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Protocol for a systematic review and evidence map: does the dose threshold for spermatogenic recovery shift with age at exposure? Age-dependent gonadal outcomes after gonadotoxic therapy in males

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ADMINISTRATIVE INFORMATION

Support - Zhejiang Provincial Natural Science Foundation Grant.

Review Stage at time of this submission - Risk of bias assessment.

Conflicts of interest - None declared.

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 30 October 2026 and was last updated on 30 October 2026.

INTRODUCTION

Review question / Objective To determine whether the association between age at exposure and post-treatment gonadal outcome in males varies by the outcome measured and the cell compartment implicated, and by the testicular dose received - that is, whether the dose threshold for spermatogenic recovery is modified by age at exposure.

PICOS

Population: males of any age at exposure who received testicular or scattered radiotherapy, alkylating chemotherapy, or total body irradiation, as cancer treatment or as conditioning for haematopoietic stem cell transplantation.
Exposure: age at exposure, analysed against a gonadal outcome; and, where reported, quantitative testicular dose in gray.

Comparator: younger versus older age at exposure; lower versus higher testicular dose.
Outcomes: any measure of gonadal function in males - semen parameters, azoospermia, gonadotrophins, testosterone or need for testosterone substitution, testicular volume, or paternity.
Study designs: cohort, cross-sectional and case-control studies, and secondary analyses of randomised trials.

Secondary objectives are to describe the direction of the reported association in each study; to distinguish age at exposure from age at survey; to map the testicular dose levels actually compared against the thresholds proposed by PENTEC; and to establish whether any study has formally tested a dose x age interaction.

Rationale Male survivors of childhood and adolescent cancer are at risk of permanent gonadal damage, and

fertility counselling after treatment depends on knowing which exposures are tolerable and which are not. The 2024 PENTEC dose-effect review quantified this for the first time, proposing a spermatogenic threshold of approximately 1 Gy and hormonal thresholds of 0.2-0.5 Gy. That synthesis modelled dose but not age at exposure, although testicular radiosensitivity is widely assumed to change with pubertal status. Conversely, the many studies that examine age at exposure seldom quantify testicular dose, and their findings conflict: some report worse outcomes in younger patients, others in older patients, and the majority report no association. The clinical question that neither literature answers is whether the dose threshold itself moves with age, which would determine whether dose-based counselling needs an age adjustment.

This review will cross-classify the direction of the reported age effect by outcome domain (semen versus hormonal) and by cell compartment, distinguish age at exposure from age at survey, and map the dose levels that have actually been studied against the postulated thresholds. We do not expect to produce a pooled estimate of effect. We expect to establish where the evidence is absent, whether the apparent disagreement between studies is explained by what they measure, and what design would be required to answer the threshold question.

Condition being studied Male gonadal dysfunction after gonadotoxic therapy. Two functional compartments are distinguished. Germ-cell failure presents as oligozoospermia or azoospermia, with reduced testicular volume and raised FSH, and may be temporary or permanent depending on dose. Leydig-cell failure presents as compensated or overt hypogonadism - raised LH with low or low-normal testosterone - and may require testosterone substitution. Both arise after testicular or scattered irradiation, after alkylating chemotherapy, and after total body irradiation used in transplant conditioning; they differ in threshold dose and in capacity for recovery, and this difference is central to the review.

METHODS

Search strategy Database: PubMed/MEDLINE, searched from inception to 28 October 2026. No language or date limits.

Strategy (as executed):
(radiotherap*[tiab] OR irradiation[tiab] OR gonadotoxic*[tiab]
OR alkylating[tiab] OR cyclophosphamide[tiab] OR busulfan[tiab])
AND
(sperm*[tiab] OR semen[tiab] OR azoospermia[tiab] OR spermatogenesis[tiab])
AND humans[MeSH]
NOT (review[pt] OR "systematic review"[pt] OR "meta-analysis"[pt])

This returned 1042 records. Because the review question requires a within-cohort analysis of age rather than a mere mention of age, screening was preceded by a restriction to records indexed as cohort, follow-up or cross-sectional studies, obtained by adding:
AND (cohort[tiab] OR survivor*[tiab] OR "follow-up"[tiab] OR longitudinal[tiab])
This subset comprised 257 records and was screened in full at title/abstract and, where relevant, at full text.

Citation searching: the reference lists of three prior syntheses were screened - the 2024 PENTEC review of testicular dysfunction after radiation therapy (PMID 37791936), a systematic review of gonadal function after haematopoietic stem cell transplantation in childhood (PMID 35644480), and a systematic review and meta-analysis of gonadal and fertility outcomes after Hodgkin lymphoma treatment (PMID 41681897). These contributed 178 records, from which 51 original studies were assessed.

No other databases were searched. Embase, Scopus, Web of Science, CENTRAL, CINAHL, PsycINFO, CNKI and LILACS were not searched; trial registers, conference proceedings and dissertation databases were not searched; and authors were not contacted. These omissions are stated so that the coverage of the search can be judged.

Participant or population Males of any age at exposure who received testicular or scattered radiotherapy, alkylating

chemotherapy, or total body irradiation, either as cancer treatment or as conditioning before haematopoietic stem cell transplantation. One included population is non-malignant (beta-thalassaemia major treated with bone marrow transplantation), retained because the gonadal exposure and outcomes are directly comparable. Studies reporting fewer than 10 male participants are excluded.

Intervention Not an intervention review. The exposure is age at exposure - the age at which gonadotoxic treatment was given - analysed against a gonadal outcome, and modelled in one of three ways: as a continuous variable, as one or more arbitrary cut-points, or as pubertal status at treatment. Where reported, quantitative testicular dose in gray is recorded as a second exposure dimension. Studies in which age entered only as an adjustment covariate, without a reported estimate for age itself, are retained but contribute no direction.

Comparator Younger versus older age at exposure; and lower versus higher testicular dose. Studies without an internal comparison are retained only if they report a within-cohort analysis of age versus outcome.

Study designs to be included Cohort, cross-sectional and case-control studies, and secondary analyses of randomised trials, reporting an analysis of age at exposure versus a gonadal outcome in males. Excluded: reviews, guidelines, animal and in-vitro studies, case reports and series with fewer than 10 males, and studies with no age-outcome analysis.

Eligibility criteria Additional criteria beyond PICOS: studies must report original data and be peer-reviewed publications; conference abstracts and unpublished reports are not eligible. Studies examining gonadal tissue obtained before treatment are not excluded, but are analysed as a separate pre-treatment stratum, because they describe baseline susceptibility rather than recovery. Age at survey or at assessment is recorded as a distinct construct rather than substituted for age at exposure.

Information sources PubMed/MEDLINE (inception to 28 October 2026). Reference lists of three prior systematic reviews

(PENTEC 2024, PMID 37791936; paediatric HSCT review, PMID 35644480; Hodgkin lymphoma meta-analysis, PMID 41681897).

No other electronic databases were searched. Trial and study registers were not searched; conference proceedings and dissertation databases were not searched; grey literature was not sought; and study authors were not contacted. No individual participant data were requested.

Main outcome(s) Direction of the reported age effect on gonadal outcome, classified as older-worse, no association, younger-worse, or undetermined. Direction is taken from each study's own reported analysis, not from re-analysis.

Timing: outcome assessed at any time after completion of treatment; time from treatment to assessment is recorded where reported.

Measurement: any reported statistical analysis of age in relation to a gonadal outcome, whether age was modelled continuously, as arbitrary cut-points, or as pubertal status at treatment.

Effect measure for synthesis: no pooled effect measure is used. Direction is tabulated as the proportion of studies in each category, overall and by outcome domain (semen versus hormonal) and by cell compartment (germ cell versus Leydig cell).

Additional outcome(s) (1) Effect estimates for the age-outcome association: odds ratios, risk ratios, hazard ratios or correlation coefficients with 95% confidence intervals. Estimates are oriented so that values above 1 denote worse outcome in older patients, and inverted where the original contrast was reversed.

(2) Testicular dose examined: the dose levels or ranges in gray actually compared within each study, mapped descriptively against the PENTEC thresholds.

(3) Whether a dose x age interaction was tested, recorded as yes or no together with the reported result.

(4) Sample size and time from treatment to outcome assessment.

Data management Records are managed in a reference manager with de-duplication before screening. Screening decisions and reasons for exclusion at full text are recorded in a screening log, and reported in a PRISMA 2020 flow diagram.

Data are extracted into a piloted electronic form held in a single version-controlled workbook, with one row per study and a fixed column structure covering study identifiers, design, population, sample size, treatment class, testicular dose, outcome definition, cell compartment, how age was modelled, direction of association, effect estimate with confidence interval, and whether a dose x age interaction was tested.

Extraction is performed by one reviewer in two passes separated by at least two weeks; discrepancies between passes are resolved against the source. The extracted dataset and the analysis scripts will be deposited in a public repository at the time of publication.

Quality assessment / Risk of bias analysis Risk of bias is assessed with ROBINS-E across seven domains: confounding; measurement of the exposure; selection of participants; post-exposure interventions; missing data; measurement of the outcome; and selection of the reported result.

Because most included studies are retrospective and small, judgements follow decision rules fixed before assessment, so that ratings are reproducible. In brief: confounding is rated low only where multivariable adjustment included an alkylating-agent or radiation dose term; exposure measurement is rated high where age at survey was substituted for age at exposure; selection is rated high where fewer than 30 participants were analysed or only a subset was evaluable; and post-exposure interventions are rated low for semen endpoints, which are unaffected by cryopreservation or testosterone substitution.

Two domains are expected to be rated some concerns for all studies: outcome measurement, because all rely on a single measurement occasion; and selective reporting, because none pre-registered its age

analysis. Assessment is performed by one reviewer.

Strategy of data synthesis Synthesis is descriptive and structured rather than statistical. For the primary outcome, the direction of each study's reported age effect is tabulated by outcome domain (semen versus hormonal) and by cell compartment (germ cell versus Leydig), and presented as an evidence map with counts and proportions. Study-level characteristics - sample size, treatment class, dose category and age construct - are summarised alongside.

A random-effects meta-analysis is pre-specified as conditional on at least three studies reporting a comparable estimate for a comparable contrast. Where that condition is not met, estimates are displayed on a common axis but not pooled, because estimates that derive from different contrasts (arbitrary cut-points versus pubertal status) or different outcome definitions cannot be meaningfully combined.

A dose-response meta-analysis is not planned. Testicular dose is quantified in a minority of included studies, and few examine the dose range in which the threshold is postulated to lie; a dose-response model could not be fitted across that range.

Certainty of the body of evidence is assessed with GRADE for two outcome domains (semen endpoints; hormonal and Leydig-cell endpoints) and for the dose-threshold question.

Subgroup analysis Direction is tabulated descriptively within the following subgroups:

- outcome domain: direct semen endpoints versus surrogate or hormonal endpoints;
- cell compartment: germ cell versus Leydig cell versus both;
- treatment class: radiotherapy, alkylating chemotherapy, haematopoietic stem cell transplantation, mixed;
- testicular dose band: below 0.5 Gy, 0.5-1 Gy, 1-6 Gy, 6-20 Gy, above 20 Gy;
- how age was modelled: continuous, arbitrary cut-points, or pubertal status.

These are descriptive stratifications. No formal test of subgroup difference is planned, because most

strata contain fewer than five studies and the review is not designed to estimate effect magnitudes.

Sensitivity analysis Eight sensitivity analyses are pre-specified:

S1 restricting to studies in which age was recorded at exposure rather than at survey;
 S2 restricting to studies with at least 50 male participants;
 S3 analysing semen endpoints and hormonal endpoints separately;
 S4 restricting to studies that specifically assessed Leydig-cell function;
 S5 where more than one study drew on the same source cohort, retaining a single study (the one with the largest analysable sample) to avoid double counting;
 S6 restricting to studies that reported a quantitative testicular dose;
 S7 restricting to studies whose age analysis was verified against the full text;
 S8 excluding any study included on the basis of its abstract only.

Direction by publication era, and a formal comparison of direction across age-modelling categories, are exploratory rather than pre-specified, and will be labelled as such.

Language restriction No language restriction was applied in the search or in screening. All included reports were published in English.

Country(ies) involved China.

Other relevant information Registration timing. This protocol is registered retrospectively, after formal searching, screening and data extraction had been completed, and before any data synthesis. Prospective registration with PROSPERO was not possible because PROSPERO does not accept initial registrations once a review has progressed beyond the data extraction stage. Following the transparency-oriented approach recommended for retrospectively registered protocols, the stage at registration is declared explicitly here and in the registration record.

What was done before registration: the search was executed and archived; screening at title/abstract

and at full text was completed; data were extracted in two passes; and the first pass of ROBINS-E

assessment was performed. What was fixed before analysis: the definitions of direction, age construct, cell compartment, outcome domain and dose completeness; the eight pre-specified sensitivity analyses;

and the decision rules for risk of bias. No synthesis had been undertaken at the time of registration.

Two design features require disclosure. First, screening was preceded by a restriction of the database

set to cohort, follow-up and cross-sectional records (1042 to 257 records); records outside those

design categories were not screened, which is a potential source of missed studies. Second, screening,

data extraction and risk-of-bias assessment were performed by a single reviewer; duplicate extraction

was performed in two passes separated by at least two weeks, but no independent second reviewer was

available.

For every potentially eligible study, the presence or absence of an age analysis was verified against the full text rather than the abstract. Studies initially classified as reporting no age analysis were re-examined, four were reclassified, and one further study was excluded after inclusion because age had been analysed only in women.

Keywords testicular irradiation; spermatogenesis; Leydig cell; age at exposure; dose threshold; male fertility; systematic review; evidence map.

Dissemination plans Submission to a peer-reviewed journal in reproductive medicine, andrology or radiation oncology; presentation at a relevant scientific meeting; and public deposition of the extracted dataset and analysis code at the time of publication.

Contributions of each author

Author 1 - Linxi Li - Conceived the review question, designed the protocol, conducted the search, screening, data extraction, risk-of-bias assessment and analysis, and drafted the manuscript. Email: lilinxi1234@163.com