Estrogen Fluctuations in the Female Menstrual Cycle

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Estrogen Fluctuations in the Female Menstrual Cycle

Senior Comprehensive Project

in partial fulfillment of

Bachelor of Science Degree

Biology

April 3, 2017

Jessie Thiessen
Estrogen Fluctuations in the Female Menstrual Cycle

April 3 2017

This work represents my own work unless otherwise cited.

This senior comprehensive project has met the minimum requirements of the Biology major for a Bachelors of Sciences degree

__________________________  _________________________
Dr. Becky Dawson                           Date

__________________________  _________________________
Dr. Chris Lundberg                           Date
The female menstrual cycle (Figure 1) is a series of changes that women go through in order to prepare for reproduction (Sharma et al., 2016). Hormones present during the menstrual cycle can fluctuate depending on a number of mental and physical health factors (Manikandan et al., 2016). Estrogen is produced in women and most present during ovulation in the menstrual cycle (Stijak et al. 2015). Estradiol is responsible for female sex characteristics and sexual functioning (Otag et al., 2016). The effect of exercise on estrogen levels in the female menstrual cycle is examined in this experiment. Two groups of human females participated – ten active females and eight females that are not active on a regular basis. Saliva samples were collected on day one and day fourteen of each participant’s menstrual cycle. On day one of their menstrual cycle, participants completed a survey to determine the demographics of each participant. An estradiol enzyme-linked immunosorbent assay (ELISA) was performed on each sample to measure the amount of estrogen in each sample. There was no significant difference between active and non-active participants on day one of sample collection (F(1) = 0.1553, P = 0.6988) (Figure 3). There was no significant difference between active and non-active participants on day fourteen of sample collection (F(1) = 0.000, P = 0.9980) (Figure 4). There was no significant difference between day one and day fourteen of the female menstrual cycle (Figure 5). The purpose of this experiment was to determine the influence of physical activity on estrogen levels in women.
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INTRODUCTION

Female humans are unique in that they undergo a monthly cycle that allows them to reproduce. The female menstrual cycle (Figure 1) is a series of changes that females go through in order to prepare for reproduction (Sharma et al., 2016). The uterine lining (endometrium) thickens once a month in order to prepare for a fertilized egg (Sharma et al., 2016). If no eggs become fertilized, the uterine lining sheds and females undergo menstrual bleeding (Sharma et al., 2016). The menstrual cycle (Figure 1) goes through two stages: the follicular phase and the luteal phase (Rodney and Sataloff, 2016). Day one of the menstrual cycle begins the follicular phase (Manikandan et al., 2016). It is also the first day of menstrual bleeding (Manikandan et al., 2016). Days seven to fourteen are considered pre-ovulation (or follicular phase) when the uterine walls are beginning to thicken again to prepare for ovulation (Rodney and Sataloff, 2016). Day fourteen is typically when ovulation occurs and an egg is released from the fallopian tubes (Rodney and Sataloff, 2016). If the egg is fertilized, it will implant into the uterine lining to begin developing into an embryo (Xiao et al., 2017). If the egg does not get fertilized, the luteal phase begins on day fifteen to twenty-eight (Manikandan et al., 2016). During the luteal phase the uterine lining continues to thicken again in preparation of a fertilized egg implant during ovulation (Rodney and Sataloff, 2016). After the luteal phase the menstrual cycle begins all over again with menstrual bleeding occurring to shed the uterine lining (Manikandan et al., 2016).
Figure 1: The Female Menstrual Cycle. The 28 days of the female menstrual cycle including the phases, hormone levels, follicle development, and uterine lining growth (Morris et al., 2013).

There are many hormones that are present throughout this cycle that help promote the process. The hormones involved in the menstrual cycle include testosterone, progesterone, luteinizing hormone, follicle-stimulating hormone, prolactin, and estrogen (Schuring et al., 2016).

Testosterone increases muscle mass and affects the quality of bone tissue (Stijak et al. 2015). Although females produce testosterone, it is found in much higher concentrations in males. Testosterone is involved in regulation of many female reproductive functions including ovulation and therefore makes it essential for women’s
health and well-being (Andersen et al., 2011). Testosterone also influences bone mass and body tissues in women (Andersen et al., 2011). The lesser amount of testosterone in women results in smaller muscle mass and fewer androgen receptors (Gould et al., 2016).

Progesterone is a hormone produced primarily in females. The role of progesterone in males is unclear (Stijak et al. 2015). In females, progesterone helps in preparation of the endometrium for implantation of the fertilized ovum (Stijak et al. 2015). Progesterone secretion is increased during high levels of stress and anxiety in both males and females (Stijak et al. 2015). Progesterone is involved in the development of female reproductive structures during embryogenesis and female secondary sexual characteristics during puberty (Rodney and Sataloff, 2016). Progesterone helps to regulate the female menstrual cycle (Rodney and Sataloff, 2016). When progesterone levels are highest, it maintains the thickened uterine lining (Rodney and Sataloff, 2016). Progesterone levels are highest during the luteal phase and lowest during the follicular phase of the female menstrual cycle (Rodney and Sataloff, 2016).

Luteinizing hormone (LH) is a hormone that stimulates the male and female gonads to secrete testosterone in males and estrogen and progesterone in females (Rodney and Sataloff, 2016). LH is present (at a constant rate) throughout the entire female menstrual cycle (Rodney and Sataloff, 2016). A surge in the LH triggers ovulation in the female menstrual cycle (Rodney and Sataloff, 2016).

Follicle stimulating hormone (FSH) is also a hormone that stimulates the male and female gonads to secrete testosterone in males and estrogen and progesterone in females (Andersen et al., 2011). FSH is present throughout the entire female menstrual cycle (Rodney and Sataloff, 2016). FSH stimulates follicle cells to begin to mature and
grow into oocytes (Rodney and Sataloff, 2016). There is also a surge in FSH during ovulation but is not as dramatic as the LH surge (Rodney and Sataloff, 2016).

Estrogen is produced in females and most present during the reproductive period of the menstrual cycle (Stijak et al. 2015). Estrogen is responsible for cell proliferation and sexual development (Otag et al., 2016). Estrogen is composed of a class of hormones such as estradiol, estriol, and estrone (Sameer et al., 2014). This experiment focused on the impact exercise has on estradiol. Estradiol is responsible for female sex characteristics and sexual functioning (Otag et al., 2016). Estradiol is important in females’ bone health (Kraemer et al., 1995). Estrogen is involved in the development of female reproductive structures during embryogenesis and female secondary sexual characteristics during puberty (Peper et al., 2011). Estrogen helps to regulate the female menstrual cycle (Rodney and Sataloff, 2016). There is a spike in estrogen concentrations right before ovulation (Hill, 1988). After ovulation, estrogen levels remain high during luteal phase of the female menstrual cycle (Kraemer et al., 1995). Estrogen affects the cardiovascular system, muscle skeletal system, immune system, and central nervous system (Otag et al., 2016). High estrogen levels helps to cause the uterine lining to thicken (Rodney and Sataloff, 2016).

Hormones can fluctuate depending on a number of mental and physical health factors (Manikandan et al., 2016). Estrogen levels fluctuate during the female menstrual cycle (Sajjad et al., 2016). Sex-hormone concentrations vary widely across days of the menstrual cycle, and normal physiologic changes in hormone concentrations can affect collagen metabolism and ligament behavior (Tourville et al., 2015). Collagen is a protein in the body that aids in tissue repair after an injury (Diegelmann, 2001). Therefore,
hormone concentrations have been found to impact injury recovery, such as ligament injuries. The behavioral expression of fear responses and anxiety may be impacted by hormonal fluctuations (Silva et al., 2016). In 2013, a study was conducted to determine reference ranges for testosterone, LH, and FSH (Radicioni et al., 2013). The reference ranges for the sex hormones evaluated needed to be adjusted for sex and age, and according to developmental stage and fertility period (Radicioni et al., 2013). In a recent study in 2016, sex hormones were measured among females (of different stages in their menstrual cycle) in order to determine reference points (Schuring et al., 2016). There was variation between subjects’ levels of sex hormones (Schuring et al., 2016). Because of the variation between sex hormones during the menstrual cycle, reference points of when hormones are increasing and decreasing are important in understanding their role throughout the female menstrual cycle. A median was determined from the samples to show that females in the luteal phase (right after ovulation) had the highest levels of estrogen (Schuring et al., 2016). In a study using female rats, estrogen and progesterone levels were highest during the luteal phase during the reproductive cycle (Matos et al., 2016). The results found in female rats from Matos et al. (2016) were similar to the findings found in human females (Rodney and Sataloff, 2016).

The effect of exercise on estrogen levels in the female menstrual cycle has been an interest of many scientists previously due to the increase in female participation in sports. Hormone changes during aerobic and resistive exercise have been examined in females in multiple studies. Hormone levels can be changed by exercise (Otag et al., 2016). Kraemer et al. (1995) found that estradiol in the luteal phase of the female menstrual cycle was slightly elevated during a low-volume resistive exercise. Estrogen
and progesterone increase after acute exercise (Otag et al., 2016). In a recent study, progesterone and estrogen secretions were suppressed by chronic exercise during the luteal phase of the menstrual cycle (Otag et al., 2016). When higher exercise intensities occur during the luteal phase of the female menstrual cycle, there was an increase in estradiol and insulin levels (Kraemer et al., 2012). The differences in study results imply that length and intensity of exercise could impact estrogen levels in the female menstrual cycle.

The purpose of this experiment is to determine the influence of physical activity on estrogen levels in females. The purpose is to also look at differences of estrogen levels among females and to verify reference ranges of estrogen during the female menstrual cycle. It is important to understand the role of estrogen in active females in order to predict and prevent possible effects that estrogen might have on the rest of the body. The female sex hormone, estrogen, may play a role in the higher risk of injury (esp. knee injuries) in females. The hypothesis for this study is that females who exercise regularly will have lower estrogen levels than females who do not exercise regularly. There will be higher estrogen levels during the pre-ovulation phase to ovulation during the female menstrual cycle.

METHODS

Participants and Samples

This study determined estrogen levels in active and non-active females to investigate estrogen fluctuations in the female menstrual cycle. Approval from the Internal Review Board (IRB) at Allegheny College was obtained to ensure safety of the participants. Human females aging from eighteen to twenty-one were used in this study.
All subjects agreed voluntarily to participate and signed a consent form. All participants received their regular monthly periods. All subjects used were taking oral birth control pills. The latter guaranteed that all subjects had normal menstrual cycles and fluctuation of hormones. 18 human females participated in this experiment. Two groups of females participated – ten regularly active females and eight females that are not active on a regular basis. Females were included in the active participant category when they indicated in the demographics survey that they exercise daily. All other participants were included in the non-active category when they indicated in the demographics survey that they exercise less than daily. Ten of the subjects were athletes or taking part in some type of physical activity on a daily basis so that the impact of estrogen on active females could be evaluated. Saliva samples were collected into small test tubes. Participants spit into a tube two times throughout their menstrual cycle. The first saliva sample was taken on day one of their monthly period. The second saliva sample was taken two weeks later (day fourteen). Participants each got a number assigned to them to keep samples confidential and organized. On day one of their menstrual cycle, participants met with me to sign the consent form and take the first saliva sample. Each participant then scheduled a time two weeks later to take the second sample. Each sample was labeled by participant number, date, and time the sample was collected. After collection of a sample, each was stored in an ultra-freezer located in Dr. Susan Rankin’s room (Room B307) in Steffee Hall of Biology.

**Demographics Survey**

A demographics survey was produced and approved by the Internal Review Board (IRB) at Allegheny College. On day one of their menstrual cycle, participants
completed a survey to determine the demographics of each participant. The one-page survey included age, age of first period, level of daily physical activity, and prior knee injuries.

**Salimetrics Enzyme Immunoassay**

Saliva was collected from each participant by having each individual spit into a small test tube (Salimetrics, 2016). At least 100 microliters of saliva was collected from every participant for each sample. The saliva samples were frozen at or below -20°C until all samples were collected. An Estradiol enzyme-linked immunosorbent assay (ELISA) was performed on each sample to measure the amount of estrogen in each sample. An ELISA is a plate-based technique to detect and quantify substances such as peptides, proteins, antibodies, and hormones (ThermoFisher Scientific, 2016). The estradiol was immobilized to a solid surface and then connected to an antibody that was linked to an enzyme (ThermoFisher Scientific, 2016). The estradiol was detected by assessing the enzyme activity (ThermoFisher Scientific, 2016).

At the time of analyzing, all samples, reagents, and the Microtitre plate were brought to room temperature. The wash buffer was prepared by diluting Wash Buffer Concentrate (10x) ten-fold with room temperature deionized water (100 mL of Wash Buffer Concentrate (10x) to 900 mL of deionized water). The HS Estradiol Standards were obtained by serial dilutions. Five polypropylene microcentrifuge tubes were labeled 2 through 6. 300 µL of HS Estradiol Assay Diluent was pipetted into tubes 2 through 6. The standard 2X was serial diluted by adding 300 µL of the 32 pg/mL standard (tube 1) to tube 2 and vortexed. After changing pipette tips, 300 µL was removed from tube 2 to tube 3 and vortexed. These methods were continued for tubes 4, 5, and 6. The final
concentrations of standards for tubes 1 through 6 were, respectively, 32 pg/mL, 16 pg/mL, 8 pg/mL, 4 pg/mL, 2 pg/mL, and 1 pg/mL. The plate layout was designed to keep all the standards, controls, and samples organized. 12 mL of HS Estradiol Assay Diluent was pipetted into the disposable tube. The tube was set aside for a later step. 100 µL of the standards, controls, and saliva samples were pipetted into the appropriate wells. 100 µL of HS Estradiol Assay Diluent was pipetted into two wells to serve as the zero. 100 µL of HS Estradiol Assay Diluent was pipetted into each NSB well. The Enzyme Conjugate was diluted 1:800 by adding 15 µL of the conjugate to the 12 mL tube of HS Estradiol Assay Diluent prepared in the earlier step. The conjugate tube was centrifuged for a few minutes to bring the liquid down to the tube bottom. Immediately, the diluted conjugate solution was mixed and 100 µL was added to each well using a multichannel pipette. An adhesive cover was placed on the plate and the plate was mixed on a plate rotator for five minutes at 500 rpm and incubated at room temperature for a total of two hours. The plate was washed four times with 1X wash buffer. Washing was done by pipetting 300 µL of the wash buffer into each well and then the liquid was discarded over a sink. After each wash, the plate was blotted on paper towels before turned upright. 200 µL of TMB Substrate Solution was added to each well with a multichannel pipette. The plate was mixed on a plate rotator for five minutes at 500 rpm and incubated in the dark (covered) at room temperature for an additional twenty-five minutes. 50 µL of Stop Solution was added with a multichannel pipette. Lastly, the plate was mixed on a plate rotator for three minutes at 500 rpm until the color of the samples turned from green to yellow. The plate was then read in a plate reader at 450 nm within ten minutes of adding the Stop Solution.
**Statistical Analysis**

The absorbance was obtained from a plate reader for my samples. Standards were developed in the plate to determine a standard curve. The average absorbance was obtained for each standard duplicate well. The standard curve was graphed in Microsoft Excel with the dilutions of estradiol (pg/mL) on the x-axis and mean absorbance on the y-axis. The equation for the trendline was obtained from the standard curve graph. The estradiol (pg/mL) was calculated by using an equation obtained from the standard curve. Results from the ELISA were analyzed using a one-way analysis of variance to see if there was a significant difference between the two groups of subjects and the amount of estrogen for day one and day fourteen of the menstrual cycle. Statistical significance was accepted for values of $p<0.05$. The one-way analyses of variance were analyzed using the programs Microsoft Excel and JMP. The subjects’ data was compared to each other to discover discrepancies between subjects’ estrogen fluctuations. The data collected on the surveys was used to compare subject’s demographics and the impact of estrogen.
RESULTS

Survey Results

All participants completed a survey to determine demographics of the study. All participants were between ages eighteen and twenty-one. Table 1 summarizes the data from the survey.

<table>
<thead>
<tr>
<th></th>
<th>Active (n = 10)</th>
<th>Non-Active (n = 8)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age (± SD)</td>
<td>19.5 (±1.18)</td>
<td>20.13 (±1.13)</td>
<td>0.2711</td>
</tr>
<tr>
<td>Mean Year of First Period (± SD)</td>
<td>2009 (±1.45)</td>
<td>2009 (±1.60)</td>
<td>0.7263</td>
</tr>
<tr>
<td>Range of years of First period</td>
<td>2007-2011</td>
<td>2007-2011</td>
<td></td>
</tr>
<tr>
<td>Knee Injury?</td>
<td>Yes 3, No 7</td>
<td>Yes 1, No 7</td>
<td>0.3749</td>
</tr>
<tr>
<td>Type of Knee Injury</td>
<td>Torn ACL; Patella Femoral Syndrome; Torn ACL (2x), MCL Sprain, Torn Meniscus (3x)</td>
<td>Torn Meniscus &amp; ACL Strain</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Demographics of Participants determined through Survey Results. Each participant completed a survey to determine level of daily activity, age, year of first menstrual period, and if they have ever had any knee injuries (if so, the type of knee injury they had). The P-value was determined for the Mean Age and Mean Year of First Period by a T-test. The P-value was determined for the Knee Injury by a Chi-squared Test.

The mean age of active participants was 19.50 (SD = 1.18) (Table 1). The mean age of non-active participants was 20.13 (SD = 1.13) (Table 1). The mean year of first period for active participants was 2009 (SD = 1.45) (Table 1). The mean year of first period for non-active participants was 2009 (SD = 1.60) (Table 1). All participants received their first menstrual period between the years 2007 and 2011 (Table 1). Out of the ten active participants, three have previously had knee injuries (Table 1). Out of the eight non-active participants, one has previously had a knee injury (Table 1). The most common knee injury was an anterior cruciate ligament (ACL) injury or meniscus injury (Table 1).
Figure 2: The Microtitre Plate showing Estradiol Absorbance. The first two columns are the standards in duplicate. Wells starting in column three are the saliva samples. The wells in the standard columns became darker shades of blue as the amount of estradiol (pg/mL) increased in dilution (0, 1, 2, 4, 8, 16, and 32 pg/mL).

All the saliva sample wells in Figure 2 were similar in color to the standard wells indicating no outliers.

On day one of sample collection ten active participants and eight non-active participants were compared to discover if there was a significant difference in estradiol (pg/mL).
Figure 3: Average Estradiol (pg/mL) between Active and Non-active Participants on Day 1 of the Female Menstrual Cycle. The mean estradiol of active participants was 2.99377 pg/mL. The mean estradiol of non-active participants was 2.86766 pg/mL. The error bars were determined by standard error of the mean. The F value with one degree of freedom was 0.1553. The p-value was 0.6988.

On day one, active participants had a slightly larger mean ($\bar{x} = 2.99377$, SE = 0.21335) of estradiol than the non-active participants ($\bar{x} = 2.86766$, SE = 0.23853). The largest amount of estradiol measured in an active participant was 4.4332 pg/mL. The smallest amount of estradiol measured in an active participant was 2.0696 pg/mL. The largest amount of estradiol measured in a non-active participant was 3.5397 pg/mL. The smallest amount of estradiol measured in a non-active participant was 1.9397 pg/mL.

Similarly, day fourteen of sample collection ten active participants and eight non-active participants were compared to discover if there was a significant difference in estradiol (pg/mL).
On day fourteen, active and non-active participants had almost the same mean of estradiol, with the non-active participants having a slightly higher mean ($\bar{x} = 2.37805$, SE = 0.26795) of estradiol than the active participants ($\bar{x} = 2.37714$, SE = 0.23966). The largest amount of estradiol measured in an active participant was 4.5371 pg/mL. The smallest amount of estradiol measured in an active participant was 1.0982 pg/mL. The largest amount of estradiol measured in a non-active participant was 3.3475 pg/mL. The smallest amount of estradiol measured in a non-active participant was 1.5034 pg/mL.
Figure 5: Average Estradiol (pg/mL) between Active and Non-active Participants on Day 1 compared to Day 14 of the Female Menstrual Cycle. The mean estradiol of active participants on Day 1 was 2.99377 pg/mL. The mean estradiol of non-active participants on Day 1 was 2.86766 pg/mL. The mean estradiol of active participants on Day 14 was 2.37714 pg/mL. The mean estradiol of non-active participants on Day 14 was 2.37805 pg/mL. The error bars were determined by standard error of the mean. The blue bars represent the active participants. The red bars represent the non-active participants.

There was a slightly larger mean of estradiol in active and non-active participants during day one of the menstrual cycle compared to day fourteen of the menstrual cycle (Figure 5).

**DISCUSSION**

The estradiol enzyme-linked immunosorbent assay (ELISA) that was performed on each sample showed that there was no significant difference between active and non-active participants on day one of sample collection (F(1) = 0.1553, P = 0.6988) (Figure 3). There was no significant difference between active and non-active participants on day fourteen of sample collection (F(1) = 0.000, P = 0.9980) (Figure 4). Therefore, there was no difference between estrogen levels in females who exercise on a daily basis and females who rarely exercise. Other studies have contradicted these findings. Hormones can be changed by exercise (Otag et al., 2016). Exercise is a stimulus to the
hypothesis in the brain, which activates the anterior pituitary gland. The anterior pituitary gland in the brain signals the secretion of many hormones, including LH (Kraemer et al., 2012). LH stimulates estrogen secretion in the female menstrual cycle (Rodney and Sataloff, 2016). In a recent study, blood samples were taken from female athletes before, immediately after, and one hour after completing a shuttle running test (Otag et al., 2016). Otag et al. found an increase in LH immediately after exercise (2016). Secretion of LH increased progesterone and estrogen immediately after acute exercise (Otag et al., 2016). However, progesterone and estrogen levels decreased to resting levels one hour after the shuttle test (Otag et al., 2016). Therefore, progesterone and estrogen secretion is suppressed by chronic exercise during the luteal phase of the menstrual cycle (Otag et al., 2016). Kraemer et al. found that estradiol in the luteal phase of the female menstrual cycle was slightly elevated during a low-volume resistive exercise (1995). When female participants had blood samples taken during and after running on a treadmill at a heavy pace (85% VO_{2}max) during the luteal phase of the female menstrual cycle, there was an increase in estradiol and insulin levels (Kraemer et al., 2012). According to these studies, exercise immediately increases estrogen levels but long-term exercise decreases estrogen levels. However, the affect of exercise in the female menstrual cycle needs to be researched further.

The estradiol enzyme-linked immunosorbent assay (ELISA) that was performed on each sample showed that there was no significant difference between day one and day fourteen of the female menstrual cycle (Figure 5). These results are conflicting with accepted knowledge of the female menstrual cycle. Day fourteen (usually ovulation) should have a sharp increase in estrogen (Hill, 1988). After ovulation, estrogen levels
remain high during luteal phase of the female menstrual cycle (Kraemer et al., 1995). Day one (menstruation) should be lower in estrogen than day fourteen (Figure 1).

In this experiment, many factors could have contributed to the lack of difference between estrogen levels in active and non-active females and the lack of differences in estrogen levels between day one and day fourteen of the menstrual cycle. Due to restricted time and requirements of participants, a small sample size was obtained for this study. Operationalizing a study where human subjects must provide saliva samples on specific days of their menstrual cycle made this experiment challenging to complete in the allotted time. The small sample size (n = 10 active and n = 8 non-active) could have allowed for an inaccurate representation of estradiol fluctuations during the female menstrual cycle. A larger sample size could have reduced uncertainty because there is more information provided to impact the results. A larger sample size provides more results to give a greater chance of detecting differences. In addition, a larger sample size is a more accurate representation of the population.

Inconsistency in the number of hours following the start of menstruation in each participant could have also affected the results of the data to be contradictory to prior research. Participants’ samples were taken on the correct days. However, the time of the day that the saliva sample was obtained was not kept consistent for the convenience of each participant. Accuracy of timing would have kept the results more consistent because each participant has a different menstrual cycle. Because of the inconsistency, each sample was technically not taken at the same time of the menstrual cycle in each participant. This may have allowed for increased variation between the levels of estrogen in each participant.
All participants in the experiment were on birth control. Participants on birth control were chosen due to the high number of females in college taking birth control pills. The role birth control has on the hormones of females who exercise regularly was of particular interest. However after completion of the experiment, it has been found that birth control may have played a role in the lack of difference between the day one and day fourteen estrogen levels in the menstrual cycle. Oral contraceptive pills decrease the fluctuation of hormones during the menstrual cycle, which reduces ovarian function (Rodney and Sataloff, 2016). Most pills are a combination of estrogen and progestin (Rodney and Sataloff, 2016). The estrogen role in birth control suppresses ovulation (Rodney and Sataloff, 2016). The suppression of ovulation may contribute to the lack of increase in estrogen on day fourteen of this study. Therefore, birth control’s effect on estrogen may be greater and cancel out any affect that exercise may have on estrogen throughout the female menstrual cycle.

In the future, a larger sample size would give a greater representation of exercise’s influence on estrogen fluctuations in the female menstrual cycle. In addition, an experiment excluding participants on birth control would portray the affect exercise has on estrogen during the female menstrual cycle because birth control would not affect the estrogen levels in participants. This experiment is also a stepping-stone for further studies examining the role of hormones on females who exercise frequently.

A continuation of this experiment would be to examine estrogen’s role in influencing knee injuries. ACL injuries are two to eight times greater in females than in males (Gould et al., 2016). Females are more at risk for an ACL injury due to anatomic, hormonal, biomechanical, and neuromuscular differences (Gould et al., 2016).
female sex hormone, estrogen, may play a role in the higher risk of ACL injury in females. Estrogen is correlated with decreased collagen synthesis and a decrease in fibroblast proliferation (Stijak et al. 2015). Thus, there is a decrease in ACL tensile strength in females (Gould et al., 2016). Table 1 shows that in ten active participants, three have had serious knee injuries. One study found an increase in ACL laxity throughout the MC (Gould et al., 2016). A second study found an increase in ACL injuries during mid-cycle when estrogen levels are highest (Gould et al., 2016). Another study found that players are more susceptible to injury during premenstrual and menstrual phases of their cycles (Biondino, 1999). All of these studies are different and hard to compare, making it difficult to assess the precise effect of circulating estrogen and related hormones on the mechanical properties of the ACL (Gould et al., 2016). However, the majority of evidence has shown that the risk of ACL injury is highest when estrogen levels are highest (pre-ovulatory phase to ovulation) (Tourville et al., 2016). Conducting a study to determine the role estrogen has on knee injuries

There were no differences between estradiol levels in active and non-active females in this experiment. However, further examination of exercise’s influence on hormone levels in females could aid to the health of many females. If consistent and successful laboratory methods can be developed for saliva collection of active and non-active females, the results could be more consistent with other studies in that exercise may play a role in hormonal changes throughout the female menstrual cycle. With the increase of females participating in sports, examining the influence of hormones on injuries could benefit many females.

REFERENCES


**APPENDIX**

**Internal Review Board Approval**

The Internal Review Board (IRB) at Allegheny College has approved this project. The project number is 2017-44.

**Consent Form**

By signing this consent, you are volunteering to take part in a research study. The purpose of this experiment is to determine the influence of physical activity on estrogen levels in women. The purpose is to also look at differences of estrogen levels among women and to verify reference ranges of estrogen during the female menstrual cycle. It is important to understand the role of estrogen in female athletes in order to predict and prevent possible effects that estrogen might have on the rest of the body. We ask that you sign below where you are comfortable and after you are sure you understand the scope,
procedures, and purposes of this project. Specifically, we want you to understand the following:

- This study will determine estrogen levels in active and non-active females to investigate estrogen fluctuations in the female menstrual cycle. You will be one of approximately 20 participants in this study.
- If you feel uncomfortable in any way during the interview session, you have the right to decline to answer any question or to end the interview.
- Your participation involves the collection of saliva into small test tubes. You will spit into a tube four times throughout your menstrual cycle (once a week for a month). Lastly, you will complete a survey about your demographics. The study will last about a month (the length of your menstrual cycle).
- The research team will not identify you by name in any reports using information obtained from the survey or saliva collection, and your confidentiality as a participant in this study will remain secure. For the purposes of this research study, your comments will not be anonymous. Every effort will be made by the researcher to preserve your confidentiality.
- This research study has been reviewed and approved by the Institutional Review Board (IRB) at Allegheny College (if you want more information, please contact Allison Connell Pensky 332-2706)

I have read and understand the explanation provided to me. I have had all my questions answered to my satisfaction, and I voluntarily agree to participate in this study. Additionally, I affirm that I am at least 18 years of age.
Survey

Estrogen Fluctuations Among Active and Non-Active Women

Researcher: Jessie Thiessen

Survey of Questions:

1.) What is your age? _________________________

2.) Date of First Menstrual Period? _________________________
3.) Level of Activity? (Circle One)
   - Exercise Daily
   - Exercise Once a Week
   - Exercise Once a Month
   - Rarely Exercise

4.) Have you ever experienced a knee injury? (Circle One)
   - Yes
   - No

If you answered “Yes” to the question above, please answer questions 5 and 6.

If you answered “No” to the question above, you have finished the survey and do not
need to answer the rest of the questions below.

5.) Type of Injury? ______________________

6.) Date of Injury? ______________________