Author's response to reviews

Title: Impact of Seminal Trace elements and Glutathione levels on Semen Quality of Tunisian Infertile Men

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Author's response to reviews:

Response to Reviewers

Dear Reviewers,

We want to say thanks for the higher interest that you showed regarding our manuscript. We have revised it, carefully, according to all your comments and suggestions. We have a great pleasure to resubmit it. The corrections as well as the additions were indicated in the current revised version by red letters.

Sincerely yours

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Responses to the Reviewers’ comments

Reviewer #1: Fanuel Lampiao

This is a good manuscript; however minor essential revisions should be made:
Introduction:
Comment 1:
Line 3: impaired or unpaired?

Response 1:
We agree with your suggestion. We revised manuscript and we corrected the term “impaired” which was replaced by “unpaired”.

Comment 2:
Response 2:
We agree with your suggestion. We revised manuscript and we corrected the term “impaired” which was replaced by “unpaired”.

Comment 3:
Response 3:
We agree with your suggestion. We revised manuscript and we corrected the term “impaired” which was replaced by “unpaired”.

Comment 4:
Response 4:
We agree with your suggestion. We revised manuscript and we corrected the term “impaired” which was replaced by “unpaired”.

Comment 5:
Response 5:
We agree with your suggestion. We revised manuscript and we corrected the term “impaired” which was replaced by “unpaired”.

Comment 6:
Response 6:
We agree with your suggestion. We revised manuscript and we corrected the term “impaired” which was replaced by “unpaired”.
The corrected word was indicated by red color in the following sentence: “ROS are highly reactive oxidizing agents belonging to the class of free radicals containing one or more unpaired electrons which are continuously being generated through metabolic and pathophysiologic processes [2].”

Comment 2:
Page 4, last paragraph, first sentence: compromising or comprising?
Response 2:
According to this comment, we correct the word “compromising” and we replaced it by the right one “comprising”.

It was written by red color in the text: “Trace elements in human semen comprising zinc and selenium have been shown to be essential for testicular development and spermatogenesis [5-8].”

Comment 3:
Page 4, last paragraph: the second sentence should be written in correct Grammar. "Responsible of" should be changed to "responsible for".
Response 3:
Done. The following sentence “Zinc (Zn) is one of the primary factors responsible of deoxyribonucleic acid (DNA) transcription and protein synthesis which are major parts of sperm development [7]” was revised. It was replaced by this sentence “Zinc (Zn) is one of the primary factors responsible for deoxyribonucleic acid (DNA) transcription and protein synthesis which are major parts of sperm development [7].”

Comment 4:
Page 5, last paragraph, first sentence does not read well. Did the authors mean the "no study in Tunisia has investigated............?"
Response 4:
Yes, we meant that there is no study in Tunisia has investigated the non-enzymatic antioxidant properties of human seminal plasma”.

According to this suggestion, we have reworded the last paragraph in page 5 in order to clarify our aim in this study. This paragraph “Despite the protective effect of seminal plasma has been well recognized, any study in Tunisia has investigated its non-enzymatic anti-oxidative properties and the possible relationship between infertility and plasmatic antioxidant defenses. On the basis of these considerations, the aim of this study is to assess the levels of the non-enzymatic antioxidants in seminal plasma from Tunisian fertile and infertile men. In order to fulfill this goal, we measured the concentrations of zinc, selenium, GSH and MDA in the seminal plasma of 250 semen samples” was replaced by the following paragraph “Keeping in view the main protection provided by seminal plasma antioxidants against oxidative damages, the purpose of our present study was to (1) evaluate the non-enzymatic antioxidants profiles...”
of idiopathic infertile men and to (2) assess their impact on semen parameters. In order to fulfill this goal, we measured the concentrations of zinc, selenium, GSH and MDA in the seminal plasma of 250 semen samples. To our knowledge, this report which investigated the repercussions of human seminal antioxidants on sperm quality constitutes the first one conducted in Tunisia”.

Comment 5:
Statistics:
Group comparisons were made using the Student's t-test, but there are more than 2 groups which were compared. Why did the author not use ANOVA?

Response 5:
We want to say thanks to the reviewer for his so instructive comment. Despite there are than 2 groups in our study, we used the Student's t-test to compare our results but each time we compared two groups separately from the other groups and we obtained significant results. But according to your advice and in order to diminish error and to better adjust our results, we have repeated the statistics of our investigation. In effect, we re-analyzed our results using SPSS.11.0 (SPSS, Chicago, IL, USA) and the differences which can exist between the evaluated study groups were re-assessed by performing an analysis of variance (One-way ANOVA) and the post-hoc test (Tukey test) to conduct pair-wise comparisons.

After revising statistics, we have indicated this in the revised manuscript in Material and Methods section; exactly in the statistical analysis paragraph (Page 9): “The Windows computing program Statistical Package for the Social Sciences “SPSS 11.0” (SPSS, Chicago, IL, USA) was used for analyzing the data. All data were expressed as mean ± standard deviation (S.D.). The differences that existed between the evaluated study groups were assessed by performing an analysis of variance (One-way ANOVA) and the post hoc test (Tukey test) to conduct pair-wise comparisons. The differences were considered to be significant at values of P<0.05”.

We have also indicated all the statistical corrections by red color in Tables 2 and 3. As well, all the additions and modifications in abstract, Results and discussion sections were marked by red color.

Comment 6:
Conclusion: Line 4, spelling of pathozoospermic.

Response 6:
Done. In the revised manuscript the term of “pathozoospermic” was replaced by another word which is “abnormal”.

The correction was marked in red color in the following sentence (conclusion, line 4): “Successfully, it was also revealed that increased MDA levels in the abnormal groups could represent the pathologic lipid peroxidation effects on sperm function”.

We have also indicated all the statistical corrections by red color in Tables 2 and 3. As well, all the additions and modifications in abstract, Results and discussion sections were marked by red color.
Reviewer# 2: Rakesh Sharma

This is an interesting study. It lends further support to establish the relevance of antioxidants in sperm quality. The study is well designed.

A few questions the authors need to address are:

Comment 1:

Was the white blood cell concentration evaluated? If yes, what was the breakdown in different groups as these can significantly impact ROS production and sperm quality and antioxidant reserves?

Response 1:

We would like to thank the reviewer for this suggestion. Effectively, we agree with you that seminal leukocytes at concentrations above the WHO cutoff value for leukocytospermia (>1×10^6 per milliliter of semen) might cause oxidative stress which is linked with imperfect antioxidant status, poor sperm quality and defective sperm function. Accordingly, we should indicate that in our investigation we excluded all patients with seminal leukocytospermia (>1×10^6 /ml) in order to highlight the powerful of seminal antioxidant properties.

For this purpose, we have added this data in the revised manuscript. It was indicated in the Material and Methods section and described in this paragraph: “Exclusion criteria: A detailed medical history was performed for all studied cases. Subjects currently on any medication or antioxidant supplementation were not included. In addition, subjects with testicular varicocele, genital infection, leukocytospermia, chronic illness and serious systemic diseases, smokers and alcoholic men were excluded from the study because of their well-known high seminal ROS levels and decreased antioxidant activity.”

Comment 2:

The abstinence time in Table 1 states 3. Was this observed in all patients?

Response 2:

We agree with your comment. It was indicated in Table 1 that duration of sexual abstinence stated 3 days. This data was also noted in Material and Methods section, exactly in the first sentence of semen analysis paragraph “Semen samples were collected by masturbation into sterile cups following 3 days of sexual abstinence”.

Yes, this abstinence time was observed for all patients selected for this study. In our study, we chose this criterion in order to eliminate the multiple effects of abstinence period on the results of semen analysis.

Comment 3:

Table 2: Some values for volume are followed by comma instead of decimal point.

Response 3:
According to this comment, the volume value in Table 2, column 3, line 3 “3,34±1;67” was corrected and replaced by “3,34±1.67”

Comment 4:
Small typing and grammatical errors need attention such as:
a- Page 2: Results, first sentence not correct.
b- Page 5: Last paragraph needs to be reworded for clarity.
c- Page 15: Altered antioxidant statue ......incorrect.

Response 4:
We would to thank the reviewer for these suggestions. We tried to revise carefully our manuscript and to correct the syntax and grammatical errors.

a- Since we repeated the statistics using One-way ANOVA test as it was suggested by the “reviewer 1”, some results were better adjusted. Consequently, the first sentence of results section in page 2 “Zinc and selenium concentrations did not differ between different groups, however, correlated positively with sperm motility and count” was replaced by the following sentence “Zinc and selenium concentrations in seminal plasma of normozoospermics were more elevated than the three abnormal groups. Nevertheless, only the zinc showed significant differences. On the other hand, zinc showed positive and significant correlations with sperm motility (P=0.03, r=0.29) and count (P<0.01, r=0.49); however selenium was significantly correlated only with sperm motility (P<0.01, r=0.36)”.

b- Done. Last paragraph in page 5 “Despite the protective effect of seminal plasma has been well recognized, there is no study in Tunisia investigating its non-enzymatic anti-oxidative properties and the possible relationship between infertility and plasmatic antioxidant defenses. On the basis of these considerations, the aim of this study is to assess the levels of the non-enzymatic antioxidants in seminal plasma from Tunisian fertile and infertile men. In order to fulfill this goal, we measured the concentrations of zinc, selenium, GSH and MDA in the seminal plasma of 250 semen samples.” was reworded and replaced by the following paragraph “Keeping in view the main protection provided by seminal plasma antioxidants against oxidative damages, the purpose of our present study was to (1) evaluate the non-enzymatic antioxidants profiles of idiopathic infertile men and to (2) assess their impact on semen parameters. In order to fulfill this goal, we measured the concentrations of zinc, selenium, GSH and MDA in the seminal plasma of 250 semen samples. To our knowledge, this report which investigated the repercussions of human seminal antioxidants on sperm quality constitutes the first one conducted in Tunisia”.

c- Done. The follow paragraph in page 15 “Altered antioxidant statue was observed in the seminal plasma of infertile men collected in our study and this may be an important risk factor of oxidant damage and makes the sperm highly susceptible to lipid peroxidation and MDA production reflects the peroxidation of membrane polyunsaturated phospholipids [30]” was also revised and replaced by
Altered antioxidant status was observed in the seminal plasma of infertile men collected in our study. This might cause oxidant damage and makes the sperm highly susceptible to lipid peroxidation and MDA production reflects the peroxidation of membrane polyunsaturated phospholipids [30].

Comment 5:
Throughout the manuscript, check spellings for oligozoospermics and teratozoospermics.

Response 5:
According to this comment, we revised the manuscript and we checked the spellings for asthenozoospermics, oligozoospermics, teratozoospermics and azoospermics. Corrected spellings were marked in red color in all the revised manuscript.

Comment 6:
Discussion is very elaborate; suggest reducing by one-third.

Response 6:
Done. We tried to reduce the discussion section by one-third without losing impact.

Comment 7:
Could update and include excellent review articles on antioxidants published recently (by Agarwal group).

Response 7:
We agree with your instructive suggestion. The review articles on antioxidants published by “Agarwal group” are very excellent and so interesting. For this reason we incorporated 3 articles published recently by “Agarwal group” among our references and they were used in “Background and Discussion” sections.

These references are:

