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Title: Mild oxidative stress is beneficial for sperm telomere length maintenance

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Authors thank the reviewers for their highly intellectual comments. We have taken the comments and modified the manuscript as per the reviewer's suggestions. The comment wise responses are as follows:

Comment1: The manuscript needs thorough revision where there is much crossing and intermingling of information between the different sections (Introduction, results and discussion), besides a few grammar and editing mistakes.
Response: The manuscript has been thoroughly revised and edited.
Introduction

i. Paragraph 4: The sentence 'Oxidative stress negatively affects the telomere length [8]' has been removed in the revised manuscript.
ii. Last Paragraph:
Previous: Study by Kumar et al [10] has ……. meditation.
Modified: Studies by Kumar et al have shown upregulation in telomerase activity[10] and decline in free radical levels, oxidized mutagenic bases following practice of yoga and meditation [11].
iii. Last line:

Previous: We have investigated…… male infertility.

Modified: In this study we have investigated the role of oxidative stress in sperm telomere length maintenance in idiopathic male infertility.

Discussion
Paragraph1
i. First line
Previous: Telomeres in ……. the eukaryotic cells.
Modified: Telomeres and telomere associated proteins play an important role in the maintenance of genomic integrity in the eukaryotic cells.
ii. Line10

Previous: ROS-induced ……. DNA by ROS.

Modified: ROS induced telomere shortening may be due to direct oxidative injury to guanine bases in telomeric DNA.

Paragraph 2

i. Previous: Oxidative stress ……. the course of DNA damage repair.

Modified: Oxidative stress induces accelerated telomere shortening by the accumulation of oxidized DNA base products (8-OHdG) in the guanine rich telomeres which further recruit DNA damage response machinary that ultimately cause telomere attrition during the course of DNA damage repair.

Paragraph 3

i. First line

Previous: It is now …… cell survival.

Modified: It is now well documented that basal levels of ROS are essential for cell survival and subsevere several physiological functions.

ii. Line5

Previous: cellular homeostasis ……. challenges

Modified: cellular homeostasis especially in cellular responses during pathological challenges

Paragraph4

i. Line1

Previous: Although basal …… and cell

Modified: Although basal levels of ROS are pivotal for redox signalling [21] and cell

Paragraph5

i. Line6

Previous: and thus can ……..stress conditions.

Modified: and thus can elicit great protective effects against severe stress conditions.

ii. The sentences 'Hence a basal level of oxidative stress…….. ultimately cellular aging [28]' have been removed from the revised manuscript as this has already been said in the previous paragraphs of discussion.

iii. The following paragraph has been incorporated at the end of the Paragraph 5.

It was documented that, when few base lesions affect telomeric DNA repeats, ……..activate DNA damage response and results in telomere shortening.

iv. Line2

Previous: and positive oxidative ……. radicals [30].

Modified: and positive oxidative stress can be induced by non lethal free radicals

v. The section "Wang et al (2010) documented that, …….telomere shortening [20]." has been removed from the revised manuscript.

Paragraph6

i. Line5

Previous: Kawanishi. S et al in 2004 [23] …….. detrimental to telomere length maintenance as is evident from the study.

Modified: Kawanishi. S et al [31] also stated that, formation of 8-OHdG at the GGG triplet in telomere sequence induced by oxidative stress could accelerate telomere shortening. Accumulation of oxidized bases beyond a certain level in telomeres may severely deplete telomere binding proteins in telomeres and result in telomere uncapping. Uncapped telomeres can become targets for ATM or ATR kinases, nucleolytic degradation eventually cause telomere shortening and cell cycle arrest and hence cause telomere shortening [26] therefore, very high oxidative stress levels are detrimental to telomere length maintenance as is evident from the study.

ii. Line13

The sentence "Extensive studies……use of antioxidants." has been removed from the revised manuscript.

Paragraph7

i. Line2

"However mild oxidative stress is……meditation into daily lifestyle." Has been incorporated in the revised manuscript.

ii. Last Line

"However the findings…..telomere length maintainance." has been removed in the revised manuscript.

Comment2: The citations are put in many paragraphs in a collective manner where the reader does not know which information fragment belongs to which reference.

Response: The references have been put at appropriate places and modifications have been done in the revised manuscript. The reference list has been modified and three references have been removed from the list. They are as follows:

17. Watson J. Oxidants, antioxidants and the current incurability of metastatic cancers.*Open Biol* 2013 Jan 8;3(1):120144. [doi: 10.1098/rsob.120144. PubMed PMID: 23303309]

23. Malinin NL, West XZ, Byzova TV. Oxidation as "the stress of life". Aging *Albany NY* 2011 Sep;3(9):906-10. Review [PubMed PMID: 21946568; PubMed Central PMCID: PMC3227455]

24. Gorrini C, Harris IS, Mak TW. Modulation of oxidative stress as an anticancer strategy.*Nat Rev DrugDiscov* 2013 Dec;12(12):931-47. [doi: 10.1038/nrd4002. Review. PubMed PMID: 24287781]

Comment3: The authors claimed increase telomere length to moderate oxidative stress level in the control group was much less than that moderate oxidative range. Therefore, the authors may correlate telomere length to oxidative stress in the control cases too and they may show that a considerable number of controls had oxidative stress levels in the moderate range.

Response: As suggested by the reviewer, we analyzed the oxidative stress levels (ROS levels) in the controls again. There were 23 controls (22.8%) that had ROS levels in the mild oxidative stress level range. When the mean telomere length in this group was compared with rest of the controls we observed a similar pattern of increased telomere length in the group of controls with mild oxidative stress (0.79±0.06) as compared to the rest of the controls with normal oxidative stress levels ((0.73±0.04) (p=0.01). The same has been incorporated in the result section of the revised manuscript.

Other changes: Table 2: The naming of the groups has been rectified.

Previous: Mild, Moderate, Severe
Revised: Normal, Mild, Severe