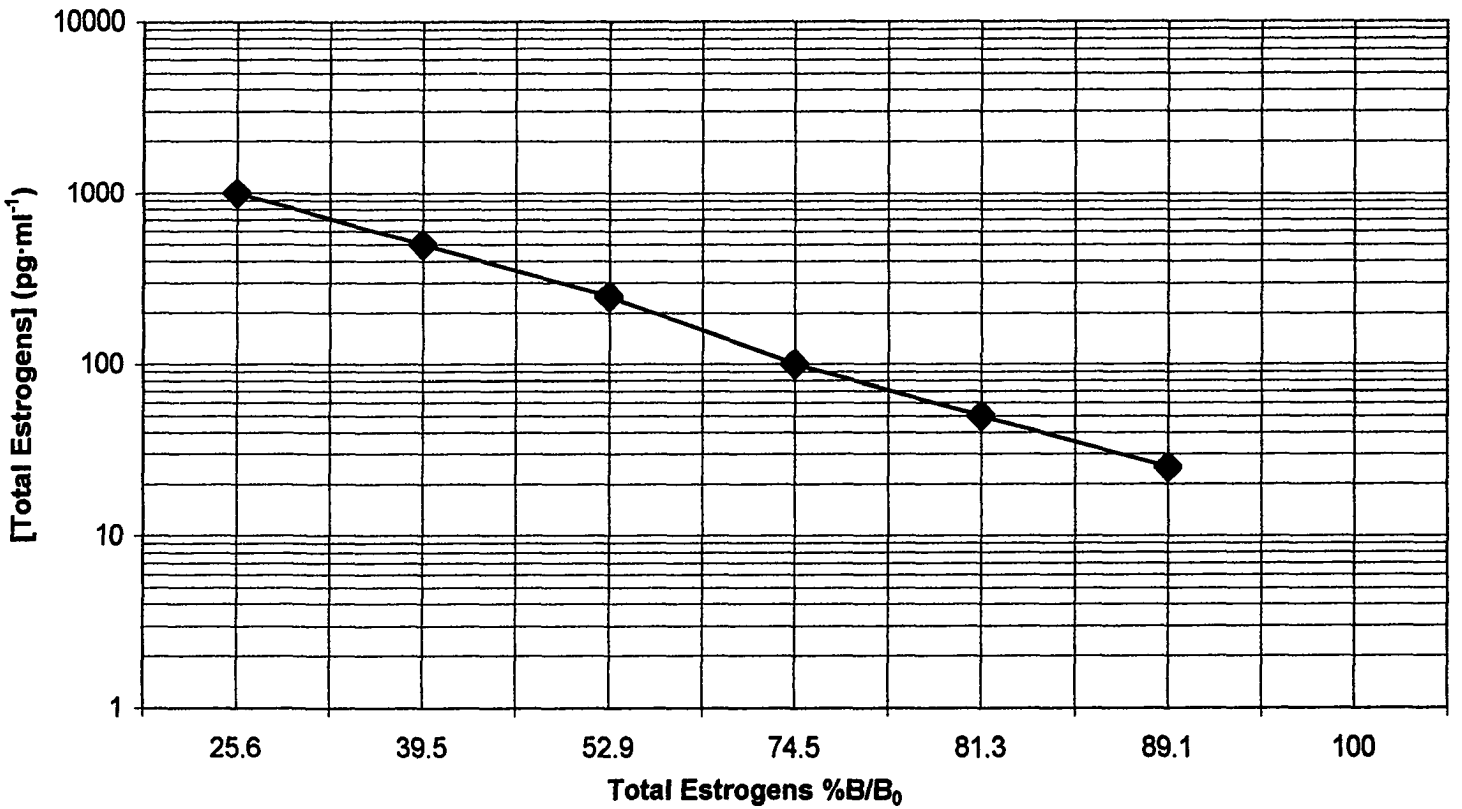


Figure 14a: Standard curve for estrogens (03/08/01).

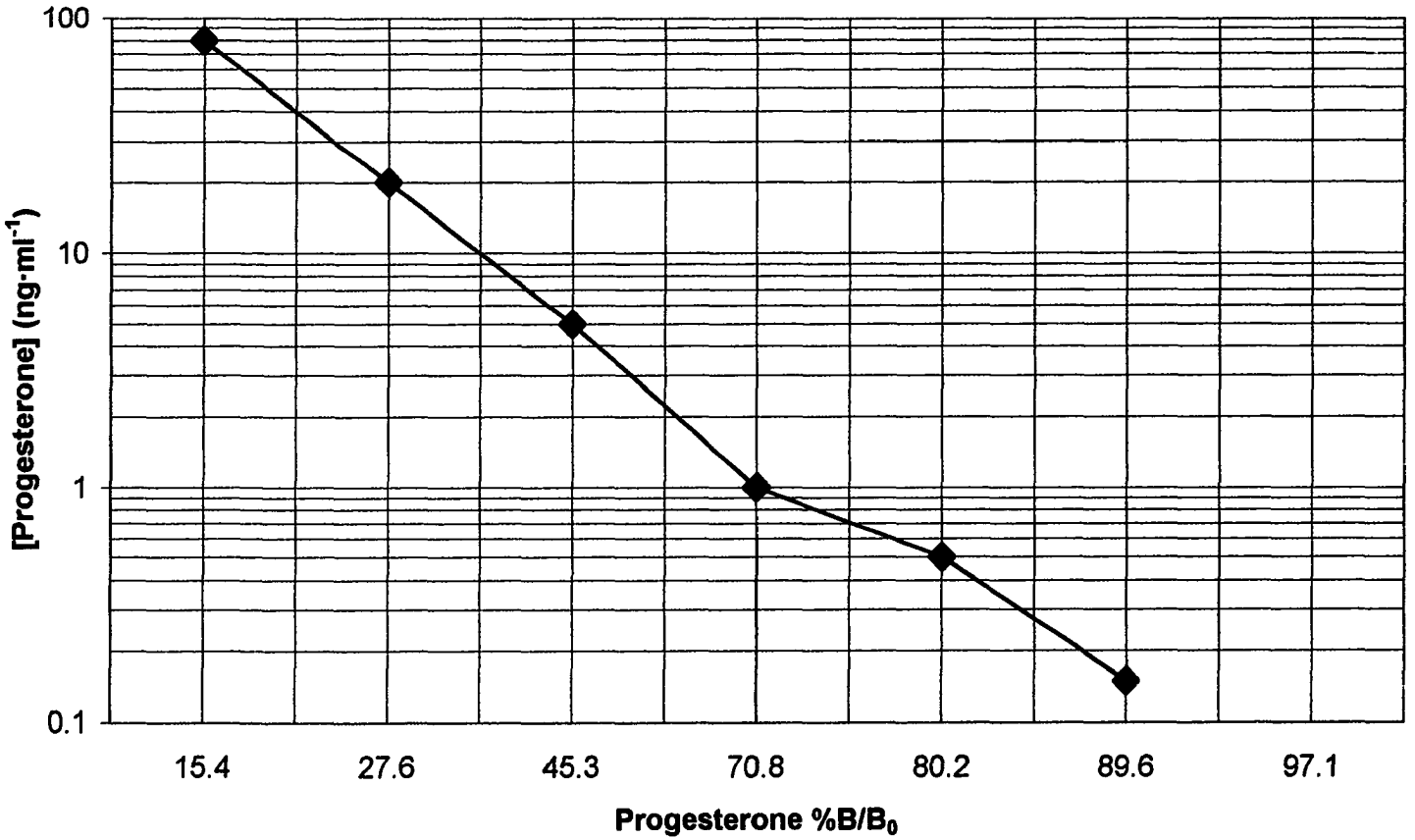


Figure 14b: Standard curve for progesterone (03/07/01).

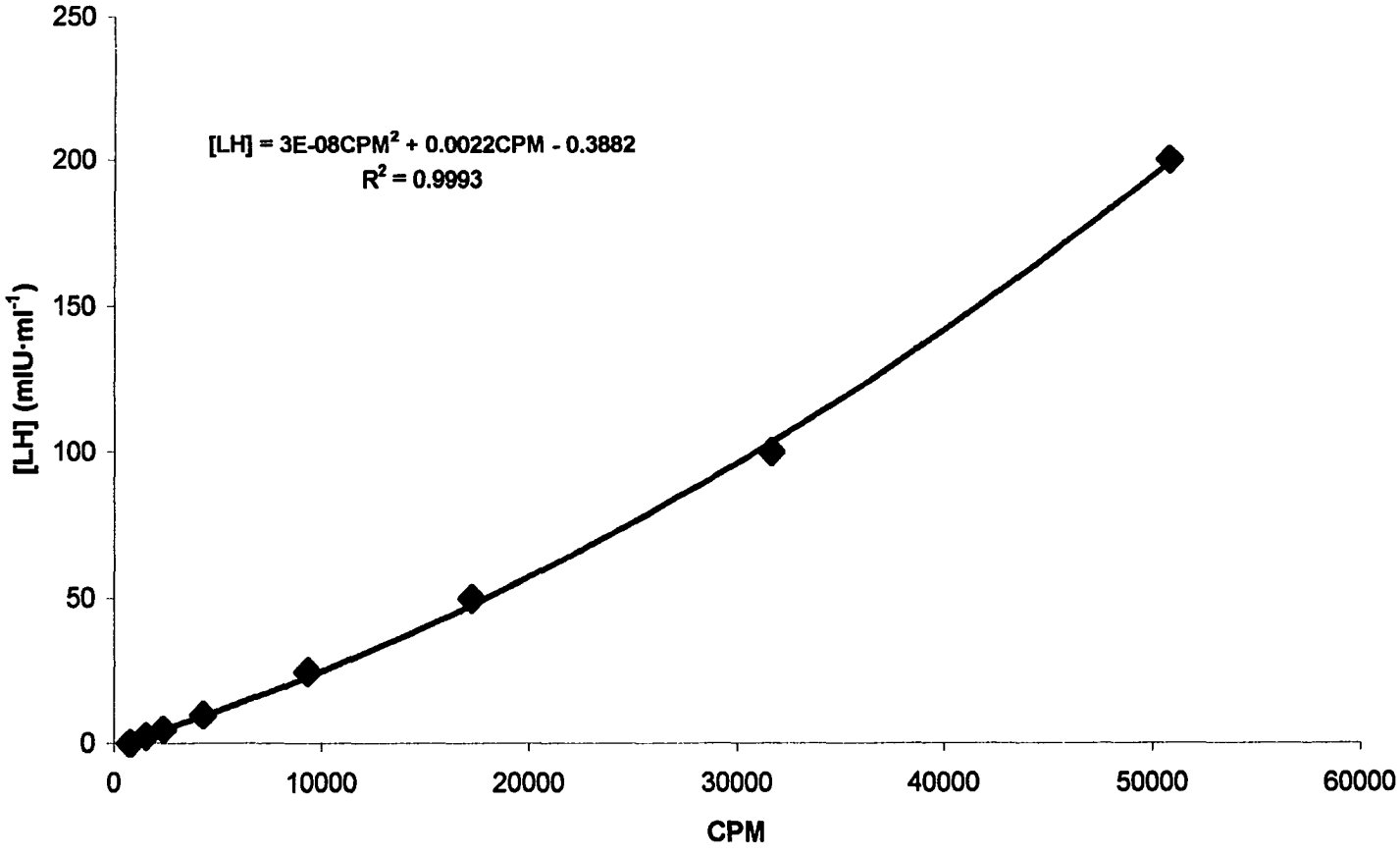


Figure 14c: Standard curve for LH (03/06/01).

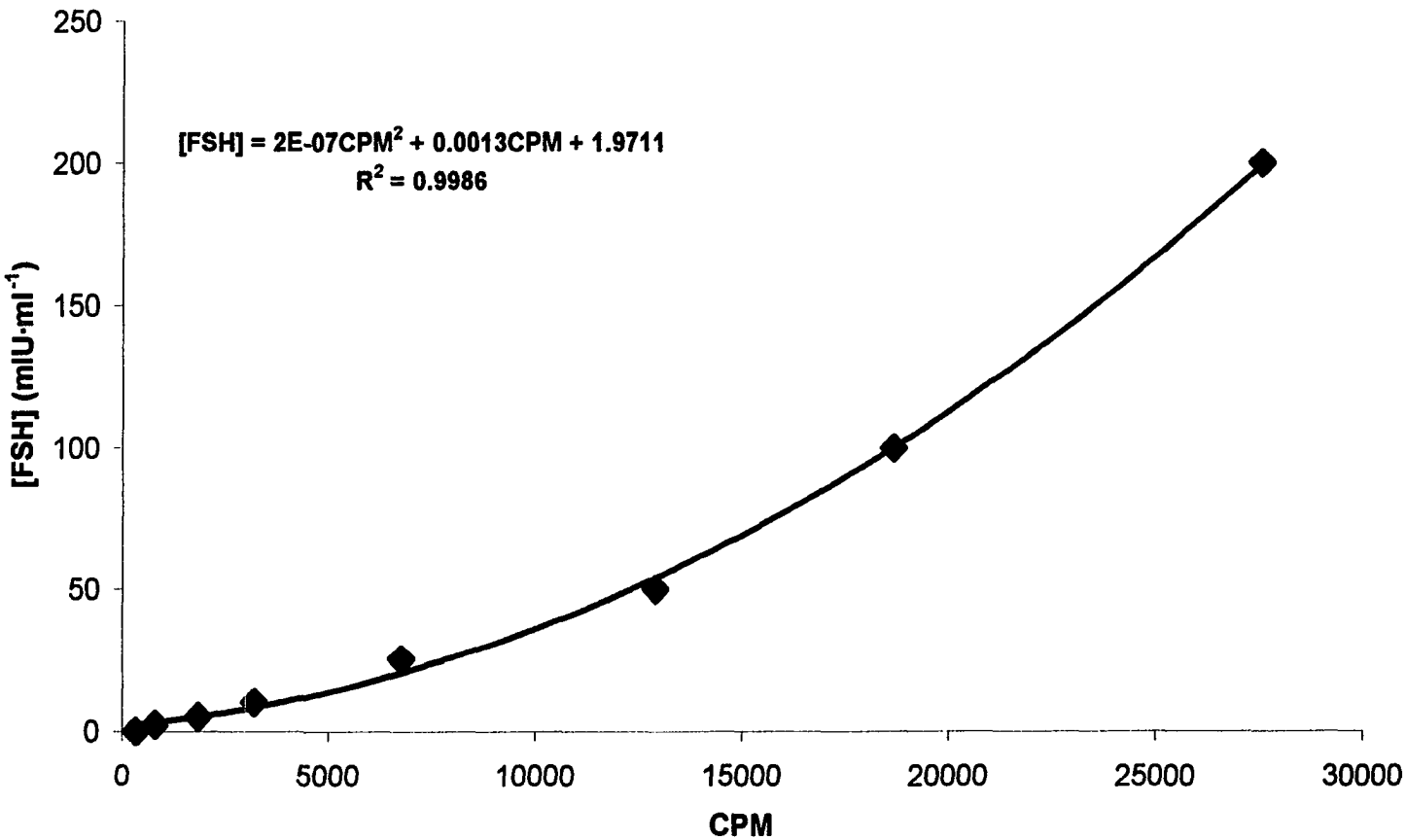


Figure 14d: Standard curve for FSH (03/06/01).

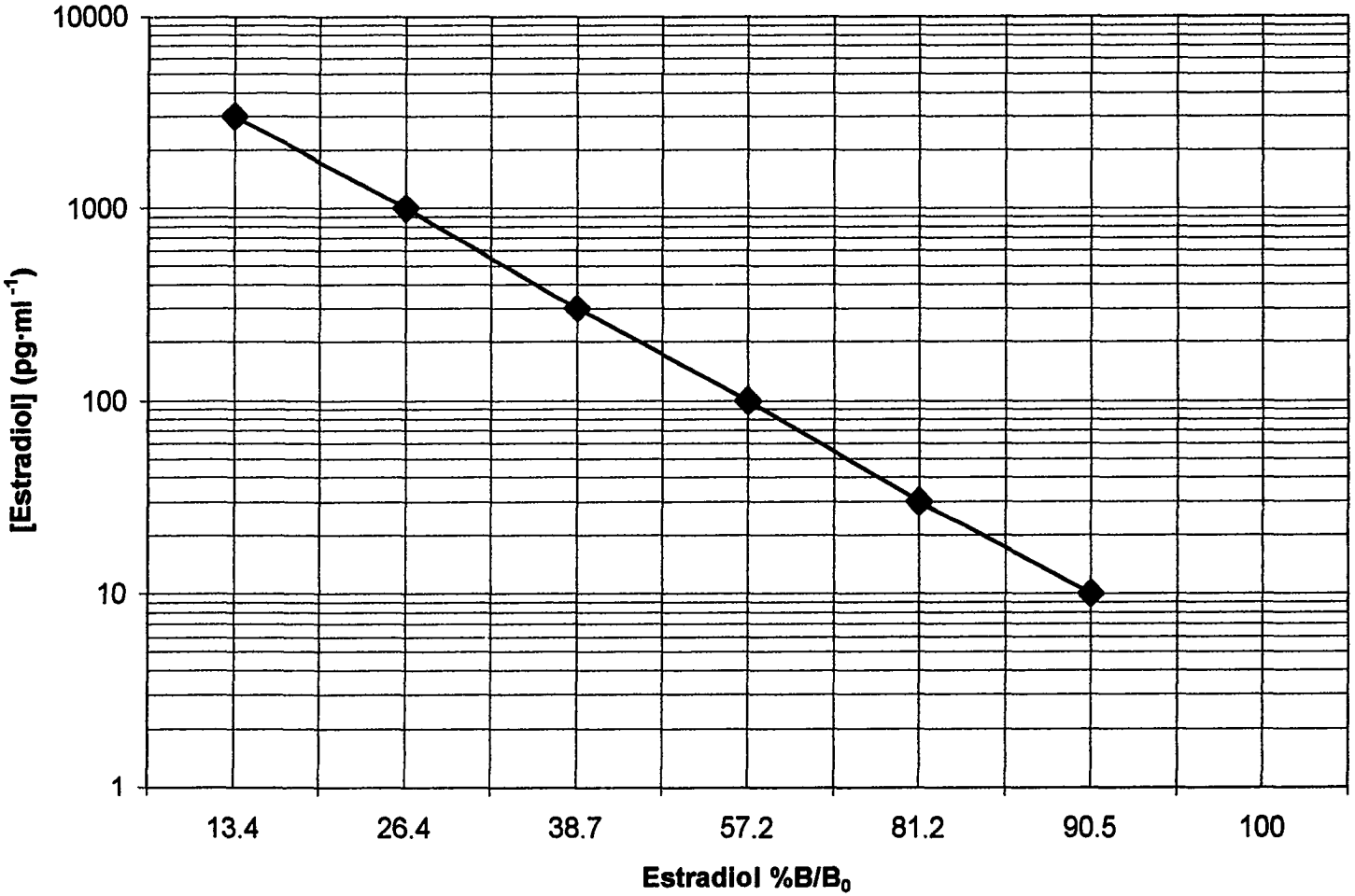


Figure 14e: Standard curve for estradiol (03/0701).

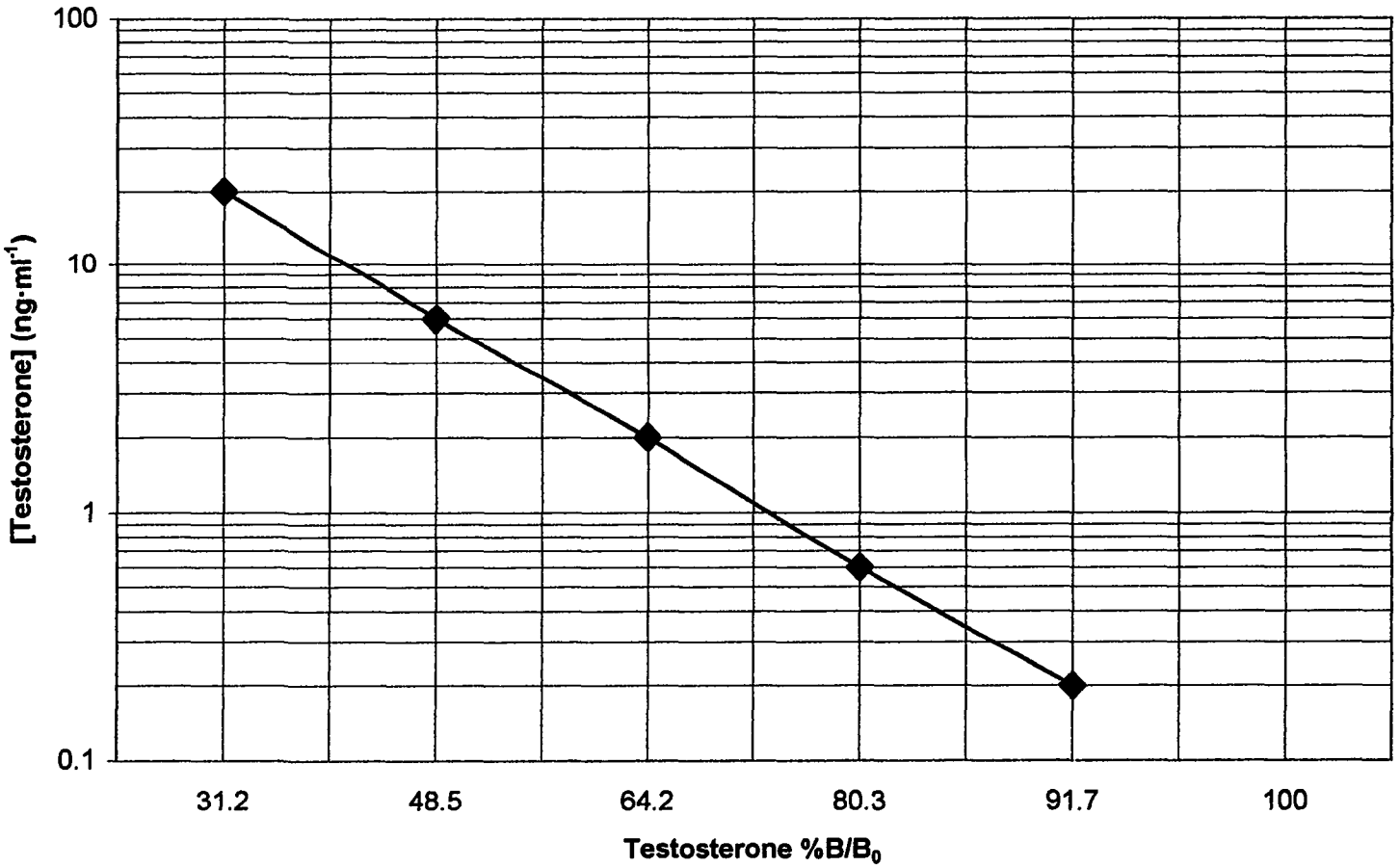


Figure 14f: Standard curve for testosterone (03/08/01).

APPENDIX G

Table 9: Raw Data for KT-2000 Intratester Reliability for Primary Examiner

Session 1	Trail 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Trial 4 (mm)	Average (mm)	SD
ID 1	1.75	2.25	4	2.75	2.69	0.97
ID 2	5.25	4.75	5		5.00	0.25
ID 3	4.25	4.5	5		4.58	0.38
ID 4	8.75	8.75	9.25		8.92	0.29
ID 5	6.25	6	6.5	6.75	6.38	0.32
ID 6	6	5.75	5.5	6	5.81	0.24
ID 7	9.75	9.75	10.25		9.92	0.29
ID 8	8.5	9	9.28		8.93	0.40
ID 9	8.25	7.75	8.25		8.08	0.29
ID 10	7.75	6.25	6.5		6.83	0.80
ID 11	1.5	2	1.75		1.75	0.25
ID 12	5.5	6	5.75	5.25	5.63	0.32
ID 13	8	8	8.25		8.08	0.14
ID 14	2.75	3	1.75		2.50	0.66
ID 15	6.5	6.25			6.38	0.18

Session 2	Trail 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Trial 4 (mm)	Average (mm)	SD
ID 1	4.25	4.5	3.75	3.75	4.06	0.38
ID 2	4	4.25	4.5		4.25	0.25
ID 3	3.75	4	4		3.92	0.14
ID 4	8.75	9.25	9.5		9.17	0.38
ID 5	6.25	6.25			6.25	0.00
ID 6	6.25	7			6.63	0.53
ID 7	6.75	7.5	7.75		7.33	0.52
ID 8	7.25	8.5	8.5		8.08	0.72
ID 9	8.75	9	9		8.92	0.14
ID 10	8.75	8.5	8.25		8.50	0.25
ID 11	2.5	2.5	2.5	2.5	2.50	0.00
ID 12	4.75	3.25	4		4.00	0.75
ID 13	9.25	9.5	8.75		9.00	0.35
ID 14	3.5	3.5	3.5		3.50	0.00
ID 15	4	3.75	3.25		3.67	0.38

APPENDIX H

Table 10: Raw Data for KT-2000 Intratester Reliability for Primary Examiner

ID #	First Reading (mm)	Second Reading (mm)	Average (mm)	Standard Deviations
1	7.38	9.25	8.32	1.32
2	.13	2.50	1.32	1.68
3	5.00	3.38	4.19	1.15
4	4.88	6.00	5.44	0.79
5	6.25	6.00	6.13	0.18
6	3.13	4.63	3.88	1.06
7	3.75	3.50	3.63	0.18
8	2.25	6.63	4.44	3.10
9	2.00	2.38	2.19	0.27
10	3.25	2.00	2.63	0.88
11	4.13	4.38	4.26	0.18
12	2.88	1.75	2.32	0.80
13	6.00	8.13	7.07	1.51
14	2.63	2.48	2.56	0.11
15	5.00	5.38	5.19	0.27
16	6.63	6.38	6.51	0.18
17	2.25	2.13	2.19	0.08
18	5.13	5.75	5.44	0.44
19	4.50	3.13	3.82	0.97
20	5.13	5.13	5.13	0.00
21	2.63	2.63	2.63	0.00
22	3.75	3.00	3.38	0.53
23	5.00	5.13	5.07	0.09
24	4.75	5.25	5.00	0.35
25	5.63	5.75	5.69	0.08
26	2.00	3.00	2.50	0.71
27	6.25	2.25	4.25	2.83
28	3.38	3.88	3.63	0.35
29	4.63	4.00	4.32	0.45
30	3.63	6.00	4.82	1.68
31	1.88	1.00	1.44	0.62
32	3.13	2.75	2.94	0.27
33	6.00	6.13	6.07	0.09
34	3.25	3.38	3.32	0.09
35	5.38	4.88	5.13	0.35
Average	4.10	4.28	4.19	0.13

APPENDIX I

Table 11: Repeated Measures ANOVA Calculations for Observing Differences in Laxity Measurement Methodologies Verses Time.

Source	Sum of Squares	df	Mean Square	F-value	P-value
Main Effect for Methodology	266.890	1	266.890	74.654	<.0001
Error (Methodology)	39.325	11	3.575		
Main Effect Over Time	1.523	2	.762	.470	.631
Error (Time)	35.647	22	1.620		
Interaction for Methodology Over Time	5.510	2	2.755	1.201	.320
Error (Methodology vs Time)	50.450	22	2.293		

APPENDIX J

Table 12: Repeated Measures ANOVA Calculations for Observing Changes in Displacement of the Lateral and Medial Compartments.

Source	Sum of Squares	df	Mean Square	F-value	P-value
Main Effect for Compartment	.014	1	.014	.001	.976
Error (Compartment)	541.924	35	15.484		

APPENDIX K

Table 13: Raw Data for Displacements of Medial and Lateral Compartments.

ID #	Medial Displacement (mm)	Lateral Displacement (mm)
1	6.50	8.25
2	3.25	3.50
3	3.00	7.00
4	2.00	11.50
5	11.00	1.50
6	1.75	8.00
7	1.50	6.00
8	8.75	-4.25
9	1.50	2.50
10	1.00	5.50
11	6.25	2.00
12	.75	4.00
13	4.00	8.00
14	4.25	1.50
15	2.25	7.75
16	3.00	10.25
17	10.00	5.50
18	5.75	4.50
19	1.75	7.25
20	9.75	.50
21	3.75	1.50
22	7.00	.50
23	4.75	5.25
24	5.00	4.50
25	1.25	10.00
26	.75	3.25
27	3.50	9.00
28	2.75	4.00
29	8.25	1.00
30	2.5	3.75
31	1.00	2.75
32	10.00	1.75
33	5.00	1.25
34	9.75	2.25
35	3.75	2.75
36	4.5	6.25

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