

ANSWERING REVIEWERS



Authors' reply to comments for manuscript number 6962

Title: Gender-related differences in IBS: Potential mechanisms of sex hormones

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6962

We thank the reviewers for the helpful and thorough analyze of our manuscript. We also thank them for their valuable time reviewing this paper, which we admit, is very long. We have carefully read their comments and revised the manuscript accordingly. Our answers to specific queries are as follows:

Responses to Reviewer #1's comments to authors:

Generally:

1. The review is way too long!!! 43 pages exceeds standards for reviews published in journals. It is beyond my understanding why the authors have submitted an abstract this long as it is not realistic to have it published in its current length. Furthermore the authors should be aware that this puts a significant workload to the reviewers.

As a consequence of the previous comment the authors seriously need to prioritize what should be included in the review. This review covers a very large subject with many aspects explained thoroughly. My proposal is to leave the basic physiological explanations out even though they are well written. Focus on the topic- the affect of the sexhormones and how this could lead to IBS symptoms.

We understand that the manuscript is very long, because of the numerous mechanisms of ovarian hormones encompassed by the review. We agree that the review should be more focused and we reduced its length accordingly.

The abstract has been reduced to one page.

The manuscript has been reduced from 10,077 words to 7977 words. Numerous part of the manuscript have been removed.

2. The authors needs to be very aware of not describing the female hormones as disease causing per se, as most women live a life without symptoms of IBS. It could be more clear and underlined more how these hormones could make women more susceptible to developing symptoms of IBS (and why they are not disease causing in most women). Because the abstract is so long and contains so much information this important point is not as clear as it should be.

We agree and are aware that ovarian hormones are a natural component of women biology and have paid special attention, throughout the manuscript, not to state that ovarian hormones cause IBS per se.

3. The authors should comment on the fact that in the Rome III questionnaire for IBS, a patient can not be defined as having IBS if symptoms only occur during menstrual bleeding. (<http://www.romecriteria.org/pdfs/IBSMode.pdf>) I know the authors do not state that sex hormones could only cause symptoms during the menstrual bleeding but more than once symptoms are described as worsened during menstrual bleeding. It should be argued from the authors how sex hormones could play a role in the development of IBS symptoms even though the mentioned exclusion criteria exists in the Rome III questionnaire.

This is a very pertinent comment and menstrual bleeding should indeed not be forgotten when assessing symptoms in patients and making diagnostic, as recommended in the Rome III questionnaire. However, our review includes studies with patients diagnosed with Rome I and II criteria as well as Rome III criteria. Consequently, we did not differentiate between the specific patient cohort recruitment criteria and analyzed the data as a whole.

Moreover, we did not state a causal link between menstrual bleeding and symptoms but only reported the findings of many studies showing worsening of symptoms during the menstrual period (and not during menstrual bleeding only).

4. The review should be readable to gastroenterologists. Phrases like "perimenses", "met/diestrus" should be explained first time they are mentioned.

All non general public concepts are now explained the first time they are mentioned.

5. An illustration of how the sex hormones potentially could lead to IBS symptoms could be very usefull. It could give a quick overview.

We provide an illustration of the potential mechanisms of sex hormones in Figure 1.

Specific comments:

1. Key words: Some of the key words are to general; e.g. human., symptoms, genes enviromen

Key words that were too general were removed from the list.

2. Abbreviations: It is standard to abbreviate Constipation predominant IBS: IBS-C and not C-IBS as written in the manuscript. Same goes for IBS-D and A. Not all abbreviations are included (e.g. HPA).

The abbreviation list has been updated and IBS-C, IBS-D and IBS-A abbreviations are now used throughout the text.

3. Abstract: The method should be described more detailed. See comment on method in general.

The abstract has been re-written.

4. The first line in the conclusion does not make any sense : "Although they do not influence clinical...": As IBS is not an organic disorder per se, the diagnosis relies on the clinical presentation, so if sex hormones do not indfluence clinical presentation of IBS are they then relevant at all? I do not think this sentence represents what is written in the manuscript.

The sentence has been removed from the conclusion.

5. Introduction: reference 1 and 2 could be more updated. There are more recent reviews on the prevalence of IBS.

References have been updated where appropriate.

6. It is argued that there is a strong correlation between dysmenorrhea and IBS, and perimenstrual exacerbation of abdominal pain have been observed. In relation to this the authors need to comment on the exclusion criteria in the Rome III questionnaire as mentioned in the general comments.

We addressed this question in the point #3 of Reviewer 1's general comments.

7. Method: This section needs to be much more detailed, see the PRISMA statement for guidelines on how the method of a systematic review should be described (<http://www.prisma-statement.org/statement.htm>) e.g. there needs to be information on number of papers included/excluded etc.

Our review does not address a specific and very delineated question but aims at presenting the current knowledge on the numerous and diverse actions of ovarian hormones and their potential influence on IBS pathophysiology. We do not focus solely on one question (e.g. "the effect of ovarian hormones on gut permeability"). Moreover, our review does not deal with quantitative meta-analysis of data. As such, our review is not a systematic review and is not appropriate for PRISMA guidelines. However, we provide more details on how we processed the papers in the methods section.

8. Female gender and influence of ovarian hormones on GI motility: Page 10. Reference 35 concludes the opposite of what the authors write. Low estrogen levels are associated to faster transit time and not reduction in transit time as written. Furthermore this reference concludes it is the upspeed in transit time that lowers the estrogen levels and not the other way around.

The correct inferences from the results of the paper have been incorporated in the manuscript.

9. It is stated that GI transit is prolonged during the third trimester of pregnancy as an argument for ovarian hormones playing a role in GI motility. Motility could also simply be prolonged as a consequence of the baby taking up space in the abdomen, making transit more difficult? This observation is not transferable to women with irritable bowel syndrome?

These possibilities have been addressed in the manuscript.

10. Page 13 The last section just before key points describes ovarian hormones and 5-HT possible impact on GI transit. This is one of the sections referred to in general comments where it needs to be more clear how this is related to disease. These hormones also fluctuate in healthy women.

This concern has been addressed and is now highlighted on page 13 of the manuscript.

11. b. Alterations of central pain processing...Page 17: In the second sentence it is written "This multimodal treatment...". What treatment is referred to? It is not clear.

For better clarity, this segment has been removed from the manuscript.

12. b. Gender differences in central visceral pain...Page 23: In the last sentence on the page it is written: "Indeed, women display differences..., such as "wind-up" in the spinal cord for instance". What does wind-up mean? This needs to be more clear!

The definition of "wind-up" has been added in the text.

13. 3. stress and female gender in IBS: In the last sentence of the section on page 29 it is hypothesised that that early stress experience can influence autonomic and HPA response even though the opposite was concluded from reference 220 and 235 on page 28?? The hypothesis appears very speculative and not very well argued for.

It was not said that stress cannot influence autonomic and HPA response but only that some studies in humans failed to show a direct correlation between women's vulnerability to stress and adverse life events and neuroendocrine responses (Ref. 143, 144 and 145 on page 23). This only suggests that early stress experience may not influence the HPA response in women (and not in humans in general) and many possible methodological confounders can explain the lack of positive findings, notably the parameters used to assess autonomic response (indirect methodologies, blood work but no biopsies). On the other hand, insights from animal studies, indeed clearly show that stress can lead to changes in hormones levels (Ref. 146-152 on page 24).

14. 4. Stress, ovarian hormones...Page 30 It is written: "These data give further support to the idea that overactivity of CRH signalling in the brain and the gut may explain the comorbidity of stress, depression and IBS in women": Again this seems like speculation as the opposite is concluded from reference 220 and 235 on page 28. Hypothesis should not be made from studies on rodents when studies on IBS patients show the opposite.

The reason why studies in humans failed to show a correlation between women's vulnerability to stressful adverse life events and neuroendocrine responses could be attributable to the fact that parameters of autonomic response assessed in these studies were indirect (e.g. skin conductance, heart rate) and stress hormones levels were determined only in plasma and saliva but not in tissue. However, in animal models, these shortcomings can be overcome, which may explain why correlations are found in animals and not in humans. However, we agree that conclusions in humans should not be made from results in rodents. We thus rephrase the sentence in a less speculative way.

15. Page 31: IBSI should be corrected to IBS

The misprint was corrected.

16. 2. Estrogens and the gut barrier. Page 32 -Reference 285 is referred to as describing colonic epithelium. This is wrong! The reference describes uterine epithelium. It is not relevant to include this reference in the review. - It is not relevant to mention ERs effect on endothelial and cervical endothelium and breast cancer cells. Focus on the topic of the review!

These irrelevant references have been removed from the text.

17. 4. Ovarian hormones... Page 34. The last sentence of the section is too long. It is too difficult to understand!

The sentence has been rephrased in order to make it easier to understand (page 29).

18. II. proposed integrative... Page 41. The first sentence of the section does not make sense. Needs to be corrected.

The sentence has been changed accordingly.

19. It is described that ovarian hormones act as a triggering factor to further sensitize this priming and ultimately contribute to onset of IBS. It does not seem likely as these hormones are a natural part of puberty. The authors should really be careful to not somatize a natural part of female life.

We paid special attention in order to draw carefully the line between normal and pathological mechanisms of sex hormones.

Responses to Reviewer #2's comments to authors:

1. The manuscripts entitled 'Gender-related differences in IBS: Potential mechanisms of sex hormones' by Meleine and Matricon provides a very comprehensive review of the literature on IBS with a focus on the importance of gender and sex hormones in the onset of the disorder.

The manuscript is generally well written, however there are numerous typographical and grammatical (problems with the use of 's' for pluralism and possession) which would benefit a review by a native English speaker.

The major comment is that the review would benefit from being more concise and focussed. In a special edition journal it's likely that the general description of factors contributing to IBS will be described by others, this manuscript should focus on the effects of gender and sex hormones on IBS. Thus, a couple of introductory sentences, rather than two paragraphs should be sufficient prior to discussing the influence of sex hormones on IBS. This will also reduce the number of references which are abundant (>300).

Clearly describe the changes in hormones during the menstrual cycle so that the reader can make conclusions regarding the changes in IBS symptoms at different stages during the cycle.

Also, only list the key points at the end of the manuscript to take all the information as a whole rather than after each section.

Include a figure to summarise the proposed integrative model.

In line with the comments of Reviewer 2, we downsized the manuscript, keeping it more focused on the effects of gender and sex hormones on IBS and on the possible mechanisms of action of sex hormones.

We provided the reader with extra explanations where appropriate to make sure that the lay readership can be familiar with the different concepts presented (e.g. menstrual cycle, wind up).

Given the structure of the manuscript, and the span of the review, which encompasses the influence of ovarian hormones at multiple levels (gut barrier, central and peripheral pain pathways, gut sensorimotor system, gut immunity), we think it is more useful for the reader to list key points at the end of each section rather than having them all combined at the end of the manuscript. We believe it also keeps the manuscript structured in a more pedagogical way.

We now provide a figure summarizing the proposed integrative model (Figure 2).

2. Minor comments:

Please complete lists. Do not end with '.....' or 'etc'.

The abstract seems quite long (>400 words).

Include running title and key words.

Pg 6: dysbiosis of what?

Pg 10: surely other factors in pregnancy such as displacement of bowel, changes in diet and reduced activity are also likely to influence GI transit time?

Pg 10: it's not clear what is meant by 'fundamental' studies – basic science?

Pg 13: male and 'female' IBS patients sounds better than 'male and women IBS..'

Pg 23: it's unclear what 'wind-up' of the spinal cord means. Please explain

The aforementioned minor comments were addressed and corrected and are highlighted in the revised manuscript.