

Format for ANSWERING REVIEWERS



January 22, 2016

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 23595-review.doc).

Title: New insights in sperm biology: How benchside results in the search for molecular markers may help understand male infertility

Author: Sara Marchiani, Lara Tamburrino, Monica Muratori, Elisabetta Baldi

Name of Journal: *World Journal of Translational Medicine*

ESPS Manuscript NO: 23595

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 We thank the editor. In the revised version of the manuscript we followed all the steps required and we provide all requested documents.

Reviewer 1: *The manuscript number 23595, entitled "new insights in sperm biology: how benchside results in search for molecular markers may help to understand male infertility" provides an insight into new biomarkers of sperm functionality that may assist with problems of infertility. The manuscript is an interesting and well-written piece - enjoyable to read, and at a mini-review level suitable to understand by others working or just interested in this field. I have no reservations recommending this for publication, pending a check through of the manuscript to correct several typos and grammatical errors.*

We thank the reviewer for nice comments and for appreciating our article. In the revised version of the manuscript we have checked carefully the manuscript and correct typos and grammatical errors. In addition, we provide a certified correction of the manuscript by a mother language person.

Reviewer 2: We thank the reviewer for helpful suggestions to improve our manuscript. *In this manuscript, Marchiani and coworkers briefly reviewed recent data in benchside studies of male infertility. This is a nice and well-written short review and will provide readers critical updated information.*

We thank the reviewer for nice comments.

Authors are welcome to address a number of issues before this manuscript is exposed to the field. 1. In the first sentence of the 2nd paragraph in introduction, words of "western countries" are inappropriately used. The word of "western" is more political, rather than life style and environment, orientated. Importantly, this statement is supported by only one article with data reported by a French group. Although no detailed information of patients' citizenship was provided, "countries" must be in question. I believe this statement can stand unless more relevant studies

from different regions/countries were reported. Dependent on the background of the relevant studies, specific term such as industries, European, North American etc should be used to avoid confusion.

We agree with the reviewer that “western country” is a political term. On the other hand, it is apparent that reproductive problems are spread world-wide and not simply confined to “western” or “industrialized” countries. However, specifically, the problem of the increase in reproductive age is particularly perceived in industrialized countries mostly because of social problems related to the difficulties in finding a job or the wishing of a career for women etc. The citation of the French paper is related to the role of advanced maternal age in reproduction and not to the general statement regarding the delay in conception. We have changed the sentence in: “The delay increase in reproductive age is becoming an important social problem, which can be particularly noted in industrialized countries”.

2. The aspect of biomarker studies is not reviewed although it is mentioned twice in the abstract. Is there any critical data that can be included, e.g., as a part of proteomic studies?

We apologise with the reviewer because, maybe, we were not enough clear in the abstract. When we talk about “biomarkers able to predict male infertility” we refer to the most promising molecular markers and diagnostic sperm fertility tests or therapeutic targets which are subsequently illustrated in the article. The sentence has been changed and we hope that now the aim of the review is clearer.

3. In aspect of epigenetic studies, is there any development regarding histone modification(s) which can be included in this review?

Until few years ago histone retention was considered as an aberrant chromatin packaging and several papers showed that protamine deficiency was associated with infertility and decrease of ART outcomes in humans (Carrell and Liu, J Androl 2001; de mateo et al, Fertil Steril 2009). Recently, it has been demonstrated that histone retention in specific sperm loci has a role in epigenetic regulation of embryo development (Hammoud et al, Nature 2009). How histone modifications impact on epigenetic regulation is still unclear. In a recent publication, histone retention in sperm, after selection by density gradient, was decreased in numerous genes respect to whole semen. However, in specific loci, such as in olfactory sensor gene family, histones increased suggesting that the histone enrichment is not casual but could play an important role in embryo development (Bolan et al, Plos one 2015). These results are interesting, as, in the future, new diagnostic tests based on specific loci of histone retention can be developed. A short sentence about these studies has been added in the revised version of the manuscript.

4. Editing should be carefully performed. For example, the successive numbers used for four reviewed aspects were 1, 2, 2, 3 instead correctly 1, 2, 3, 4.

We checked carefully and corrected the text. In particular, paragraph numbers have been deleted.

Reviewer 3: We thank the reviewer for helpful suggestions to improve our manuscript.

The manuscript serves a nice and concise introductory review of male infertility covering widely the genetic, epigenetic and protein aspects of the issue, and should therefore be of interest to researchers who may want to make a foray into the field.

We thank the reviewer for nice comments.

The only problem of this manuscript is still the English, the level of which remains below the standard for publication. This reviewer has indeed found it difficult to understand the manuscript in places, and had to refer to the citations to grasp what the authors meant to say. The manuscript is sprinkled with errors of grammar and punctuation, poor choice of words, supplemented with occasional sentences which are bordering on incomprehensibility, or are otherwise in poor writing style.

The revised version of the manuscript has been checked carefully and corrected by a mother language person. In addition a language editing certificate has been provided.

Oddly, the problematic English seems to occur more frequently in the barely two and a half pages of the introduction section including the abstract, in which at least 18 errors of all sorts can be readily identified. For example, why should the word 'augment' be used in the abstract, instead of a simpler but eminently more suitable word such as 'increase'?

In the revised version of the manuscript the word "augment" has been changed in "increase". Other corrections were made by the mother language person who corrected the MS.

The use of anecdote about some female employees having had the fees for freezing their oocytes paid by their companies in the US is not appropriate in a serious scientific review. By the way, the word 'employees' was written as 'employers' in the manuscript.

Such anecdote was included as an example of how life style habits and desire to carrier, often encouraged by the employers, influence the age of conception in women. However, if the reviewer retains that such sentence is not appropriate, we can eliminate it from the article.

In the same paragraph, the last sentence is both ambiguous and grammatically wrong. The 'male age' at its position in the sentence could refer to the age of the offspring, and the word where the 'contribute' is should be a noun. This sentence should therefore be rewritten. One possible sentence structure could be the following: In addition, it has been demonstrated that the higher number of de novo mutations found in the offspring of increasing paternal age is mostly contributed by the paternal transmission.

The sentence has been modified as "In addition, it has been demonstrated that the higher number of de novo mutations found in offspring of increasingly older fathers can mostly be attributed to paternal transmission" according to reviewer's suggestion.

The use of 'above all' in the last sentence of the page is entirely out of place.

In the revised version of the manuscript "above all" has been replaced by "especially in case of".

Direct quoting of a statement from a paper (e.g. lines 6-7 from the bottom of page 3) is not commonly practised, unless the statement is generally regarded as classic or extraordinary in some sense. The authors should express the message of the statement in their own words.

The sentence has been changed in the revised version of the manuscript.

Note that these are just a few examples. The authors are urged to revise the English of the

manuscript once again. The article is otherwise worth publishing on the grounds of scientific merit.
As mentioned above a careful reading and revision of the text was performed by a native English speaker and a certificate is provided.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Translational Medicine*.

Sincerely yours,

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