

Peer Review File

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Reviewer A

The purpose of this manuscript was to perform an "updated systematic review and meta-analysis aims to evaluate whether couples with high level of SDF will benefit more from Testi-ICSI as compared to Ejac-ICSI."

1. The authors note on line 533 that "the included studies were retrospective." What about studies 4,7,8 and 9 which are listed as prospective in Table 1 of included studies?

Reply 1: Two researchers (Guicheng Zhao and Yi Zhen) re-examined full manuscript of the included studies, confirm that only studies 4,7,8 and 9 were prospective, and the others were retrospective. What we want to present is that most of the included studies are retrospective studies. Changes in the text: we have modified our text as advised (see Page 19, line 563-564 "the included studies were retrospective (only studies 4,7,8 and 9 were prospective.")

2. Line 99: "was not registered." Why not?

Reply 2: We strongly agree with the requirement and importance of registration for meta-analysis. However, meta-analysis registration is not a mandatory requirement, we did not understand the requirement to register this trial before it was conducted, and we did not register the study. Admittedly, one important limitation of this study was that the current study was not registered and small biases may exist, we performed this meta-analysis strictly abided by the Preferred Reporting Items for the Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines and the PICOS strategy. We decided to delete "This SRMA was not registered", added "Admittedly, one important limitation of this study was that the current study had not been registered and small biases may exist." in the subheading section "limitations of this study".

Changes in the text: we have modified our text as advised (see Page 4, line106; Page 19, line 578-579)

3. The authors excluded studies with severe oligozoospermia and gave reasoning. Since as they note this group often has high SDF why not include it as clinically these are often patients where the question of whether to use testicular or ejaculated sperm is important?

Reply 3: Because SDF cannot be measured accurately in Severe oligozoospermia ($< 5 \times 10^6/ml$) using SDF assay Kit. In the other hand, severe oligozoospermia ($< 5 \times 10^6/ml$) may be strongly associated with high SDF, due to intratesticular apoptosis induced by impairment in sperm maturation that leads to early DNA damage, not only factor about oxidative stress from

reproductive pathways. However, testis-ICSI was an attempt to overcome SDF caused by oxidative stress from epididymis and other reproductive pathways. So, we excluded studies with severe oligozoospermia.

Changes in the text: None.

4. The authors note that their primary outcome was "paired SDF levels between ejaculated and testicular sperm." So why include studies that did not include SDF for testicular sperm?

Reply 4: Although there are several methods to evaluate the SDF, they are relatively limited for testicular sperm. There are only a limited number of studies investigating the SDF of testicular sperm. According to the search strategy, only 4 studies related to "paired SDF levels between ejaculated and testicular sperm" in this meta-analysis. The main focus of this study is that the SDF of testicular sperm may be lower in those males with high level of SDF in ejaculated semen, so as to explore whether testicular sperm used for ICSI can improve ART outcomes. Therefore, those studies which presented the comparative ICSI pregnancy outcomes of ejaculated sperm with high SDF and testicular sperm, although the SDF of testicular sperm was not measured, included in this meta-analysis.

Changes in the text: None.

5. The authors note that data extraction was performed independently. Did the 2 authors who extracted the data enter it into two separate databases? Were the 2 databases compared to identify any data entry errors? What did the authors do to ensure accuracy of data recording and entry? What did they do if there was missing data? Please expand on discussion.

Reply 5: In this study, two researchers (Guicheng Zhao and Yi Zhen) extracted the data enter it into two separate databases, and any disagreement in selecting studies or assessing risk of bias was resolved by obtaining consensus with the opinion of a third observer (Dingming Li). Another researcher (Hengzhou Bai) re-examined full manuscript of the all included studies to ensure accuracy of data recording and entry. When studies did not have all the necessary information available, we contacted the corresponding authors to request the missing data as being needed. These studies were excluded only if no data were provided or no reply was received by the time of the meta-analysis. We further elaborate on the above information again in the part of Methods.

Changes in the text: we have modified our text as advised (see Page 5, line 139-146 "Two researchers (Guicheng Zhao and Yi Zhen) evaluated each title, abstract and full manuscript of the articles, extracted and entered the data into two separate databases, and any disagreement was resolved by consensus with the opinion of a third researcher (Dingming Li). Another researcher (Hengzhou Bai) re-examined the full manuscripts of all included studies to ensure accuracy of data collection and entry. When studies did not have all the necessary information, we contacted the corresponding authors to request the missing data. These studies were excluded if no data were provided or no response was received by the time of meta-analysis.")

6. Line 354: "extremely high DFI". Please define extremely high DFI.

Reply 6: we define “extremely high DFI” as $DFI \geq 30\%$ in the text.

Changes in the text: we have modified our text as advised (see Page 13, line 372).

7. Line 436: spelling error: ICIS?

Reply 7: The abbreviations "ICIS" is indeed a miss-pelling, we have reduced redundancy to the minimum in the subheading section “The difference level of SDF between testicular and ejaculated sperm” and decided to delete this sentence.

Changes in the text: we have modified our text as advised (see Page 16, line 453-456).

8. Line 448: "ORP" please spell out

Reply 8: The abbreviations "ORP" have been spelled out “oxidation reduction potential, ORP”. We have reduced redundancy to the minimum in the subheading section “The debate about SDF testing methods and thresholds” and decided to delete this sentence.

Changes in the text: we have modified our text as advised (see Page 16, line 468).

9. In Figure 4: under SCD column: disadvantage: "Number of analysed sperm cells". Is this low number?

Reply 9: Yes! SCD analyses only 300–500 spermatozoa manually under the microscope.

Changes in the text: we have modified our text as advised (see Figure 4: under SCD column: disadvantage: “a low number of analysed sperm cells”).

10. The discussion section is long and redundant in many errors. Could the authors please shorten and reduce redundancy?

Reply 10: Thank you for the valuable comment, we have shorten and reduced redundancy in the discussion section. The details of the modification are as follows:

In the first part of the discussion section (Lines 306-325), we repeat the same information provided in the “Introduction” section. So, this part are deleted;

we have reduced redundancy to the minimum in the subheading section “The difference level of SDF between testicular and ejaculated sperm”, the subheading section “the different types of sperm DNA breaks related to ICSI outcomes”, and the subheading section “The debate about SDF testing methods and thresholds”, focusing on the discussion of our own findings.

Changes in the text: Completed as requested.

Reviewer B

The authors conducted a SRMA to investigate whether couples with high levels of SDF will benefit more from Testi-ICSI as compared to Ejac-ICSI. In fact, this is an interesting topic. However, the authors need to address the following issues:

Introduction

- Introduction section is lengthy and redundant. It should be more concise. The following parts can be removed:
 - o Lines 52-59 “Infertility affects nearly 7% either in the ejaculate semen or testicular tissue”.
 - o Lines 70-74: “There are several well-known techniques role of testicular sperm for the patients concerned”.

Reply Comment on Introduction: Thank you for the valuable comment about Introduction, we decide removed the parts of “Lines 52-59” and “Lines 70-74”.

Changes in the text: we have modified our text as advised.

Methods

- Line 99: The authors state that “This SRMA was not registered”. Authors have to either delete this or explain why it was not registered.

Reply Comment on Methods: We strongly agree with the requirement and importance of registration for meta-analysis. However, meta-analysis registration is not a mandatory requirement, we did not understand the requirement to register this trial before it was conducted, and we did not register the study. Admittedly, the current study was not registered and small biases may exist, we performed this meta-analysis strictly abided by the Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines and the PICOS strategy. We decided to delete “This SRMA was not registered”, added “Admittedly, one important limitation of this study was that the current study had not been registered and small biases may exist.” in the subheading section “limitations of this study”.

Changes in the text: we have modified our text as advised (see Page 4, line106; Page 19, line 578-579)

Discussion:

- Generally, the discussion section is redundant and poorly written. Here are some examples:
 - In the first part of the discussion section (Lines 306-325), the authors repeat the same information provided in the “Introduction” section. So, this part should be deleted.

- In the subheading “The difference level of SDF between testicular and ejaculated sperm” the authors wrote a long narrative on the pathogenesis of SDF and methods of selection of a healthy sperm for ICSI. Similarly, in the subheading “the different types of sperm DNA breaks related to ICSI outcomes”, the authors elaborate on the mechanisms of sperm DNA damage and the techniques used for its evaluation. These parts need to be reduced to the minimum, and alternatively the authors should focus on the discussion of their own findings.
- Additionally, the subheading “The debate about SDF testing methods and thresholds” is not directly relevant to the scope of this study. I suggest reducing this part and just including a brief summary of it in the limitations of the study.

Comment on Discussion: In the first part of the discussion section (Lines 306-325), the authors repeat the same information provided in the “Introduction” section. So, this part should be deleted.

Reply: Thank you for the valuable comment, we decide removed the parts of “Lines 306-325”.

Changes in the text: we have modified our text as advised.

Comment on Discussion: In the subheading “The difference level of SDF between testicular and ejaculated sperm” the authors wrote a long narrative on the pathogenesis of SDF and methods of selection of a healthy sperm for ICSI. Similarly, in the subheading “the different types of sperm DNA breaks related to ICSI outcomes”, the authors elaborate on the mechanisms of sperm DNA damage and the techniques used for its evaluation. These parts need to be reduced to the minimum, and alternatively the authors should focus on the discussion of their own findings.

Reply: Thank you very much for the kind suggestions and comments, we have reduced redundancy to the minimum in the subheading section “The difference level of SDF between testicular and ejaculated sperm” and the subheading section “the different types of sperm DNA breaks related to ICSI outcomes”, focusing on the discussion of our own findings.

Changes in the text: we have modified our text as advised (see the subheading section “The difference level of SDF between testicular and ejaculated sperm” and the subheading section “the different types of sperm DNA breaks related to ICSI outcomes”).

Comment on Discussion: Additionally, the subheading “The debate about SDF testing methods and thresholds” is not directly relevant to the scope of this study. I suggest reducing this part and just including a brief summary of it in the limitations of the study.

Reply: Thank you very much for the kind suggestions. The precise diagnosis methods and management of SDF thresholds for the prediction of ICSI-related pregnancy are lacking unified consensus so far. We think that the heterogeneity of SDF testing methods and thresholds in the included studies may might affect to value the outcomes comparison of ICSI, so we want to keep this part and have reduced redundancy to the minimum in this subheading section.

Changes in the text: we have modified our text as advised (see the subheading section “The debate about SDF testing methods and thresholds”).

Additional minor comments

- Authors need to revise all abbreviations in the manuscript. Here are some examples:
 - o the term DNA fragmentation index (DFI) has been abbreviated twice in the “Introduction” section.
 - o the term “sperm DNA fragmentation” is abbreviated “SDF” in the “Introduction” section (line 63). The acronym “SDF” should be used throughout the text.
 - o reactive oxygen species (ROS) in lines 337 and 340.

Reply: Thank you for the above valuable comment, we have re-examined full of text and revised all abbreviations in the manuscript.

Changes in the text: we have modified our text as advised (see Lines 93; Line223; Line 225; Line 329; Line 342).

- Lines 316-319: The authors state that “Up to now, numerous clinical studies and systematic reviews about the utility of SDF in ICSI management”. However, only one reference is cited (20).

Reply: In the first part of the discussion section (Lines 306-325), we repeat the same information provided in the “Introduction” section. Thanks for your above valuable advice “Comment on Discussion” that this part should be deleted. So, we decided to delete this sentence.

Changes in the text: we have modified our text as advised (see Page 12, line 333-336).

- Line 362: The word “anesthetic” needs to be corrected “anesthesia”.

Reply: We have corrected this word in the text.

Changes in the text: we have modified our text as advised (see Page 3, Line 364).

Reviewer C

I would like to congratulate the authors for the article and proposed methodology. Subsequently, some suggestions will be made in order to improve the evidence present in the manuscript.

Abstract:

Lines 29-30: The terms intracytoplasmic sperm injection (ICSI) and sperm DNA fragmentation (SDF) are abbreviated... usually one avoids using abbreviations in this topic. If possible modify.

Reply: We strongly agree that it usually avoids using abbreviations in the Abstract. Considering that many parts of the abstract need to use these two abbreviations to explain the content, which may lead to too many words in the abstract, we still consider using the above two abbreviations.

Changes in the text: None.

Method:

Line 99: Suggestion... In order to guarantee the methodological reliability and also the non-repetition of researches with the same focus, the registration of the systematic review is recommended. Did the authors decide not to register or was it done and there was no approval time for submission?! (If possible, insert reason)

Reply: We strongly agree with the requirement and importance of registration for meta-analysis. However, meta-analysis registration is not a mandatory requirement, we did not understand the requirement to register this trial before it was conducted, and we did not register the study. Admittedly, the current study was not registered and small biases may exist, we performed this meta-analysis strictly abided by the Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines and the PICOS strategy. We decided to delete “This SRMA was not registered”, added “Admittedly, one important limitation of this study was that the current study had not been registered and small biases may exist.” in the subheading section “limitations of this study”.

Changes in the text: we have modified our text as advised (see Page 4, line106; Page 19, line 578-579)

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Lines 102-105: PICOS inclusion and exclusion criteria are quoted, but not shown in table or figure form. If possible insert as an attachment or in the text itself.

Reply: We have inserted the Supplementary Table S1 “The PICOS design structure for literature search strategy”, which can be found attached to the submission.

Changes in the text: Completed as requested.

Line 116-119: The text mentions additional research in databases recording ongoing research, however, how was this manual search performed using specific terms?! If I wanted to replicate this search in the databases, I would find the same result. Insert if possible.

Reply: We have inserted the search terms used for performing manual search in databases recording ongoing research in the manuscript.

Changes in the text: we have modified our text as advised (see Page 4, Lines 124-126 “using the search terms as follows: “sperm DNA fragmentation”, “sperm DNA damage”, “sperm chromatin integrity OR damage”, “testicular sperm”, “ejaculated sperm”, “intracytoplasmic sperm injection”, with the filter “human” in any language”).

Line 127: Define relevant steps. Relevance is a rather subjective analysis. Didn't they agree with what was proposed?

Reply: Of course, they agreed with what was proposed! The requirements for the relevant outcomes are covered in the eligibility criteria. So, we have corrected “excluding articles that did not meet the eligibility criteria or that did not include relevant outcomes” to “excluding articles that did not meet the eligibility criteria”.

Changes in the text: we have modified our text as advised (see Page 6, Lines 137).

Line 136: PICOS eligibility criteria are again inserted if possible insert a table or figure showing the criteria for readability

Reply: We have inserted the Supplementary Table S1 “The PICOS design structure for literature search strategy”, which can be found attached to the submission.

Changes in the text: Completed as requested.

143-149: Something that drew attention was the lack of delimitation of a time period. We know that older articles have less sensitive methodologies and can lead to a somewhat biased analysis. If possible, discuss or insert the reason in materials and methods.

Reply: Yes, some of the older included articles may have less sensitive methodologies and can lead to a somewhat biased analysis. However, the studies on the SDF measurement of ejaculated sperm are more, but on the SDF test of testicular sperm are relatively fewer. The relatively older researches provided important data on SDF test in the testicular sperm, these older articles were also included in this meta-analysis. We have inserted the reason in methods section.

Changes in the text: we have modified our text as advised (see Page 6, Lines 164-167).

Line 165 - 167: Suggestion: Insert the mean age of the patients listed in the articles, as this is also important information.

Reply: We have inserted the mean age of the patients listed in the articles in the Methods section and table 1.

Changes in the text: we have modified our text as advised (see Page 7-8, Lines 184-185, table 1).

Results:

216: Inserting the number of patients allocated in the study in table 1 is interesting information to show the representativeness of the sample.

Reply: We have inserted the number of patients allocated in the study in table 1 and in the Methods section.

Changes in the text: we have modified our text as advised (see Page 7-8, Lines 184-185, table 1).

Reviewer D

The authors are to be commended for their extensive efforts reviewing numerous published articles. While the conclusions suggest possible better PG outcomes with testicular sperm, the low number of studies using many different lab procedures leads them to conclude that more studies are required.

Reply: We extend many thanks to anonymous reviewer for the above valuable comment and the constructive comments and during the manuscript review. We re-examined full of text and have modified some parts of the paper, shorten and reduced redundancy in the discussion section.

Changes in the text: we have modified our text as advised.