Author's response to reviews

Title: Effects of sex hormones on bronchial reactivity during the menstrual cycle

Authors:

Maria Matteis (maria.matteis@unina2.it)
Francesca Polverino (fpolverino@partners.org)
Giuseppe Spaziano (giuseppe.spaziano@unina2.it)
Fiorentina Roviezzo (roviezzo@cds.unina.it)
Carlo Santoriello (carlo.santoriello@virgilio.it)
Nikol Sullo (nikol.sullo@unina2.it)
Maria Rosaria Bucci (mrbucci@cds.unina.it)
Francesco Rossi (francesco.rossi@unina2.it)
Mario Polverino (mariopolverin@gmail.com)
Caroline A Owen (cowen@rics.bwh.harvard.edu)
Bruno D'Agostino (bruno.dagostino@unina2.it)

Version: 4
Date: 2 April 2014

Author's response to reviews:

Dear Dr Catia Cornacchia,

We thank you for your interest in our manuscript “Effects of sex hormones on bronchial reactivity during the menstrual cycle”.

We have prepared a revised version of the article based on the reviewers’ comments, and we hope that our revisions address all of their concerns.

Please find below point-by-point responses to the reviewers’ comments and to all the issues raised in your letter, copying the pertinent sentences or word fragments from the reviewers’ critiques and your letter immediately above each of our responses. We have numbered each point as comment 1 (C1), response 1 (R1), and so on.

Our revised manuscript includes:

(a) A “Marked-up” version of our previous manuscript (we used red font to indicate the revised portions of the manuscript).

(b) A “Clean” version of our revised manuscript (we used black fonts)

In particular the specific answers to reviewers are as follows:

Reviewer: n° 1

Comments to Authors

The authors have investigated the effect of the menstrual cycle and sex hormone levels on bronchial reactivity (BR) in a group of PMA women. The main findings are that BR is increased in the follicular phase of the menstrual cycle in women and associated with lower cAMP levels in sputum samples, which may contribute
to bronchoconstriction. I have the following comments:

Major Comments:

C1: Background, page 4/5. The authors aimed to investigate the effects of the menstrual cycle in a group of PMA women. To this end, 36 asthmatic women were included. Importantly, a lot of information on the clinical characteristics of these patients is missing.

A.- Did the subjects really have asthma? This is important, because many patients seem to have a PC20 methacholine > 8 mg/ml or even 25 mg/ml.

- Did they use inhaled corticosteroids/LAMAs/LABAs?

- I would suggest to include a table with the baseline characteristics and outcome of the measurements during the follicular and luteal phases.

R1: A) We would like to thank the referee for his comments. As suggested by the referee, in the new version of the manuscript we have inserted a table with the baseline characteristics and outcome of the measurements during the follicular and luteal phases. Please see page 27.

B.- Perimenstrual worsening of asthma has been reported in 30-40% patients. Importantly, the subjects included in the present study are referred as ‘PMA patients’, at least in the methods section. However, were they really all PMA patients? This appears not to be the case, since they did not have a perimenstrual increase in symptoms or BHR. This should be made more clear throughout the manuscript, as it is currently confusing.

R1: B) We agree with the referee that in the original version of the manuscript there is a lack of clarity about whether all the subjects had PMA. Therefore, in the marked-up version of the manuscript we have added text to make this issue more clear. For example, please see the revised text on page 2, 8 lines from the bottom of the page; 1st line on page 5; page 6, 10 lines from the bottom of the page.

C. With respect to the study design: When exactly were the measurements performed during the menstrual cycle, i.e. PFTs, PC20, blood collection? At day 1 of menstruation and 14 +/- 1 days after? Obviously this is crucial information which is currently lacking. R1: C) We apologize for this omission. The exact timing of the measurements (PFTs, PC20 and blood collection) has been added in the revised version of the paper to page 10, 11 lines from the bottom of this page.

C2: The authors did not find any difference in the severity of BR between the luteal and follicular phase. Nevertheless, they argue that 72% of patients had a lower PC20 FEV1 in the follicular compared to the luteal phase. Was there a difference when analyzing the data in this way? Although I do not feel the data to be very convincing, I would describe these results in results section and then make a clearer decision in the discussion as to whether or not the severity of BR should be considered to be more severe the follicular phase.

R2: Following your suggestion, we have clarified the differences in PC20 FEV1 in the follicular compared with the luteal phase. Indeed, patients with the lowest
PC20 FEV1.0 comprised ~30% of subjects (not 70%), which is precisely the opposite of what we stated in the first version of the paper, due to a transcription error. We have corrected this statement in the result and discussion sections of the revised manuscript.

C3: The discussion with respect to testosterone is too long.
R3: The discussion with respect to testosterone has been shortened in the revised manuscript.

Minor comments:
C1: PMA is described as an exacerbation of symptoms. I would suggest to change exacerbation to worsening in this context throughout the ms.
C2: Methods. Study population. This section includes results which should be described in the results section.
C3: Measurements of IgE mediated hypersensitivity. Very lengthy description and I am not sure why this is relevant.
C4: An IgE level > 100 was considered as high probability of allergy. Do the authors mean total IgE? In that case, I do not agree as patients with non-allergic asthma often also have increased total IgE levels.
C5: FEV1 or FEV1.0?
C6: The direction of the associations is often very confusing.
Example 1, page 14. “Our study documented a significant inverse correlation between BR and serum testosterone levels:”
- First, according to figure 1, I think the authors mean a positive correlation rather than inverse correlation.
- Second, does an increase in BR refer to an increase in the PC20 value or a more severe BR, i.e. lower PC20 value?
Example 2, page 15 “Although sputum levels correlated indirectly with sputum PDE levels”: What does this mean? A higher sputum testosterone is associated with lower sputum PDE? Please describe the results more clearly throughout the ms.
C7: Results. Page 7. Start with the alinea describing the allergic profile? Why is this information important? R: In agreement with your suggestion, all of the above listed minor points have been addressed in the revised manuscript.
R1: see page 2 8 lines from the bottom of the page and page 5 1st line from the top of the page.
R2: see page 12.
R3: see page 7 14th line from the top of the page.
R4: see page 8, 2nd line from the top of the page.
R5: see page 2, line 13 lines.
R6: see page 15 line 16 and page. 16, line 14.
R7: see page 12.

Reviewer n° 2

Comments to Authors

Major Revisions:

This is an original investigation on the role of hormonal changes in young women affected with pre-menstrual asthma. The study has been correctly and interestingly carried out and in my opinion no compulsory revisions should be done. The demonstration here of the role of testosterone in this respiratory alteration is a breakthrough for eventual studies on the role and ways of action of this hormone.

Minor revisions:

C1) -The first citation of PDE, while the acronymus should be well known, must be preceded by the extended name phosphodiesterase;

C2) - It seems that a discrepancy does exist between the percentage of women with alteration of respiratory function results between the text and the legend of the fig.1. This issue must be clarified.

C3) - When reporting the results, Authors indicate at first the title human study, but there is not an eventual section entitled "in vitro study" as already done in method section. We thank you for comments. In agreement with your suggestion, all of these minor points have been addressed by revisions to the text in the revised manuscript.

R1: see page 2, 6 lines from the bottom of the page;

R2: the patients with lower PC20 FEV1.0 were about 30% and not 70%, precisely the opposite of as stated in the first version of the paper, due to a transcription error (see page 12, 3 lines from the bottom of the page;

R3: see page 14, 1st lines from the bottom of the page.

Reviewer n° 3

Comments to Authors

Major points:

C1. Lack of quantitative data in the abstract.

R1: As suggested, as you can see in the revised version of the manuscript we have included quantitative data in the abstract.

C2. The novelty of the study is not clearly stated in the Introduction section. Authors should provide that.

R2: We would like to thank the referee for his comment. We have outlined the novel findings more clearly in our revised paper in the Background section and added a new sentence on the novel aspects of our study to page 4, 5 lines from the bottom of the page.

C3. In the Results section, the authors show the allergic profile of the subjects of the study. For simplification, I suggest that the authors convert this data into a
chart or table.

R3: Following a suggestion made by another reviewer (see above), we have moved the allergic profile of the subjects to the methods section. Therefore, we think it is not necessary, in the new version of the manuscript, to convert this data into a chart or table.

C4. English needs to be corrected throughout the text (mainly Materials and Methods section).

R4: We have corrected the English in all sections of the revised manuscript.

C5. The discussion seems too long, authors should consider to reduce it and make it easier to follow.

R5: Following your suggestion, the discussion of the manuscript has been substantially shortened.

Minor points:

C1. The format of some references should be reviewed.

C2. The description of functional methods is unclear.

C3. Revise all the figures’ captions.

R3: All of these minor points have been addressed by making revisions to the revised manuscript.

Reviewer n° 4

Comments to Authors

In the present study, the authors investigate the effect of sex hormones on bronchial reactivity during the menstrual cycle using a cohort of 36 pre-menopausal women. This manuscript is well-written and well-organized, and the results are clearly summarized. However, the study would benefit from a more rigorous description of the study population demographics as well as the statistical methods used. Specific comments are as follows:

Major Comments

C1. Study participants demographic characteristics and additional information provided for the participants during the interview at the time of enrollment needs to be summarized in the results section of the manuscript.

R1: We have addressed your concern and those of other reviewers by adding a table containing the baseline characteristics of the study population. Results of the measurements during the follicular and luteal phases have been added in the text. Please see page 27.

C2. A description of the statistical analyses carried out by the authors needs to be included in the methods section of the manuscript. Statistical methods used in comparison, covariates included in the statistical models, and statistical power of the study need to be defined in detail.

R2: Addressing your comment and those from another reviewer, we have included a detailed description of the statistical analyses used in the Methods section of the revised manuscript. Please see page 11.
C3. In the results section the authors reported not significant differences in the PC20FEV10 values, LH and PRL plasma levels and ESTR and PGR plasma levels during the follicular and luteinic phases. Could this lack of statistical significance be due to a sample size issue?

R3: Even if we do not have a large sample size, this is unlikely to be the cause of the lack of significant difference between the PC20FEV10 values, LH and PRL, plasma levels and ESTR and PGR plasma levels during the follicular and luteinic phases. Indeed, with the same sample size, we demonstrated significant differences in the PC20FEV10 values and testosterone plasma levels.

Minor comments

C1. P values are missing for comparisons in Table 1 and Fig 2-4 and Fig 8.

C2. The terms “intermittent asthma” and “persistent asthma” (results section, allergic profile) need to be defined.

R: To address your concerns, all of the above-listed minor points have been addressed by additions to the text in the revised manuscript.

R1: see page 28 and pages 29-30.

R2: see page 6 line 5 and 8 lines from the bottom of the page.

Reviewer n° 5:

Reviewer's report:

Discretionary Revisions.

C) Add the description of the statistical analysis which is not adequately reported in the section Methods.

R: To address your concern and those of the other reviewers, we have included in the methods section of the manuscript a detailed description of the statistical analyses used.