Author’s response to reviews

Title: Role of Calcifying Nanoparticles in the Development of Testicular Microlithiasis in Vivo

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Version: 1 Date: 11 Jan 2017

Author’s response to reviews:

Dear editor and reviewers,

Thank you for the comments on our manuscript. We have carefully prepared a revised manuscript to address all the questions raised by the reviewers, and hope that our replies and new revision are satisfactory to you and the referees, and that the manuscript is now acceptable for publication in BMC Urology.

Please let me know if you have any questions.

Sincerely yours,

Best regards.

Qing-hua Zhang,

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ARRIVE guidelines

In accordance with BioMed Central editorial policies (http://www.biomedcentral.com/submissions/editorial-policies#standards+of+reporting), could you please ensure your manuscript reporting adheres to the ARRIVE guidelines (http://www.nc3rs.org.uk/page.asp?id=1357) for the reporting of animal experiments. This is so your methodology can be fully evaluated and utilised. Can you please include a completed ARRIVE checklist as an additional file when submitting your revised manuscript.

Answers:

Thank you for your helpful suggestions. We promise our manuscript reporting adheres to the ARRIVE guidelines, and have included a completed ARRIVE checklist as an additional file with our revised manuscript.

Comments:

Please add email addresses for all authors to the title page, and move the description of ethical approval to the declarations section.

Answers:

Thank you. We have added email addresses for all authors to the title page, and moved the description of ethical approval to the declarations section.
Reviewer 1

Comments for the Author...

The aim of this study was to test the hypothesis that human-derived NPs could invade the seminiferous tubules and induce TM phenotype. The authors adopted the conventional methods to evaluate it. Their results indicated that human-derived NPs could invade the seminiferous tubules and induce TM phenotype. The writing and grammar of this paper is good.

Comments :

1. The authors should put at least one manuscript to each used technique, to reference it. Despite of some of them being well known, it is necessary to inform details to someone that want to use it at the first time.

Answers:

According to your helpful suggestion, we have added the following reference regarding the technique in the Methods section of the revised manuscript (page 6, line 9).


Comments :

2. In the statistical analysis the authors says "Results are presented in the form of mean ± SD." Which results they refers to? The work has only description of the figures.

Answers:

We apologize for this mistake in the statistical analysis. According to your helpful suggestion, we have deleted "Results are presented in the form of mean ± SD" from the Statistical analysis section (page 8, line 2).

Comments :

3. Also, authors should review the colors of the arrows in the figures with those cited in the text.
Answers:

Thank you for pointing out our mistake. We have corrected the wrong colors of the arrows in the figures with those cited in the text (page 15, line 7-14).

Reviewer 2

Comments for the Author...

Although original, I suspect this manuscript has low appeal to the general readership. TM is ill-defined phenomena, and this study brings us no closer to the potential aetiology.

The paper is well written and experiments well described.

Comments

1. Fig 1A - does not add anything to the paper and should be removed.

Consider adding patient demographics and how patients with TM were chosen.

Answers:

Thank you for your kind suggestion. We have removed Fig 1A in the revision.

We have added "Referring to our previous research methods, nine patients with TM (multiple foci < 3 mm in diameter in testicular parenchyma with sonography) were recruited." in the Methods section (page 6, line 8-9).

Comments

2. Fig 2 - no scale bars

In Fig 2 to identify the leucocyte population, specific IHC should be used.

Answers:

We are really sorry for our mistake of no scale bars in Fig 2. We have added scale bars in Fig 2.

Thank you for your expert opinion. Specific IHC used to identify the leucocyte population could bring a big power to support our conclusion. In our previous study design, we believed that it
was not absolutely necessary to compare the difference of leucocyte population between group B and group C. Therefore, we have ignored this technique in the present study. We will perform relevant experiments in future studies.

Comments

3. TM is patients is a radiological finding. How well does the model recapitulate this (i.e. need US scans of the rat's testes)

Answers:

TM is an uncommon condition of unknown etiology with multiple tiny calcifications present within the seminiferous tubules, which is formed by the intratubular deposits consisting of calcified central cores surrounded by multiple concentric layers of cellular debris, glycoprotein, and collagen within the seminiferous tubules. In clinic, a surgical biopsy is an effect method to detect whether or not a patient to have TM. However, this method is an invasive procedure with potential complications and few enrolled patients agreed to undergo TESE or testicular biopsy. Therefore, most patients with TM are only radiological findings in clinic.

In this study, possible pathological calcifications are assessed by histopathologic examination, transmission electron microscopy and scanning electron microscopy. Furthermore, promising results have recently been obtained in this study.

Reviewer 3

Comments for the Author...

The present manuscript showed that human semen nanoparticles is involved in the formation of testicular microlithiasis by morphological studies. This is a descriptive study, not a mechanistic study. The present study can be accepted after a modification stated below.

Comments

1. What is rationale of injecting E Coli in rat? It is not discussed in the text.
Answers:

Thank you for your suggestive opinion. In order to discuss whether E Coli injection in rat induce calcificationas or not, we used it as a control. According to your suggestion, we have added relevant discussion in the revised manuscript (page 11, line 14-18).

Comments :

2. Authors should discuss the possible mechanism (a short paragraph) of calcification within the seminiferous tubules stimulated by nanoparticles in the discussion section.

Answers:

According to your suggestion, we have added a short paragraph of the seminiferous tubules stimulated by nanoparticles in the discussion section (page 10, line 7-14).

Comments :

3. The bars of figures 2 and 4 are missing.

Answers:

Thank you for your helpful suggestion. We have added the bars of figures 2 and 4 in the text.