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Section II – Exercise Physiology and Sports Medicine

Effects of Urinary Sex Hormones during the Menstrual Cycle on Anterior Cruciate Ligament Laxity in Female Adolescent Soccer Players

by
Yipei He [1], Dongliang Shi [1,2], Bingyin Liang [3], Rong Su [1], Xian Zhang [1], Li Cui [4], Han Meng [5], Yinan Shi [1], Yan Qi [1,]*

The aim of this study was to investigate the relationship between urinary sex hormone levels and anterior cruciate ligament (ACL) laxity in female adolescent soccer players, and to explain the relationship through establishing regression equations. Urinary aliquots were collected daily from 15 healthy subjects over a continuous period of 27–34 days. Knee laxity at 67, 89 and 133 N was assessed using a standard KT-1000 knee arthrometer. Urinary metabolite levels of estradiol, progesterone and testosterone were tested using UPLC-Xevo TQ MS. Greatest ACL laxity was recorded during the luteal phase, followed by ovulatory and follicular phases ($p < 0.05$). In addition, there were significant correlations between ACL laxity and estradiol or progesterone ($p < 0.05$). Estradiol, progesterone, and their interaction in the model were significant predictors of knee laxity ($p < 0.05$). In conclusion, ACL laxity was significantly and positively correlated with urinary estradiol and progesterone levels. The menstrual cycle (especially during the luteal phase) had a significant effect on ACL laxity. The regression equations we developed to analyze female adolescent soccer players accounted for 7.39% of the variation in ACL laxity with urinary sex hormones.

Keywords: *progesterone; estradiol; gonadal steroid hormones; female athletes*

Introduction

Soccer carries a high risk of injury (Ekstrand et al., 2021), exhibiting an incidence rate of approximately 6.6 injuries per 1,000 player-hours. Among these, ACL injuries are particularly prevalent. A cohort analysis in Serie A documented an ACL injury incidence of 0.062 per 1,000 exposure hours (Grassi et al., 2020). Notably, female athletes were reported to have a 2- to 8-fold higher ACL injury incidence compared to males participating in comparable competitive activities (Crossley et al., 2020; Fox et al., 2020), with female soccer athletes exhibiting a 2.1-fold elevated injury probability (Schilaty et al., 2018).

The etiology of this sex-based disparity is multifactorial, and several hypotheses have been proposed to elucidate the underlying mechanisms (Edison et al., 2022; Larwa et al., 2021). Evidence has implicated cyclical sex hormone variations—particularly peri-pubertal estrogen-progesterone dynamics—as key modulators of knee laxity in female athletes (Hartman et al., 2024). ACL laxity is a primary determinant of knee laxity, as it directly increases anterior translation of the tibia, thereby contributing significantly to overall knee instability. Multiple investigations have documented significant correlations between knee laxity and menstrual cycle phases (Maruyama et al., 2021; Park et al., 2009; Zazulak et al., 2006),

[1] *Shanghai YangZhi Rehabilitation Hospital (Shanghai Sunshine Rehabilitation Center), School of Medicine, Tongji University, Shanghai, China.*
[2] *Department of Pediatric Surgery, Shanghai Jiahui International Hospital, Shanghai, China.*
[3] *Department of Rehabilitation Medicine, Shanghai East Hospital, Tongji University School of Medicine, Shanghai, China.*
[4] *Shanghai Jinyang Community Health Service Center, Tongji University School of Medicine, Shanghai, China.*
[5] *Institute of Sports Medicine, Fudan University, Shanghai, China.*
* Correspondence: 1410937@tongji.edu.cn

indicating that variations in estradiol, progesterone, and testosterone might influence ligamentous laxity (Dos'Santos et al., 2023; Hansen and Kjaer, 2016; Shagawa et al., 2023). Among these hormones, estradiol demonstrated the strongest association with knee laxity (Gilmer et al., 2025; Lee et al., 2013). Knee laxity was identified as a contributing factor to the increased risk of the ACL rupture (Hoffman et al., 2008; Shultz et al., 2011). Following ACL injury, athletes often experience diminished subjective performance and compromised neuromuscular control during dynamic tasks such as landing, deceleration, and directional changes. Thus, preventing ACL injury is critical for maintaining athletic performance (Raj et al., 2023). Enhancing understanding of the relationship between knee laxity and the menstrual cycle, and developing more straightforward methods of detection are imperative.

Conventional methodologies utilizing intermittent blood sampling have yielded inconclusive evidence regarding menstrual cycle effects on laxity. Daily venipuncture proves impractical for athletic populations due to procedural discomfort and potential iatrogenic nutrient depletion. Consequently, the analysis of urine aliquots is preferable to blood sampling when evaluating the levels of estradiol, progesterone and testosterone in female soccer players, with the objective of preventing sports injuries through urinary hormone monitoring. However, whether knee laxity varies with urinary sex hormones throughout the menstrual cycle remains controversial for female adolescent soccer players.

Therefore, the objective of the present study was to examine the association between urinary hormone levels and ACL laxity in female adolescent soccer players. The investigation sought to elucidate the underlying relationship by developing regression equations to facilitate prediction. To address this knowledge gap, the present investigation aimed to (1) establish relationships between dynamic fluctuations in urinary sex hormone concentrations (estradiol, progesterone, testosterone) and anterior cruciate ligament (ACL) laxity metrics across menstrual cycle phases in female adolescent athletes; (2) develop multivariate regression models quantifying hormone-laxity interactions; and (3) propose a clinically actionable, non-invasive urinary monitoring protocol for ACL injury prevention programming.

Methods

Participants

A total of fifteen healthy adolescent female soccer players participated in this study (mean age: 14.12 ± 1.6 years; body mass: 52.0 ± 3.3 kg; body height: 163.88 ± 3.7 cm). Data were collected over one complete menstrual cycle for each participant, during which daily urine samples and anterior knee laxity measurements were obtained. All participants reported regular menstrual cycles (mean cycle length: 30.75 ± 1.9 days) with minimal inter-cycle variation (±1 day), as confirmed by three months of basal body temperature tracking and menstrual charting prior to data collection. None of the participants reported the use of oral contraceptives.

All participants were physically active, engaging in recreational soccer training for 10–20 hours per week over the preceding six months. None had a history of lower extremity injuries, systemic illnesses, smoking, or connective tissue disorders. Written informed consent was obtained from both the participants and their legal guardians. The study protocol was approved by the ethics committee of the Shanghai YangZhi Rehabilitation Hospital (Shanghai Sunshine Rehabilitation Center), School of Medicine, Tongji University, Shanghai, China (protocol code: 2023-001; approval date: 17 January 2023).

ACL Laxity

ACL laxity was assessed using KT-1000 knee arthrometers (MEDmetric Corp, San Diego, California, USA). Each participant was positioned supine on a firm testing platform, with both lower limbs stabilized at 90° knee flexion using a popliteal support. An integrated foot support apparatus was employed to ensure symmetrical foot alignment and minimize tibial external rotation.

To enhance participants' comfort and measurement consistency, the knee angle was adjusted to 30° via a thigh strap that also controlled hip rotation. Anterior tibial translation was induced using three standardized force levels (67 N, 89 N, and 133 N). Each force level was applied in triplicate, and the average displacement values were recorded for subsequent analysis. All

assessments were performed by a single trained investigator to minimize inter-rater variability (Figure 1).

Sex Hormones

Urinary concentrations (pg/mL) of estradiol, progesterone, and testosterone metabolites were quantified via liquid chromatography-tandem mass spectrometry (LC-MS/MS), selected over ELISA for its enhanced sensitivity and precision in detecting trace-level hormonal analytes. Freshly voided morning urine specimens were systematically collected in 50 mL sterile polypropylene tubes, with urine specific gravity immediately measured using a handheld refractometer to account for hydration variability prior to aliquot storage at –80°C in an ultra-low-temperature freezer.

Urine aliquots (0.8 ml) were pipetted into a 96-well plate by reverse solid phase extraction; 10 μl of internal standards working solution was then added, followed by 10 μl of β-glucuronidase. Enzymatic hydrolysis was conducted through incubation at 55°C for duration of three hours. Thereafter, the samples were subjected to vacuum concentration using a designated apparatus, followed by the introduction of 1 ml of pure water. Following the evaporation of the water, the 5% methanol/water solution was added using a multichannel pipetting gun. Next, the samples were subjected to further complete evaporation in order to dry them. Thereafter, the remaining mass was transferred into a 96-well receiving plate using a pipette, eluted with 0.5 ml of pure methanol, and then evaporated to dryness one final time using the vacuum constriction device. The 96-well receiving plate was subsequently positioned on the automatic sampler for the purpose of LC/MS-MS analysis.

Standard samples of β-estradiol and testosterone, in addition to β-glucuronidase, were purchased from Sigma-Aldrich (St. Louis, Missouri, US), while the progesterone came from Fisher Scientific (Ottawa, Canada); 17β-estradiol (−2, 4, 16, 16-d_4), progesterone (−2,2,4,6,6,17α,21,21,21-d_9), and testosterone (−16,16,17-d_3) were purchased from CDN isotopes (Pointe-Claire, Canada).

Subsequent to the collection of all samples, concentrations of estradiol, progesterone and testosterone metabolites in the urine were quantified using a UPLC-Xevo TQ MS (Waters Corporation, Milford, MA). Thereafter, the concentrations were standardized by the specific gravity of the urine. Separations were performed using a HSS T3 column (100 mm × 2.1 mm, 1.8 μm). Solvent A was 5% methanol containing 10 mmol/L ammonium acetate, and solvent B was 95% methanol containing 10 mmol/L ammonium acetate. The samples were eluted with a linear gradient at a flow rate of 0.5 mL/min. The gradient was started with 95% A, held for 1 min, then linearly decreased to 30% A over 1 min, then linearly increased to 95% A over 2 min. After washing with 95% A for 3 min, the column was re-equilibrated with 95% A for 2 min prior to the next injection.

The MS operating conditions were as follows: metabolites of estradiol and progesterone were detected in the negative ion mode, with an evaporator pressure of 50 psi, the nitrogen flow rate of 11 mL/min, 450°C, cone voltage of 45 V, and capillary voltage of 4500 V. The detection of positive ions was performed using testosterone metabolite, with an evaporator pressure of 60 psi, the nitrogen flow rate of 11 mL/min, 400°C, cone voltage of 55 V, capillary voltage of 4000 V. The injection volume was 5 μl.

Statistical Analysis

Descriptive statistics and all inferential analyses were performed using STATA version 15.0 (StataCorp, College Station, TX, USA). One-way repeated measures analysis of variance (ANOVA) was employed to evaluate changes in urinary hormone concentrations and ACL laxity across menstrual cycle phases. LSD post hoc tests were applied for pairwise comparisons. Mixed factorial ANOVA was used to assess the interaction effects of hormone levels and cycle phases on knee laxity.

Pearson's r correlation coefficients were computed to examine bivariate relationships between hormone concentrations and laxity measures. To explore predictive relationships, multiple linear regression models were constructed with hormone levels as independent variables and knee laxity (at 133 N) as the dependent variable. The threshold for statistical significance was set at $p < 0.05$. The regression equation is presented below:

$$\text{Knee Laxity} = A + B * (\text{Estradiol}) + C * (\text{Progesterone}) + D * (\text{Testosterone}) + E * (\text{Estradiol} * \text{Progesteron}) + F * (\text{Estradiol} * \text{Testosterone}) + G * (\text{Progesterone} * \text{Testosterone}) + H * (\text{Estradiol} * \text{Progesteron} * \text{Testosterone})$$

Results

Daily anterior knee laxity measurements and corresponding urinary hormone concentrations were collected over a 30-day period for each participant, thereby minimizing potential selection bias and capturing the full spectrum of menstrual cycle variability. Normality tests indicated that the distributions of estradiol, progesterone, testosterone, and knee laxity values deviated significantly from a normal distribution ($p < 0.05$).

To account for inter-individual variability in knee laxity measurements, data were normalized using the following transformation:

$$X' = (X - \text{Xmin})/(\text{Xmax} - \text{Xmin})$$

where X' represented the normalized value, X was the original measurement, and X_{min}/X_{max} corresponded to minimum and maximum observed values respectively.

Longitudinal analysis of knee laxity across three anterior loading conditions (67 N, 89 N, and 133 N) revealed a consistent pattern of variation throughout the menstrual cycle. Specifically, the highest mean ACL laxity was observed during the ovulatory phase, followed by progressive reductions during the luteal and follicular phases (Table 1, Figure 2).

Pearson correlation analyses demonstrated statistically significant positive associations between ACL laxity (at all three force levels) and urinary concentrations of estradiol and progesterone (Table 2, Figure 3). No significant correlations were detected between testosterone levels and knee laxity at any force magnitude.

Although we also obtained displacement values for the bilateral knee joints at 67 N and 89 N, preliminary graphical and statistical comparisons revealed that changes in knee laxity across these force levels and 133 N were similar. These findings supported the conclusion that cyclical changes in laxity were independent of force magnitude. Therefore, we constructed a regression equation with reference to the results of Shultz et al. (2004) and selected the knee laxity of the left knee at 133 N as the criterion variable for this study. The results are shown in Table 3.

The resulting regression model was as follows:

$$\text{Knee Laxity} = 0.4590203 + 0.38251 * (\text{Estradiol}) + 0.0278159 * (\text{Progesterone}) - 0.0039217 * (\text{Estradiol} * \text{Progesteron})$$

Regression analyses revealed that the three hormones collectively accounted for 7.39% of the variance in knee laxity ($R^2 = 0.0739$). Critically, the estradiol-progesterone interaction significantly predicted laxity, whereas estradiol-testosterone and progesterone-testosterone interactions demonstrated negligible contributions.

Table 1. Changes in anterior knee laxity during the menstrual cycle.

	Folicular Phase	Ovulation Phase	Luteal Phase	FP vs. OP	FP vs. LP	OP vs. LP
Knee laxity (after normalization)	Mean ± SD	Mean ± SD	Mean ± SD	*p*	*p*	*p*
67 N (L)	0.368 ± 0.213	0.617 ± 0.142	0.801 ± 0.151	0.000†	0.000†	0.000†
67 N (R)	0.499 ± 0.260	0.646 ± 0.204	0.711 ± 0.217	0.000†	0.000†	0.040*
89 N (L)	0.384 ± 0.210	0.624 ± 0.146	0.792 ± 0.149	0.000†	0.000†	0.000†
89 N (R)	0.504 ± 0.262	0.651 ± 0.207	0.712 ± 0.216	0.000†	0.000†	0.059
133 N (L)	0.384 ± 0.228	0.606 ± 0.178	0.704 ± 0.196	0.000†	0.000†	0.000†
133 N (R)	0.497 ± 0.254	0.639 ± 0.217	0.683 ± 0.214	0.000†	0.000†	0.285

*FP: folicular phase; OP: ovulation phase; LP: luteal phase; * statistically significant at $p < 0.05$; †: statistically significant at $p < 0.01$*

Table 2. Correlation between urinary metabolites of sex hormones and knee laxity at 67, 89, and 133 N.

Sex Hormones	Pearson	67 N (L)	67 N (R)	89 N (L)	89 N (R)	133 N (L)	133 N (R)
Urinary estradiol†	COA	0.117	0.168	0.133	0.138	0.151	0.121
	p	0.009	0.000	0.004	0.002	0.001	0.008
Progesterone*	COA	0.125	0.151	0.118	0.142	0.115	0.151
	p	0.006	0.001	0.009	0.002	0.011	0.001
Testosterone	COA	0.045	−0.288	0.056	−0.045	0.076	−0.066
	p	0.328	0.527	0.216	0.325	0.094	0.145

*COA: coefficient of association; * statistically significant at $p < 0.05$; †: statistically significant at $p < 0.01$*

Table 3. Urinary metabolites of sex hormone variables and predictive equations.

	β [95% CI]	*p*
E	0.0383 [0.0178, 0.0588]	0.000*
P	0.0278 [0.0145, 0.0411]	0.000*
T	0.0030 [−0.0153, 0.0212]	0.7510
E*P	−0.0039 [−0.0066, −0.0012]	0.004*
P*T	−0.0015 [−0.0048, 0.0018]	0.3870
E*T	−0.0039 [−0.0086, 0.0007]	0.0970
E*P*T	0.0006 [0.0000, 0.0012]	0.0590
_cons	0.4590 [0.4003, 0.5178]	0.000*
Adjusted R^2	0.0739	

*E: Estradiol; P: Progesterone; T: testosterone; * statistically significant at $p < 0.05$*

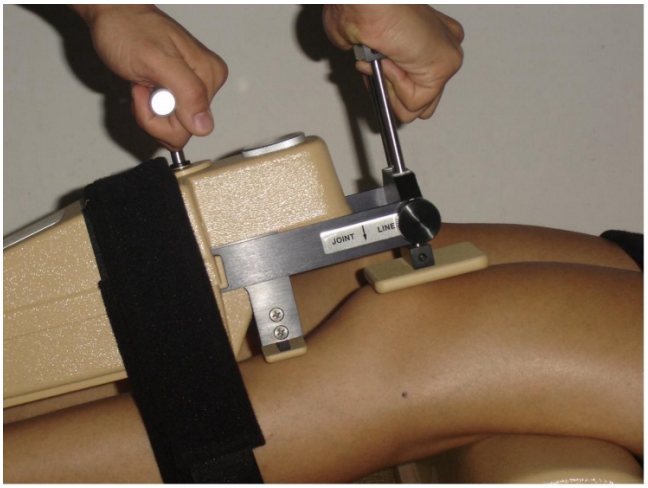

Figure 1. Experimental setup for measuring ACL laxity by applying anteriorly directed force to the tibia with the knee fixed at 30° of flexion.

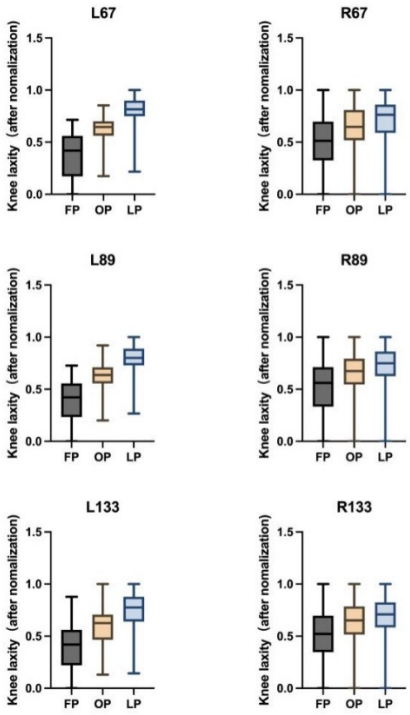

Figure 2. Anterior cruciate ligament laxity during the menstrual cycle.
FP: folicular phase; OP: ovulation phase; LP: luteal phase

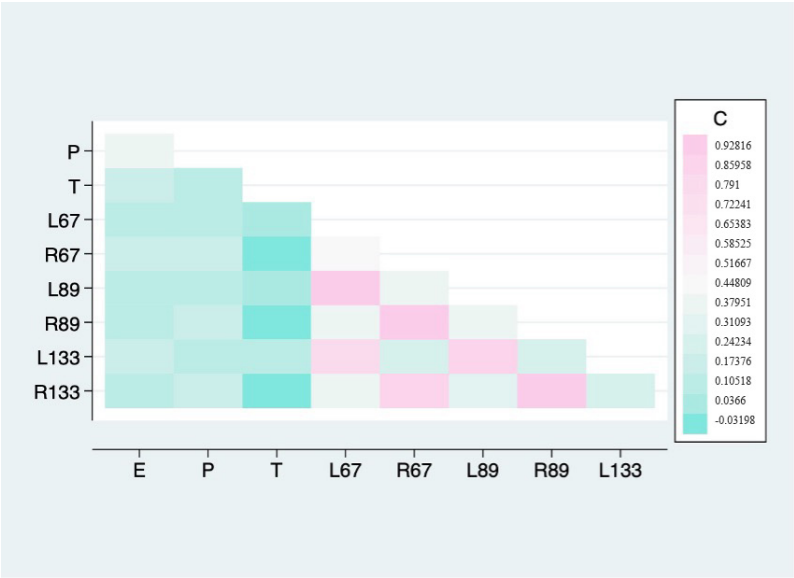

Figure 3. Correlation coefficient matrix heatplot between urinary metabolites of estradiol, progesterone, testosterone and knee laxity at 67, 89, and 133 N for each subject.
E: Estradiol; P: Progesterone; T: Testosterone

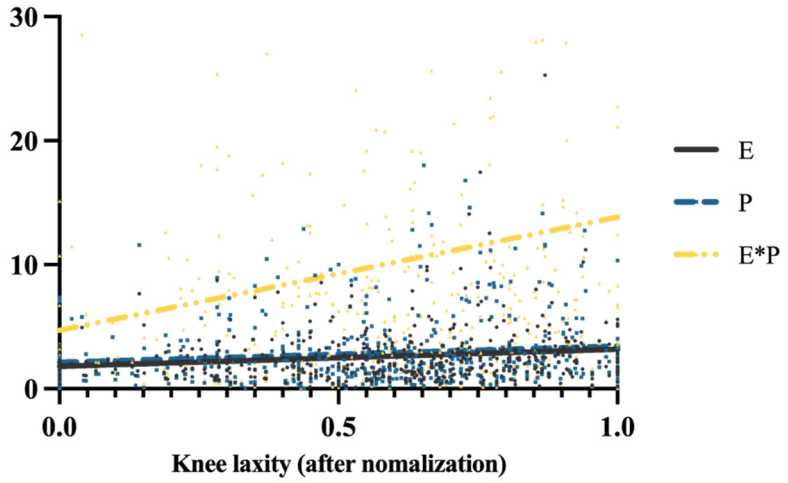

Figure 4. Scatter plot with the fitting line of sex hormones and knee laxity at 133 N. The equations of knee laxity: Knee laxity = 0.4590203 + 0.38251 * (Estradiol) + 0.0278159 * (Progesterone) − 0.0039217 * (Estradiol * Progesterone)
Adj R-squared = 0.0379
E: Estradiol; P: Progesterone

Discussion

As the influence of the menstrual cycle on exercise performance may be mediated by fluctuations in female steroid hormones, serum estrogen and progesterone levels have been recommended as the gold standard for research. Salivary hormone measurement is convenient and non-invasive, but has limitations due to the low concentrations of estrogen and progesterone in saliva (Janse et al., 2019). For estradiol and progesterone, testing with a urine sample is a good alternative to serum testing (Newman et al., 2019). In several instances, measured ACL laxity values lagged behind the expected values based on urinary sex hormone concentrations. Conversely, values for mean estrone conjugates lagged behind concentrations of serum estradiol by up to one day, whereas daily urinary pregnanediol-3-glucuronide profiles lagged behind serum progesterone by one to two days (Stanczyk et al., 2018). Existing studies suggest that relying solely on hormonal changes at limited time points (e.g., two time points) to determine menstrual cycle phases may compromise accuracy (Brown et al., 2025). Such imprecise phase classification could lead to substantial hormonal variability within the same phase due to potential contamination by samples from different stages. In this experiment, to address these variations, we implemented multipoint monitoring of urinary sex hormone metabolites throughout a complete menstrual cycle to minimize lag-induced errors. In summary, urinary sex hormone metabolites appeared to be more appropriate than serum-based measures for investigating the correlation with ACL laxity.

This study recorded the concentrations of estradiol, progesterone and testosterone metabolites in the urine of 15 youth athletes and the corresponding laxity of the ACL each morning for a 27–34 day period. The findings demonstrated a statistically significant positive correlation between ACL laxity and both estradiol and progesterone concentrations, while no significant correlation was observed with testosterone. In

summary, the findings of the present study demonstrate a positive correlation between the levels of estradiol and progesterone, on the one hand, and the degree of laxity of the ACL, on the other. This relationship suggested a hormonally mediated mechanism contributing to increased ligamentous laxity. These findings align with previous studies using serum sex hormones, which suggested that urinary sex hormone measurements could also be used to predict ligament laxity (Gilmer et al., 2025; Shagawa et al., 2021; Shultz et al., 2010). Due to the young age of the study participants and the fact that they were all adolescent females with low testosterone levels, quantifying testosterone's relative impact remains challenging for the ACL laxity compared to estradiol or progesterone. Collectively, the data suggested that ACL responsiveness to sex hormones varied among individuals. This is one possible reason for the lack of significant correlations between testosterone and ACL laxity in all subjects. In addition to hormonal influences, factors such as genetic predisposition, training habits, the lifestyle, and previous trauma may also impact ACL laxity. It has been suggested that hormone-induced increases in knee laxity may not be directly related to ligament stiffness and may not directly affect the risk of injury, but may have an indirect effect by impairing postural control (Legerlotz and Nobis, 2022).

A regression model was developed to predict knee laxity based on individual hormone concentrations in female adolescent athletes. The regression model identified that both estradiol and progesterone could be used for prediction. Estradiol suppresses type I and III procollagen production in ACL fibroblasts in vitro, compromising the mechanical strength of the ACL (Gilmer et al., 2025). Progesterone has been shown to counteract the inhibitory effect of estradiol on collagen production (Yang et al., 2023). A reduction in collagen generation has the potential to compromise connective tissues, encompassing tendons, ligaments, and muscles. Shultz et al. (2004) suggested that the interaction of estradiol and testosterone was a significant predictor of knee laxity. Contrary to the findings of Shultz et al. (2004), the present study found that the combination of estradiol and progesterone, rather than testosterone, significantly explained the variability in ACL laxity. Although testosterone may be an agonist that increases knee laxity, the low levels of testosterone in adolescent females did not permit the demonstration of this effect in the present experiment.

This study identified statistically significant variations in ACL laxity throughout the menstrual cycle of all 15 study participants. The results demonstrated the greatest laxity in the ACL during the luteal phase, followed by the ovulatory phase and then the follicular phase. This finding indicates that the menstrual cycle and the level of sex hormones exert a substantial influence on anterior knee laxity. A literature review found three studies that reported the greatest anterior laxity of the knee during the luteal and ovulatory phases, which was significantly greater than during the follicular phase (Balachandar et al., 2017). However, further six studies reviewed failed to find any statistically significant effect of the menstrual cycle on ACL laxity. It should be noted that those studies only collected samples for a single day and did not consider sex hormones. Further studies found greater knee laxity during the ovulatory phase and reported that knee laxity was greater before ovulatory than after ovulatory (Balachandar et al., 2017; Herzberg et al., 2017; Lee and Petrofsky, 2018; Westin et al., 2018). Maruyama et al. (2022) concluded that female athletes had significantly higher prevalence of genu recurvatum in the late follicular, ovulatory, and luteal phases than in the early follicular phase, although there were no significant differences between the heavy and light menstrual groups. The study's findings indicate that female athletes may potentially face an elevated risk of ACL injury from the late follicular phase to the luteal phase. The findings of the aforementioned studies indicate that the menstrual cycle exerts a substantial influence on the laxity of the ACL and on anterior laxity of the knee during luteal and ovulatory phases.

The prevailing view maintains a direct association between knee laxity and ligament injuries, with heightened ligament injury susceptibility correlating with increased knee laxity (Ye et al., 2023). This conclusion, however, contrasts with an earlier systematic review demonstrating the peak ACL injury incidence between the post-menstrual and pre-ovulatory phases (Balachandar et al., 2017). According to the current study, during this term, knee laxity is at its

lowest point (stiffest), which would typically offer better joint stability and a reduced risk of ligamentous injury. Consequently, knee laxity may not serve as a comprehensive indicator of knee injury predisposition. Conversely, in cases of increased ligament laxity, greater joint flexibility is advantageous. Thus, joint injury incidence decreases. ACL has tensile and viscoelastic properties. Tensile strength facilitates joint mobility and restricts excessive displacement under loading. Viscoelastic properties govern ligament deformation behavior under loading, and they include strain, hysteresis, and stress relaxation. The tensibility is the base of the ligament to stabilize the joints and viscoelastic properties can protect the ligaments to a certain extent (Cui et al., 2022; Oftadeh et al., 2018). On the other hand, as reported in related studies listed above, the level of sex hormones in the body has an effect on the flexibility of the knee, and likewise for soft tissues. This is beneficial in that the increased joint flexibility can reduce the risk of ligamentous injury. In conclusion, ACL laxity was significantly and positively correlated with urinary estradiol and progesterone levels. The menstrual cycle (especially during the luteal phase) had a significant effect on ACL laxity. The regression equation developed in this study to analyze female adolescent soccer players accounted for 7.39% of the variation in ACL relaxation with urinary sex hormones.

However, two primary limitations should be acknowledged. First, the study population comprised female adolescents with largely regular menstrual cycles, while athletes in this age group are susceptible to menstrual dysfunction as part of the female athlete triad, which can influence hormone metabolism and confound findings. Future research should include participants with menstrual irregularities to evaluate the generalizability of results. Second, the sample size was relatively small, potentially limiting the power to detect subtle hormone-laxity interactions. Subsequent studies with larger cohorts are warranted to validate and expand upon these findings.

Practical Implications

This study utilized direct measurements of urinary hormone concentrations to determine menstrual cycle phases based on observed fluctuations. Further research is needed to investigate prospectively the long-term relationship between sex hormone levels and ACL laxity or ACL injury in female adolescent athletes and to determine an optimal level of sex hormones in the body and an optimal ACL condition for reducing the risk of injury. Further prospective research is needed to investigate the long-term relationship between sex hormone levels and ACL laxity or injury risk in competitive adolescent female athletes. Given the non-invasive and convenient nature of urinary sex hormone testing, this method may offer a novel and practical strategy for injury prevention. In this study, the detection of sex hormones in urine demonstrated comparable efficacy as in serum. Based on these findings, the development of a urine-based diagnostic kit could assist adolescent female athletes in monitoring ACL laxity and tailoring training regimens to mitigate injury risk.

Author Contributions: Conceptualization: Y.Q. and D.S.; methodology: Y.Q.; software: Y.H. and B.L.; validation: Y.H., D.S. and B.L.; formal analysis: Y.H. and B.L.; investigation: D.S. and B.L.; resources: Y.Q.; data curation: D.S.; writing—original draft preparation: Y.H., D.S. and B.L.; writing—review and editing: R.S. and X.Z.; visualization: L.C., H.M. and Y.S.; supervision: Y.Q.; project administration: Y.Q.; funding acquisition: Y.Q. and D.S. Additionally, Y.H., D.S. and B.L. are co-first authors. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted following the principles of the Declaration of Helsinki, and approved by the ethics committee of the Shanghai YangZhi Rehabilitation Hospital (Shanghai

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Informed Consent: Informed consent was obtained from all participants included in the study.

Conflicts of Interest: The authors declare no conflict of interest.

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