Author's response to reviews

Title: Reproductive life disorders in Italian celiac women. A case-control study.

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Author's response to reviews: see over
Dear Editor,

Now, we have revised our manuscript according with your demands and reviewers’ requests. The corrections were highlighted in yellow in the revised text. Here reported point-by-point reply.

All authors have approved the revised manuscript.

With my Best Regards

Rosa Prato

Corresponding Author

**Editorial Request**

In addition to the concerns raised by the referees, please also ensure you document ethical approval. Experimental research that is reported in the manuscript must have been performed with the approval of an appropriate ethics committee. Research carried out on humans must be in compliance with the Helsinki Declaration (http://www.wma.net/e/policy/b3.htm), and any experimental research on animals must follow internationally recognized guidelines. A statement to this effect must appear in the Methods section of the manuscript, including the name of the body which gave approval, with a reference number where appropriate.

We better stated the ethical approval procedure in the methods section.
Point-by-point

Reviewer: Jonas Ludvigsson

Q.1 – This paper concerns an important paper but could be improved. I have reviewed this paper for another journal before. Since then it has been improved, but further revisions are needed. This is especially true regarding the description of the statistics. I have recalculate some of the ORs and these do not turn out as in the paper. This worries me and the authors need to explain this. e.g. main outcome measure amenorrhea: abstract OR is 33. When I calculate it based on the data 12-50 (cases) and 4-182 (controls) I get 11 (?). And similarly when I calculate the odds ratio for at least one pregnancy complication I get 2.5 instead of 4.1. I cannot rule out that I have misunderstood something but I would be interested to know how the authors calculated these ORs. Did the use a matched approach (they do not state that), or?

A.1 – Sorry for this mistake. We used a matched approach to calculate the ORs and their 95% CI but we forgot to state this in the Methods section. We clarified the used approach in the revised text.

Q.2 – I appreciate that the authors have included ORs, confidence intervals and p-values in their presentation (including in the abstract). Another change of major importance is that “oats” is not included among the grains containing gluten. I also urged the authors to define their main outcomes.

A.2 – We chose to report the investigated outcomes in the Appendix 1 for not over sizing the Methods section. Now we inserted a sentence in the revised text.

ABSTRACT:

Q.3 – Two outcomes are reported with ORs etc in the abstract, “amenorrhea” and “at least one complication during pregnancy”. However in your methods section in your abstract you list five main outcome measures. Although I feel five main outcome measures is a lot, I accept it, but urge you then to present ORs for all these main outcome measures. Presenting more detailed data (ORs+95% CI+p-value if you like) will occupy some space and I suggest you skip the list of all other outcomes.
A.3 – As suggested, we reported only the two main outcomes in the revised text.

Q.4 – "in a sample of childbearing age women" is not specific enough, please state the number of women and their ages (median and range). Was this a matched case-control study? If so, state that and for what the women with or without CD were matched (age?, other?) smoking?).

A.4 – We reported the number of women and their ages (median and range) in the results section. We clarified the used approach in the revised text.

Q.5 – Conclusion. You state that the correlation between CD and reproductive disorders could explain fertility problems in CD. However, what is very important is that the largest study until this date (Tata et al) actually found not association between CD and infertility! Actually we do not know if there is any link between CD and infertility... Hence I suggest that you do not refer to infertility in your conclusion.

A.5 – Thank you for your suggestion.

INTRODUCTION.

Q.6 – The prevalence on different continents is not necessary, neither the absolute number of individuals with CD in the United States.

A.6 – As suggested we removed the sentence.

I suggest skipping the references by Baldassare and Malamut. These were not original works. If you want a review on the prevalence of CD, try Dube et al 2005.

A.7 – The above references were skipped and the suggested reference was introduced in the revised text.

Q.8 – You cite a paper by Ciacci (reference 17), please note that the shorter breastfeeding duration in mothers with untreated CD was not statistically significant (p-value <0.10 but not <0.05). The same was true for the increased risk of low birth weight that was not stat significantly increased in the Ciacci paper (p-value above 0.05, but below 0.10).

A.8 – These citations were deleted from the revised text.

METHODS

Q.9 - Omit the words about privacy law watchdog

A.9 – The sentence was removed.

Q.10 - What proportion of women had undergone biopsy, and for women with
biopsy did you require villous atrophy for the diagnosis?
A.10 – 41.9% of the interviewed women referred to have undergone biopsy. The questionnaire did not include a specific query on villous atrophy.

Q.11 – Enrolling women from an Italian association of CD meeting is risky. It is possible that participants at such a meeting either have: a more severe disease (why else would you engage in such an organization), or that they are socially more talented, or less often have children (because than you cannot travel as freely as if you have children....)?
A.11 – Thank you for your observation. We stated this as one of the major limit of our study in the discussion. Really we know that the Italian Association of Celiac Disease (AIC) meets patients with different degree of CD to promote the assistance of them and their families and to stimulate scientific research.

Q.12 – What were the educational levels of women with and without CD?
A. 12 – We apologize for the lack of this information. The questionnaire we used was just tested by AIC in a previous own survey.

Q.13 – I am afraid that the sampling was inappropriate, that you were able to enroll all participants at the Apulia meeting and then had a 100% response rate is extraordinary. compare: 85% of controls accepted to participate.
A. 13 – See A.11.

Q.14 – Regarding the statistics, it may be that you should have used Fisher’s exact test since the expected number of women in the CD group should have been less than "5" on several occasions??
Q.14 – As stated in the Methods, Fisher’s exact test was performed when an expected variable value was less than 5.

Q.15 – Did you use a conditional logistic regression when you evaluated the case-control study? controls were matched for cases. A clear description of the statistics is very important.
Q.15 – No, we did not performed a conditional logistic regression. See A. 1.

RESULTS
Q.16 – Either the OR or the 95% CI of your data on miscarriages (page 7) is incorrect, >OR cannot be 1.2 if the 95% CIs are 0.9-4.6 (2.1 ??).
A.16 – Sorry, there was a typo. The right OR value was 2.1.

Q.17 – In the results section you present a large number of absolute data (e.g. 25
women had diarrhea, 11 had vomiting etc. Please write: (n=25) and not only “(25)” since the latter may be misinterpreted as a reference even if BMC uses [xx].

A.17 – Your suggestion was inserted in the revised text.

**DISCUSSION**

Q.18 – You suggest that women with menstrual disorders should be screened for CD. But do we know (and do you show??) that a gluten-free diet will lead to less menstrual disorders?

A.18 – GFD effects on menstrual disorders were not investigated even if during our survey we observed (and reported) that “almost 70% of celiac women with menstrual cycle disorders have reported that they did not follow a gluten free diet”.

Q.19 - Another weakness was that only women that could be reached by phone were included as controls. You did not report how many could not? This is of some importance since cases were not reached by phone. It should also be noted that data from cases and controls were obtained in different ways (or???). Cases were interviewed at the Apulian meeting while controls were interviewed over the phone. It cannot be ruled out that phone interviews were shorter, that participants did not have the time to recall all negative pregnancy outcomes and menstrual disorders and therefore underreported such events. That will lead to an overestimation of relative risks among women with celiac disease diagnosis.

A.19 – Thank you for your comment. This could represent another limit of the study. Now we better discussed this in the revised text.

Q.20 – Another weakness is that you did not consider smoking status. In most studies, smoking is negatively associated with CD (although Ludvigsson et al in their study in Clinical Gastroenterology Hepatology 2005 vol. 3 (9) pp. 869-74 found no association between CD and smoking in pregnant women). If there is no association between CD and smoking then lack of smoking data is no problem (as in the Ludvigsson paper – not the same paper that you have already referred to, published in Gastroenterology), but if there is a negative association this could have influenced your risk estimates. Please comment on this.

A.20 – We inserted the comment you suggested in the discussion.

Q.21 – Finally it should be commented that only 41.9% of the women with CD in this study had undergone small intestinal biopsy. That is a weakness.

A.21 – Only 41.9% of the women referred to have undergone small intestinal biopsy when
they were asked for the diagnosis. We agree with you that this could be a weakness and we have better commented in the revised text.

TABLES
Q.22 - Please include the data on at least one pregnancy complication in Table 2, or better create a new table (third table, see below). These pregnancy complications should also be defined. Did the interviewed women themselves define “severe anemia”? or did you have a certain hemoglobin cut-off? What was “threatened abortion” – did the women themselves define this? And what is uterine hyperkinesias? Gestational hypertension. All these concepts should be defined in the methods section, or you should state that they were self-reported and not confirmed by patient charts. If so, this should also be listed among the limitations of this paper, in the discussion section.
A.22 – All the definitions used while interviewing were reported in Appendix 1.

Q.23 – Third table. I also suggest that you create a third table for pregnancy complication.
A.23 – As suggested, we inserted a third table for pregnancy complications.

FIGURES
Q.24 – The figures are really nice. But please define the outcomes of your study, e.g. threatened abortion?
A.24 – All the definitions used while interviewing were reported in Appendix 1.

REFERENCES
Q.25 – Since you wrote your paper, an excellent paper has been published based on Danish data: Khashan et al. PMID. 19939833. (at least I believe it is published) Please refer to that paper. Several of the references in your manuscript are misspelled. E.g. title of ref 11. Journal of ref 1 etc. Please check all the references again.
A.25 – Thank you for your good suggestion. The suggested reference was added. References list was checked.

LANGUAGE
Q.26 – A professional language review of this paper would improve the paper.
A.26 – A mother language reviewer revised the text.
Reviewer: Lorete Maria M da Silva Kotze

Q.1 – Why only 41.9% of the patients were submitted to intestinal biopsy?
A.1 – Only 41.9% of the women referred to have undergone small intestinal biopsy when they were asked for the diagnosis. This could be a weakness and we have better commented in the revised text.

Q.2. Why there is no mention or relation to the severity of celiac disease and nutritional status of the patients and the disturbances?
A.2 – The questionnaire did not include specific queries on these relations. This could be a limit of our study. Really we know and stated in the revised text that the Italian Association of Celiac Disease (AIC) meets patients with different degree of CD to promote the assistance of them and their families and to stimulate scientific research. GFD effects on menstrual disorders were not investigated even if during our survey we observed (and reported) that “70% of celiac women with menstrual cycle disorders have reported that they did not follow a gluten free diet”.

Q.3 – There is no mention about the same complaints before and after a gluten-free diet, and no report about differences for the adherent and non-adherent women.
A.3 – The aim of our study was to explore the association between celiac disease and reproductive disorders. To celiac women with reproductive disorders we asked who of them followed a GDF. To analyze the same complaints before and after a gluten-free diet could be the aim of a future study.

Q.4 – The crucial point is that the authors do not try to explain the findings: mechanisms or theories about the subject!
A.4 – We reported the most supported hypothesis in the discussion.
Reviewer: Eyal Sheiner

Q.1 – I strongly advise the authors to consult an epidemiologist. Since celiac here was the exposure and not the outcome, this is a cohort study and not a clear case-control (such as studies investigating risk factors for preterm labor for example).


Q.2 – Also, there are mistakes in the results: for example the OR for amenorrhea is NOT 33, and the 95% CI is NOT 7.17-151.8, but rather the OR is 10.9; 95% CI 3.1-42.2, P<0.001!!!!!

A.2 – Sorry. We used a matched approach to calculate the ORs and their 95% CI but we forgot to state this in the Methods section. We clarified the used approach in the revised text.

Q.3. I recommend consulting with Obstetricians, since the lower weight at birth is actually birthweight etc.

A.3 – It was a problem in language revision by native speaker. Now, we corrected the term in the revised text.

Q.4 – The tables should be revised: Table 2: why do we need the "no" line? Just put the complications (amenorrhea etc). Every reader can than calculate the "no" if he wishes to do so…

A.4 – Tables 2 and 3 follows the double-entry contingency tables (2x2) design.

Q.5 – Is 49 years old or even 45 years old part of the "childbearing ages"???

A.5 – Age 15-49 is the interval considered fertile in demography by Italian National Institute of Statistic (ISTAT).

Q.6 – No need to repeat the results of the tables in the results section.

A.6 – As suggested, we removed these results from the revised text.

Q.7 – The first sentence of the abstract should be deleted.

A.7 – As suggested, we removed the sentence.

Q.8 – Use actual p values and not bigger than 0.05. P=0.06 and P=0.999 are both
bigger than 0.05 but give different illustration of the results.

A.8 – As suggested, actual p values were reported.

Q.9. English needs revision by a native speaker.

A.9 – A mother language reviewer revised the text.

Q.10 – Several studies exist regarding this issue, which surprisingly were not included in the discussion and introduction of this study. The study of Sheiner et al (Eur J Obstet Gynecol Reprod Biol. 2006 Nov;129(1):41-5. ) found higher rates of IUGR, and in a subsequent article (Arch Gynecol Obstet. 2009 Jan;279(1):1-3.) whether screening for celiac is necessary. Nevertheless, these studies were not even mentioned by the authors.

A.10 – Thank you for your good suggestion. The suggested reference was added.

Q.11 – Discussion is too long and unfocused.

A.11 – We have tried to address to all the comments of the 3 reviewers. Several points needed to be clarified in the Discussion. Nevertheless, we shortened the section.