Effect of menstrual cycle phase, menstrual irregularities and hormonal contraceptive use on anterior knee laxity and non-contact anterior cruciate ligament injury occurrence in women: a protocol for a systematic review and meta-analysis

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ABSTRACT
Exercising women report three to six times more ACL tears than men, which happen, in the majority of cases, with a non-contact mechanism. This sex disparity has, in part, been attributed to the differences in reproductive hormone profiles between men and women. Many studies have shown that anterior knee (AK) laxity and the rate of non-contact ACL injuries vary across the menstrual cycle, but these data are inconsistent. Similarly, several studies have investigated the potential protective effect of hormonal contraceptives on non-contact ACL injuries, but their conclusions are also variable. The purpose of this systematic review and meta-analysis is to identify, evaluate and summarise the effects of endogenous and exogenous ovarian hormones on AK laxity (primary outcome) and the occurrence of non-contact ACL injuries (secondary outcome) in women. We will perform a systematic search for all observational studies conducted on this topic. Studies will be retrieved by searching electronic databases, clinical trial registers, author’s personal files and cross-referencing selected studies. Risk of bias will be assessed using the Newcastle Ottawa Quality Assessment Scale for Cohort and Case–Control Studies. Certainty in the cumulative evidence will be assessed using the Grading of Recommendations Assessment, Development and Evaluation approach. The meta-analyses will use a Bayesian approach to address specific research questions in a more intuitive and probabilistic manner. This review is registered on the international database of prospectively registered systematic reviews (PROSPERO: CRD42021252365).

BACKGROUND
The participation of girls and women in sport has increased worldwide, both in recreational and professional practice. This growth in and development of women’s sport has resulted in a growing number of reports regarding the nature and rate of injuries sustained by sportswomen. Depending on the age-group, the
sport and the level of practice, women report different rates of musculoskeletal, sports-related injuries than their male counterparts.\(^1\) As one of the most prominent musculoskeletal injuries, exercising women report three to six times more ACL tears than men,\(^3\) which occur, in the majority of cases, via a non-contact mechanism.\(^5\) Most non-contact ACL injuries happen during fast-paced multidirectional activities (eg, snow skiing, netball, football, rugby, gymnastics).\(^7\) The sex disparity for non-contact ACL injuries starts at the adolescent growth spurt and peaks during adolescence.\(^8\) This sex difference has been attributed to several factors that also emerge at this time, namely: anatomical (eg, laxity, body composition), physiological (especially hormonal), biomechanical, neuromuscular recruitment patterns\(^9\) and gendered factors present in the developmental environment.\(^10\) The potential impact of hormones on the mechanisms underpinning non-contact ACL injuries deserves greater attention given the numerous differences in the concentration of reproductive hormones between sexes and the time course of reproductive endocrinology, especially in women.

Ovarian hormone profiles vary between and within women and are not stable over a woman’s lifespan (eg, they change across phases of the menstrual cycle, as a result of hormonal contraceptive use, during pregnancy and following menopause). Ovarian hormones influence the structure of all soft tissues (ie, muscles, tendons, and ligaments) by determining their collagen metabolism (Liu et al\(^4\); data from rabbits; Yu et al\(^2\) and Konopka et al\(^5\); data from human ACL cells), and structural integrity (Konopka et al\(^6\); data from human ACL cells; Lee et al\(^4\); data from engineered ligaments). Alterations of the ACL structure, caused by fluctuations in ovarian hormone levels, may increase the risk for potential ligament failure (Lee et al\(^4\); data from engineered ligaments; Yu et al\(^2\); data from human ACL cells). Indeed, it has been suggested that women’s ACLs and musculoskeletal systems react to changes in the reproductive hormone milieu, thus changing their properties at certain points of the lifespan corresponding to different hormonal profiles.\(^15\)

In the last two decades, many studies have shown that anterior knee (AK) laxity\(^16\) and the rate of non-contact ACL injuries\(^17\) change during different phases of the menstrual cycle in eumenorrheic women, although the findings from studies in this area are inconsistent. Several studies have also been conducted on the potential protective effect of hormonal contraceptives, especially oral contraceptive pills (OCPs), on non-contact ACL injuries, due to their users having a consistently downregulated endogenous ovarian hormone profile, although these data are also inconsistent.\(^18\) These inconsistencies in findings (ie, menstrual cycle phase and OCP use) might be due to poor methodological quality, especially with regards to the definition and confirmation of menstrual cycle phases and the heterogeneity of hormonal contraceptives used in these studies (for a comprehensive overview of methodological issues see Elliott-Sale et al\(^19\)).

A systematic review and meta-analysis published in 2017\(^18\) concluded that the quality of evidence (ie, data published up to August 2016), on the effect of the menstrual cycle and hormonal contraceptives on the laxity of the ACL and the occurrence of non-contact injuries to the ACL, was ‘very low’, due to numerous methodological shortcomings affecting the eligibility of the participants. Our systematic review and meta-analysis will expand the review by Herzberg et al\(^18\) by: (i) including studies published up to and since August 2016, (ii) performing a meta-analysis on the injury data and not just the laxity data, (iii) employing different inclusion/exclusion criteria and (iv) including women with menstrual irregularities. In addition, our review will adopt a different statistical method (ie, a Bayesian approach) to allow for a more intuitive and probabilistic synthesis and interpretation of existing data. Therefore, the purpose of this systematic review is to identify, evaluate and summarise the effects of endogenous and exogenous ovarian hormones on knee joint laxity and occurrence of non-contact injuries of the ACL in women.

We hypothesise that: (i) AK laxity will differ in response to the fluctuations in endogenous ovarian hormones that occur at different phases of the menstrual cycle, leading to an increased occurrence of non-contact ACL injury, (ii) AK laxity and the occurrence of non-contact ACL injury would be greater in non-hormonal contraceptive users.

**METHODS**

The protocol for this aetiology systematic review and meta-analysis follows the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols\(^20\) and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) for Searching (PRISMA-S)\(^21\) and is registered with the International Prospective Register of Systematic Reviews (PROSPERO) registration number CRD42021252365.

**Eligibility criteria**

Studies will be selected according to the PECOS (ie, participants, exposures, comparator, outcomes, study designs) criteria (table 1). There will be no restrictions on the time frame or setting of the studies. Studies reported in English, French, Spanish, Portuguese and German languages will be considered. A list of possibly relevant titles in other languages will be provided as an appendix if relevant.

**Information sources**

Search strategies will be developed using text words related to the population, exposures and outcomes. Five electronic databases will be searched from their inception onwards: PubMed Central (includes MEDLINE), SPORTDiscus (via EBSCOhost interface), Scopus, the Cochrane Central Register of Controlled Trials and ProQuest Central: Health and Medical Collection; Nursing and Allied Health; Research Library: Health
Table 1 Overview of PECOS eligibility criteria

| Participants     | Human female athletes (defined as one who takes part in an individual or organised team sport wherein: (i) they compete regularly against others; (ii) excellence and achievement are emphasised and (iii) systematic intensive training is required\textsuperscript{35} and female exercisers (defined as one who engages in physical activity with the will to: (i) augment their fitness level; (ii) improve their health; (iii) ameliorate their physique and (iv) acquire or improve skills\textsuperscript{35}) of reproductive age (ie, postmenarche and premenopausal) will be included. Specifically, eumenorrheic, naturally menstruating women, women with menstrual irregularities (eg, oligomenorrhoea, polymenorrhoea, amenorrhoea, anovulatory and luteal phase deficient cycles) and hormonal contraceptive users (eg, combined and progestogen-only OCPs, injections, implants, patches, intra-uterine systems) will be included; with pregnant and perimenopausal women excluded. Participants must not be using any form of medication known to affect ovarian hormone profiles (with the exception of hormonal contraceptives) or the musculoskeletal system. |
| Comparators      | Where relevant, hormonal contraceptive users will be compared with non-hormonal contraceptive users. |
| Exposures        | Outcomes relating to the physical assessment of AK laxity (primary outcomes) and the occurrence of non-contact ACL injuries (secondary outcomes). The primary outcomes are focused on micro changes (ie, physiological changes to the AK laxity that potentially occur due to changes in ovarian hormone concentrations) and the secondary outcomes are focused on macro changes (ie, number of non-contact ACL injuries that may potentially occur due to micro changes). AK laxity refers to the degree of tightness/looseness of the AK in a sagittal plan; in the knee, ligaments are present to connect and stabilise the various bones that are present by keeping the knee joint flexible enough to move but also firm enough to provide support. It is measured using (i) clinical examination (eg, Lachman test—manual test to assess the AK laxity; subjective measure) and (ii) equipment designed to evaluate the AK laxity by quantifying the anterior displacement of the anterior tibial tubercle relative to the femur when a predefined anteriorly directed force is applied, from the upper calf (eg, arthrometers; objective measure). |
| Study designs    | Observational studies will be considered for inclusion if they meet the following inclusion criteria: (i) published, in full, in a peer-reviewed journal; (ii) have the objective of assessing changes in AK laxity in response to phases of the menstrual cycle, menstrual irregularities and/or hormonal contraceptive use and (iii) report the incidence of ACL injuries aligned with phases of the menstrual cycle, menstrual irregularities and/or hormonal contraceptive usage. Cohort studies and case–control studies will be included when reporting primary outcomes (ie, the physical assessment of AK laxity). Cross-sectional studies, cohort studies and case–control studies will be included when reporting secondary outcomes (ie, the occurrence of non-contact ACL injuries). Case studies, review articles, protocol papers, editorials, conference abstracts and commentaries will be excluded. |

AK, anterior knee; OCPs, Oral contraceptive pills.

and Medicine. The electronic database search will be supplemented by searching for trial protocols through three registers: Clinical Trials (www.clinicaltrials.gov), EU Clinical Trials Register (www.clinicaltrialsregister.eu) and International Standard Randomised Controlled Trial Number (ISRCTN) (www.isrctn.com). To ensure literature saturation, the reference lists of included studies or relevant reviews identified, which may have been identified through the initial search strategy, will also be hand searched. All authors will search their personal files to make sure that all relevant material has been identified.

Search strategy

The PubMed Central search strategy will be developed with input from all authors using the Peer Review of Electronic Search Strategies standard.\textsuperscript{22} In addition, the search strategy will be peer-reviewed by a research librarian who has expertise in systematic review searching and is not otherwise associated with the project. A draft search strategy for PubMed Central is included in online supplemental file 1. Once the PubMed Central search strategy is finalised, the search strategy will be adapted to the syntax and subject headings of the other databases. The search will be updated toward the end of the review, prior to publication, to retrieve any articles published during the interim period.

Study record

All search results will be uploaded and stored in a systematic review management platform (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia), which will be accessible to all reviewers. Covidence will automatically remove duplicates by checking the following fields: titles, year, volume, authorship. Two reviewers will independently check the duplicates removed by Covidence and verify their accuracy.

Titles and abstracts will be independently screened by two reviewers, guided by the inclusion and exclusion criteria. Disagreements will be resolved with a consensus-based discussion, and, when in doubt, articles will be carried forward to full-text review. The full text of eligible papers, based on the titles and abstracts, will be downloaded and independently screened. If the reviewers are not in agreement, a third reviewer will be consulted and will provide recommendations. The reviewers will use the annotation facility on the decision dashboard to explain their decision and inform further discussions. If a study is reported in more than one publication, the multiple
reports will be collated. When in doubt regarding the
eligibility criteria of a study, the reviewers will contact the
authors; with a maximum of three attempts, two emails
and one phone call (if possible), over a 4-week period.
Any ongoing trials, which have not yet been reported, will
be recorded, so that they can be added to the ongoing
studies table. A PRISMA flowchart detailing the search
and selection process will be included (see online supple-
mental file 1 for a draft template); as well as a list of all
full-text studies excluded, detailing the specific reason
for exclusion.
A data extraction template will be created based
on those used in similar meta-analyses. Data will
be extracted by two reviewers. To ensure consistency
across reviewers, calibration exercises will be conducted
before starting the data collection process (ie, the data
extraction form will be pilot-tested by each reviewer on
five randomly selected studies). When outcome data are
not reported in a usable format (ie, in a figure instead
of a numerical format) specialist software will be used to
extract the data from the figure (eg, WebPlotDigitizer
Version 4.4).
In order to avoid double-counting data, records will be
scrupulously compared, for example, juxtaposing author
names, treatment comparisons, sample sizes and/or
outcomes. If the same study data are reported in more
than one publication, all publications will be treated
as one dataset rather than multiple datasets. When we
extract data, we will prioritise the following criteria:
greatest number of participants, longest follow-up and
primary reports where the primary outcome assessed
is most relevant to our research questions. If the data
differ across publications, it will be noted, investigated,
and the authors contacted for more information; with a
maximum of three attempts, two emails and one phone
call, over a 4-week period.
Disagreements will be recorded and resolved with a
consensus-based discussion between the two reviewers.
Any disagreement that cannot be resolved will be
referred to a third reviewer who will provide a recom-
mendation. Study authors will be contacted if there are
any doubts about the extracted data, with a maximum
of three attempts, two emails and one phone call, over
a 4-week period. If any disagreement cannot be resolved
(i.e., either through discussion between the reviewers or
with the authors) the disagreement will be reported in
the review.

Data items
Reviewers will extract data on the following: (i) study
characteristics (ie, design, location, sources of funding,
study aim), (ii) participant characteristics (ie, eligibility
criteria, age, height, body mass, body mass index, training
status, etc), (iii) exposure and comparison characteristics
(i.e., type, dosage, and duration of hormonal contraceptive
use, menstrual cycle phase, type of menstrual irregular-
ity, methods of determining participants’ ovarian
hormonal status, etc), (iv) outcome characteristics for
AK (ie, method of assessment, assessment characteris-
tics, etc) and occurrence of ACL injuries (ie, method(s)
used to confirm the injury, profile of the injury (injury
mechanism, context of the injury, primary or recurrent
injury, isolated ACL injury or other collateral structures
injured), etc).

Outcomes and prioritisation
The primary outcomes are the physical assessment of AK
laxity and the secondary outcomes are the occurrence of
non-contact ACL injuries.

Risk of bias assessment
Risk of bias will be initially evaluated at the individual
study level, using the Newcastle Ottawa Quality Assess-
ment Scale for Cohort or Case–Control Studies. The
Newcastle Ottawa Quality Assessment Scale is a domain-
based risk of bias tool that comprises eight items within
three categories to assess the key bias domains: (i) selec-
tion; (ii) comparability and (iii) outcome/exposure. We
have developed coding systems, that are very similar to
formerly published work in our research area, according
to our outcomes (ie, anterior knee laxity and ACL injury
occurrence) and ensured that the assessment is specific
to each outcome. We have opted for using the Newcastle
Ottawa Quality Assessment Scale without the star-rating
system, as the PRISMA explanation and elaboration
states that presenting assessments for each domain in
the tool is preferable to reporting an overall ‘quality score’
because it enables users to understand the specific
domains that are at risk of bias in each study. Accord-
ingly, we will separate the key bias domains covered by
the Newcastle Ottawa Quality Assessment Scale when
assessing bias and we will present the results in a table.

Certainty in cumulative evidence
Certainty will be assessed by two independent reviewers
using a strategy based on the recommendations of the
Grading of Recommendations Assessment Development
and Evaluation working group. Any differences
between reviewers will be resolved by discussion and, if
needed, in consultation with a third reviewer. Certainty
in cumulative evidence will be based on consideration
of five domains, namely risk of bias (assessed using the
NOS as described above), indirectness, inconsistency,
imprecision or evidence of publication bias. Directness
will be ascertained based on the methods used to iden-
tify and confirm menstrual cycle phase, along with injury
confirmation. This information is considered essential,
given that if unconfirmed, any result observed cannot be
directly attributed to the phase under investigation. This
will be evaluated based on the response to two questions:
(Q1) Was the ovarian hormone profile confirmed?

If the authors provide a definition for the sampled
population and report using blood samples to confirm
ovarian hormone status, the a priori rating will be main-
tained, if not the study will be downgraded a level (eg, a
study that is classified as ‘high’ quality, would be downgraded to ‘moderate’ quality).

(Q2) Was the injury medically diagnosed either as part of the study or prior to the study?

These questions are based on methodological conclusions made in previous studies.18 23

Consistency will be ascertained using the meta-analysis results and will be based on visual inspection of effect size and variance estimates across the different levels (eg, within study variation, between study variation and between outcome variation). Precision will be judged based on the number of outcomes available (with outcomes based on <3 data points downgraded) and on interpretation of width of the credible intervals (CrIs). Small-study effects (ie, publication bias) will be visually inspected with funnel plots and quantified with a multi-level extension of Egger’s regression-intercept test.29 Collectively, these procedures will result in a final level of certainty for each outcome (table 2): namely of ‘high’, ‘moderate’, ‘low’ or ‘very low’. This certainty appraisal strategy will not be used to exclude any study.

Data synthesis

Data will be presented in summary tables, which will describe the study characteristics and outcomes. A Bayesian framework was chosen over a frequentist approach as it provides a more flexible modelling approach that will enable results to be interpreted intuitively through reporting of subjective probabilities rather than null hypothesis tests or frequentist CIs.28 For the approach that will enable results to be interpreted intuitively through reporting of subjective probabilities rather than null hypothesis tests or frequentist CIs.

By including both AK laxity and the occurrence of non-contact ACL injuries in women, we will provide an up-to-date, detailed summary and interpretation of the current state of the art of this topic. Furthermore, this meta-analysis will examine the strength of the outcomes and indicate methodological considerations for future research. The findings of this review will have practical implications for female athletes (elite to recreational) and for those working with active women.

DISCUSSION

This systematic review and meta-analysis will synthesise evidence to evaluate the effects of various levels of endogenous and exogenous ovarian hormones on AK laxity and the occurrence of non-contact ACL injuries in women. By including both AK laxity and the occurrence of non-contact ACL injuries in naturally menstruating women, women with menstrual irregularities and hormonal contraceptive users, we will provide an up-to-date, detailed summary and interpretation of the current state of the art of this topic. Furthermore, this meta-analysis will examine the strength of the outcomes and indicate methodological considerations for future research. The findings of this review will have practical implications for female athletes (elite to recreational) and for those working with active women.

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Table 2  Significance of the four certainty of evidence categories

| Level     | Description                                                                 |
|-----------|-----------------------------------------------------------------------------|
| High      | Confident that the true effect lies close to that of the estimate of the effect |
| Moderate  | Moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different |
| Low       | Confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect |
| Very low  | Little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of the effect |
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