ABSTRACT

Introduction Infertility is a focal issue in public health and affects human reproduction and survival. Notably, an increasing number of studies in recent decades have found that sperm DNA integrity plays a critical role in the development of healthy embryos. Among the multiple pathogenic factors of sperm DNA fragmentation, oxidative stress has proven to be predominant. Coenzyme Q10 supplementation, which has been used for the treatment of male infertility, has shown good clinical efficacy due to its oxidation resistance, but its efficacy as measured by the sperm DNA fragmentation index remains controversial. To address this issue, we will perform a systematic review and meta-analysis to evaluate the efficacy of coenzyme Q10 for male infertility patients with a high sperm DNA fragmentation index.

Methods and analysis The PubMed, Embase, Cochrane Central Register of Studies and Web of Science databases will be comprehensively searched from inception to 31 December 2022 to identify relevant studies published in the English language using appropriate search strategies. The search terms will be derived from the following concepts: sperm DNA fragmentation, coenzyme Q10 and randomised controlled trials. Two review stages, that is, title and abstract screening and full-text screening, will be performed by two reviewers. The risk of bias, publication bias and evidence grade of the included studies will be assessed using a standardised protocol. Data will be used to calculate effect sizes. Heterogeneity among the studies will be evaluated graphically. Subgroup analysis and sensitivity analysis will be performed if necessary to validate the results.

Ethics and dissemination No ethical approval will be needed, as there will be no participants in this study. We will follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines to disseminate the findings through publication and conference presentation.

PROSPERO registration number CRD42022293340.

INTRODUCTION

It is well known that normal embryonic development depends on the integrity of complete genetic material from both sperm and oocytes. Among the factors that influence the normal development of embryos and fetuses, the integrity of sperm DNA is important. Multiple studies have demonstrated that sperm DNA integrity plays a crucial role in embryo development and has the capacity to predict the final pregnancy outcome. However, the current evaluation of infertile men commonly relies on semen analysis, which still has a limitation of inaccuracy in predicting male fertility potential and final pregnancy outcome from assisted reproductive technology (ART). The Sperm DNA Fragmentation Index (SDFI), as an indicator reflecting sperm DNA integrity, can offset this limitation.

Oxidative stress (OS) is defined as ‘an imbalance between oxidants and antioxidants in favour of the oxidants, leading to a
disruption of redox signalling and control and/or molecular damage. This biological process participates in the onset of dozens of diseases at the molecular level, especially sperm DNA fragmentation (SDF). Hence, there is an urgent need to identify an effective treatment for male infertility patients with high SDFI by regulating OS status in vivo.

Coenzyme Q10 (CoQ10), a well-known exogenous antioxidant supplement, is an isoprenylated benzoquinone that works by transporting electrons from complexes I and II to complex III. The antioxidant effect of CoQ10 manifests as preventing membrane phospholipid peroxidation and free radical oxidation. Given its excellent antioxidation, CoQ10 supplementation has been applied for decades to improve semen parameters with good clinical efficacy. Although a certain number of clinical studies have confirmed that coenzyme Q10 can improve sperm quality, whether oral CoQ10 can improve SDFI remains controversial. Therefore, we hypothesised that oral coenzyme Q10 alone could improve the SDFI and increase the probability of conception in male infertility patients. The purpose of this study is to systematically review the clinical evidence that CoQ10 supplementation improves SDFI and assess its clinical effect on SDFI through meta-analysis.

This systematic review and meta-analysis will be completed and reported in accordance with the Cochrane Handbook for Systematic Reviews of Interventions and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement.

METHODS AND ANALYSIS
Patient and public involvement
This study will have no patient or public involvement.

Protocol and registration
In accordance with guidelines, this protocol will be conducted by following the PRISMA-Protocols (PRISMA-P) (online supplemental table 1) and registered with the International Prospective Register of Systematic Reviews (PROSPERO) on 14 August 2022 (registration number CRD42022293340).

Criteria for study selection
Inclusion criteria
This study will include published randomised controlled clinical trials on male infertility patients with a high SDF index. It will exclude animal studies, conference papers, case reports and reviews.

Comparators
The intervention will be administration of coenzyme Q10 supplementation alone, in any form (eg, tablet, powder, capsule). Trials whose only intervention combined coenzyme Q10 with other antioxidants (L-carnitine, vitamin E, Zn, etc) will be excluded. The control will be placebo or no intervention.

Literature Search
Two authors will independently search the PubMed, Embase, Cochrane Central Register of Studies and Web of Science databases from inception to 31 December 2022 to identify relevant articles written in the English language. The two authors will also manually review the reference lists of the selected articles to identify additional eligible articles. There will be no limits on publication date. The search strategy will include medical subject heading terms combined with free words (online supplemental table 2).

Article selection
The titles and abstracts will be screened independently by two reviewers in accordance with the inclusion criteria. Next, the full texts of the remaining studies will be carefully read to determine whether they meet the inclusion criteria. Any disagreement between investigators will be resolved through discussion. We will list the reasons for excluding articles (see online supplemental figure 1).

Data extraction and analysis
Data extraction
Two reviewers will perform data extraction independently using a standard data collection form (online supplemental table 3). Excel 2019 software will be used for data recording. Initially, two or three potentially eligible articles will be piloted tested. Divergence will be resolved by consensus. If there is no consensus, a third reviewer will be consulted to resolve the inconsistency.

The following study characteristics will be extracted:
- Study information: author(s) name, title, publication year, lower limit of SDFI (if provided).
- Study methods: study design, total duration of the study, study centres (country).
- Study participants: sample size, age, race, semen parameters at baseline.
- Study intervention: dosage forms of coenzyme Q10, type of oral dose and frequency.
- Study outcomes: primary outcome—SDFI (measured by the sperm chromatin structure assay, terminal deoxynucleotidyl transferase 2’-deoxyuridine 5’-triphosphate (dUTP) nick end labelling and sperm chromatin dispersion); secondary outcome—spouse pregnancy rate (if reported), sperm concentration, normal morphology, total motility and reactive oxygen species level.

Measure of treatment effect
As a change in SDFI is a continuous variable, the treatment effect will be measured using mean difference (MD) with 95% CI. The standardised MD and 95% CI will be used to evaluate effect size because indicators differed in detection methods and units used to measure raw data (such as reactive oxygen species).

Missing data management
If relevant data are missing, corresponding authors will be contacted to obtain, if possible, any data, including source data not presented in the publication. If the data...
are not available on request, we will use informative missingness ORs\textsuperscript{23} to attribute missing data.

**Risk of bias assessment**

Two researchers will evaluate the risk of bias (ROB) from the included studies with the Cochrane Collaboration’s tool for assessing ROB V.2.0\textsuperscript{24} (online supplemental table 4). The main items needed for evaluation will be as follows:

- Bias arising from the randomisation process.
- Bias due to deviations from intended interventions.
- Bias due to missing outcome data.
- Bias in outcome measurement.
- Bias in selection of the reported result.

The possible ROB on each of the five domains based on the extracted information will be rated as ‘high risk’ or ‘low risk’. If there was insufficient detail reported in the study, we judged the ROB as ‘unclear’.

**Publication bias assessment**

We will perform the assessment of publication bias using a visual inspection of the funnel plot asymmetry and Egger’s test of asymmetry.\textsuperscript{25} If there are fewer than 10 studies associated with one outcome, the power of the assessment would be too low to be implemented according to the Cochrane recommendations. Egger’s test of asymmetry is also invalid when the number of included studies is fewer than 20.

**Evidence evaluation**

We will use the Grading of Recommendations Assessment, Development and Evaluation system\textsuperscript{26} to assess the strength of evidence from each included study.

**Data synthesis**

We will extract and summarise the study characteristics and present them in text descriptions and baseline tables. The synthesis describes the characteristics of each of the included studies and shows information about the effective measures for outcomes and quality of study.

A meta-analysis will be performed based on the availability of data, including the MD or OR. The heterogeneity between the included studies will be approached graphically.\textsuperscript{27} The metafor package for R (V.4.0-0) will be used to pool the data from the included studies.\textsuperscript{28} The final analysis data will also be presented in a forest plot by using the metafor package for R.

**Subgroup analysis**

Subgroup analysis will be conducted when we have adequate data for each of the following variables:

1. The dosage of coenzyme Q10 supplementation.
2. Duration of intervention.
3. Patient ages.
4. Detection methods of the SDFI.

If an adequate number of included studies (n>2) included an outcome, subgroup analyses will be extended to random effects meta-regression analyses. We will choose MD as the effect size for the data from the included studies.

**Sensitivity analysis**

A sensitivity analysis will be conducted to account for the ROB through a leave-one-out method operated in the metafor package for R (V.4.0-0).

**DISCUSSION**

This study will provide a repeatable and transparent procedure to comprehensively explore the efficacy of CoQ10 supplementation in male infertility patients. In this systematic review, the strengths and weaknesses of the included studies will be identified. Additionally, this review will provide estimates for the effectiveness of interventions in terms of improving SDFI and OS status. The different doses, durations and detection methods selected may cause significant heterogeneity among the included studies. To solve this problem, a narrative summary will be presented to provide valid data if possible.

**Conclusion**

The findings of this review can offer clinicians a new therapeutic approach to treat patients with high SDFI. Considering the higher cost of the combination of antioxidants, administration of CQ10 alone may significantly reduce the healthcare costs of patients.

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**Contributors** TX is the guarantor. YZ drafted the manuscript. YZ, XZ, GZ, RM, QG, BO and TX contributed to the development of the selection criteria, the risk of bias assessment strategy and data extraction criteria. XZ developed the search strategy. GZ provided statistical expertise. All authors read, provided feedback and approved the final manuscript.

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**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

**Patient consent for publication** Not applicable.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Supplemental material** This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those
REFERENCES
Identification of studies via databases and registers

**Identification**
- Records identified from:
  - Databases \(n=\) 
  - Registers \(n=\)
- Records removed before screening:
  - Duplicate records removed \(n=\)
  - Records marked as ineligible by automation tools \(n=\)
  - Records removed for other reasons \(n=\)

**Screening**
- Records screened \(n=\)
- Records excluded \(n=\)
- Reports sought for retrieval \(n=\)
- Reports not retrieved \(n=\)
- Reports assessed for eligibility \(n=\)
- Reports excluded:
  - Reason 1 \(n=\)
  - Reason 2 \(n=\)
  - Reason 3 \(n=\)
  - etc.

**Included**
- Studies included in review \(n=\)
- Reports of included studies \(n=\)
### Supplementary Table 1: PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

<table>
<thead>
<tr>
<th>Section and Topic</th>
<th>Item No</th>
<th>Checklist item</th>
<th>Page No.</th>
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<td>Identify the report as a protocol of a systematic review</td>
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<tr>
<td>Update</td>
<td>1b</td>
<td>If the protocol is for an update of a previous systematic review, identify as such</td>
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<td>If registered, provide the name of the registry (such as PROSPERO) and registration number</td>
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<tr>
<td>Contact</td>
<td>3a</td>
<td>Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author</td>
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<td>Contributions</td>
<td>3b</td>
<td>Describe contributions of protocol authors and identify the guarantor of the review</td>
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<td>Amendments</td>
<td>4</td>
<td>If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments</td>
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<td>Sources</td>
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<td>Indicate sources of financial or other support for the review</td>
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<td>Sponsor</td>
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<td>Role of sponsor or funder</td>
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<td>Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol</td>
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**Introduction:**

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<table>
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<tr>
<th>Rationale</th>
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<th>Describe the rationale for the review in the context of what is already known</th>
<th>4</th>
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<tbody>
<tr>
<td>Objectives</td>
<td>7</td>
<td>Provide an explicit statement of the question(s) the review will address with reference to participants, interventions/exposures, comparators, and outcomes (PICO/PECO)</td>
<td>4</td>
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</table>

**Methods:**

| Eligibility criteria | 8 | Specify the study characteristics (such as PICO/PECO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review | 5 |
| Information sources | 9 | Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage | 5 |
| Search strategy | 10 | Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated | Supplementary Table 2 |

**Study records:**

<p>| Data management | 11a | Describe the mechanism(s) that will be used to manage records and data throughout the review | 6-7 |
| Selection process | 11b | State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis) | 5-6 |
| Data collection process | 11c | Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators | 6-7 |
| Data items | 12 | List and define all variables for which data will be sought (such as PICO/PECO items, funding sources), any pre-planned data assumptions and simplifications | 6 |</p>
<table>
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<tr>
<th>Outcomes and prioritization</th>
<th>13</th>
<th>List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale</th>
<th>6</th>
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<tr>
<td>Risk of bias in individual studies</td>
<td>14</td>
<td>Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis</td>
<td>7</td>
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<td>Data synthesis</td>
<td>15a</td>
<td>Describe criteria under which study data will be quantitatively synthesised</td>
<td>7-8</td>
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<td>15b</td>
<td>If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I², Kendall’s τ)</td>
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<td>15c</td>
<td>Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)</td>
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<td>15d</td>
<td>If quantitative synthesis is not appropriate, describe the type of summary planned</td>
<td>7-8</td>
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<tr>
<td>Meta-bias(es)</td>
<td>16</td>
<td>Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)</td>
<td>7</td>
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<tr>
<td>Confidence in cumulative evidence</td>
<td>17</td>
<td>Describe how the strength of the body of evidence will be assessed (such as GRADE)</td>
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*N/A- Not Applicable

Search strategy

**Pubmed**

(((Infertility, Male[MeSH Terms]) OR (male infertility>Title/Abstract))) OR (Male Infertility>Title/Abstract)) OR (Sterility, Male>Title/Abstract)) OR (Male Sterility>Title/Abstract)) OR (Subfertility, Male>Title/Abstract)) OR (Male Subfertility>Title/Abstract)) OR (Sub-Fertility, Male>Title/Abstract)) OR (Male Sub-Fertility>Title/Abstract)) OR (Sub Fertility, Male>Title/Abstract)) AND ((sperm DNA fragmentation index>Title/Abstract)) OR (sperm DFI>Title/Abstract)) OR (sperm dfi>Title/Abstract)) AND (((Coenzyme Q10[MeSH Terms]) OR (CoQ 10>Title/Abstract)) OR (co-enzyme Q10>Title/Abstract)) OR (ubidecarenone>Title/Abstract)) OR (ubiquinone 50>Title/Abstract)) OR (ubiquinone Q10>Title/Abstract)) OR (ubiquinone Q10>Title/Abstract)) OR (Bio-Quinone Q10>Title/Abstract)) OR (ubisemiquinone radical>Title/Abstract)) OR (Q-ter>Title/Abstract)) OR (ubisemiquinone>Title/Abstract)) AND (randomized controlled trial)

**Embase**

'infertility, male'/exp OR 'male infertility':ti OR 'sterility, male':ti OR 'male sterility':ti OR 'male subfertility':ti OR 'male sub-fertility':ti OR 'male sub fertility':ti AND 'coenzyme q10'/exp OR 'co-enzyme q10':ti OR ubidecarenone:ti OR 'ubiquinone 50':ti OR 'ubiquinone q10':ti OR 'ubiquinone 10':ti OR 'sperm dna fragmentation index':ti OR 'sperm dfi':ti AND 'randomized controlled trial':ti

**Web of science**

(((TS=(Infertility, Male)) OR TS=(male infertility)) OR TS=(Male Infertility)) OR TS=(Sterility, Male)) OR TS=(Male Sterility)) OR TS=(Subfertility, Male)) OR TS=(Sub-Fertility, Male)) OR TS=(Male Subfertility)) OR TS=(Sub Fertility, Male)) AND (((TS=(Coenzyme Q10)) OR TS=(co-enzyme Q10)) OR TS=(ubidecarenone) OR TS=(ubiquinone 50)) OR TS=(ubiquinone Q10)) OR TS=(Bio-Quinone Q10)) OR TS=(ubiquinone 10) OR TS=(CoQ10) OR TS=(ubisemiquinone radical)) OR TS=(Q-ter)) OR TS=(ubisemiquinone) AND ((TS=(sperm DNA fragmentation index)) OR TS=( sperm DFI) OR TS=( sperm dfi) AND TS=(randomized controlled trial)

**Cochrane library**

(Infertility, Male or male infertility or Male Infertility or Male Infertility or Male Sterility or Subfertility, Male or Male Subfertility or Sub-Fertility, Male or Male Sub-Fertility or Sub Fertility, Male):ti AND (Coenzyme Q10 or co-enzyme Q10 or ubidecarenone or ubiquinone 50 or ubiquinone Q10 or Bio-Quinone Q10 or ubiquinone 10 or CoQ10 or ubisemiquinone radical or Q-ter or ubisemiquinone):ti AND (sperm DNA fragmentation index or sperm DFI or sperm dfi):ti AND (randomized controlled trial):ti
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