Author’s response to reviews

Title: A preliminary evaluation of influence of body mass index on in vitro fertilization outcome in non-obese endometriosis patients. An observational cross-sectional study

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Author’s response to reviews:

Dear Editor and Reviewers:

Subject: Submission of the revised paper "A preliminary evaluation of the influence of body mass index on in vitro fertilization outcome in non-obese endometriosis patients. An observational cross-sectional study" (Manuscript number: BMWH-D-17-00006)

Dear Editor,

First, I would like to express our sincerest thanks for your response letter and for the reviewers' comments on our manuscript entitled "A preliminary evaluation of the influence of body mass index on in vitro fertilization outcome in non-obese endometriosis patients. An observational cross-sectional study" (Manuscript number: BMWH-D-17-00006). We are pleased to resubmit for publication the revised version of our manuscript. We appreciate the constructive criticism of the reviewers, and the reviewers’ comments were valuable, insightful and helpful for revising our paper and enabled us to improve the quality of our manuscript. We have studied the
comments carefully and have made the corrections which we hope meet with approval. Revised parts of the manuscript are marked in red in the document.

Below we respond to the specific comments / suggestions / queries point by point.

Major comments

Edgardo Somigliana (Reviewer 2):

"The sample size is absolutely insufficient to draw meaningful conclusions."

"A sample size calculation is lacking and should be added."

Paola Vigano (Reviewer 3):

"The Authors did not provide a sample size calculation based on a primary endpoint. Therefore, it is highly likely that the study is underpowered."

"A more reliable message could be obtained including a higher number of patients."

Response to Major comments

The most critical concern we identified was related to the sample size calculation. We sincerely appreciate the comments of reviewers Edgardo Somigliana and Paola Vigano. We hope the reviewers and the Editor will appreciate the effort we have made to evaluate the situation at hand and arrive at conclusions which would guarantee the quality of our manuscript.

As stated in the title of our manuscript, our retrospective observational study is a “preliminary study”, and we hope to have accomplished some of the aims of this type of study. They are most often defined as the evaluation if a new idea is worth developing; initial assessment of the issue (that previously was at a too preliminary stage for a full-scale definitive study) that could be address in future prospective studies; addressing the topic where no study has ever been done before; providing sufficient information to design a definitive study; providing up-to-date data, often used for the calculation of the sample size for the future, main trials (A) [Smith PG. Preliminary studies and pilot testing. In: Smith PG, Morrow RH, Ross DA, editors. Field Trials of Health Interventions: A Toolbox. 3rd edition. Oxford (UK): Oxford University Press Oxford; 2015. p.216-222.]. However, we are aware that some statements that we made were more ambiguous than intended, and we have adjusted the text to be clearer (Please see lines 33-34, 50, 78-81, 230-231, 234-236, 256-264, 257-259, 390, 394). We hope our revision and explanations (further in this response to reviewers) regarding sample size will improve the paper to a level of their satisfaction.
Additional explanations:

Previously published literature provide very few data on the prevalence of under-, normal and overweight patients who underwent IVF. Also, only one study provided this information specifically for women with endometriosis. Moreover, these studies (included in the discussion and quoted in the references section as references No 11, 12, 13 and 14) were published more than 15 years ago. On the other hand, today’s lifestyle and behavior choices are often sedentary and unhealthy, which could lead to a redistribution of women between populations of underweight, normal weight, overweight, and obese women. We delivered up-to-date information on the specific prevalence of those populations among infertile women with endometriosis undergoing IVF, which adds value to our paper. The importance of such data lies in the fact that an estimate of prevalence is needed for sample size calculation. Since the literature often delivers several different prevalences, up-to-date facts from the most recent preliminary studies with similar study design and population are very valuable [Naing L, Winn T, Rusli BN. Practical Issues in Calculating the Sample Size for Prevalence Studies. Archives of Orofacial Sciences 2006; 1: 9-14.].

In our effort to improve the manuscript, we have encountered an accepted opinion that preliminary studies per se, do not require sample size calculation and formal calculation may even not be appropriate [Billingham SA, Whitehead AL, Julious SA. An audit of sample sizes for pilot and preliminary trials being undertaken in the United Kingdom registered in the United Kingdom Clinical Research Network database. BMC Med Res Methodol. 2013; 20; 13:104.]. Furthermore, only a small proportion of published preliminary studies and pilot trials report study sample size calculations and most journal editors state that it is not a mandatory criterion for their publication [Arain M, Campbell MJ, Cooper CL, Lancaster GA: What is a pilot or feasibility study? A review of current practice and editorial policy. BMC Med Res Methodol 2010, 10:67.].

With this in mind, sample size calculation was not performed initially. However, a highly homogenous group of study participants could eliminate almost all confounding factors that may lead to bias. Nevertheless, we tried to be very careful in the interpretation of our results. Results were discussed only in the context of similar studies, without drawing any strong or limiting conclusion about the issue. Therefore, we believe that the discussion in that framework was not overstated. To further address this issue, the Discussion section contains a sentence: "We are aware that the strict inclusion criteria applied lead to a relatively small number of underweight participants and we acknowledge this as a limitation of our study." and similarly in the Conclusions: "We believe that consideration of our results could initiate further evaluation of this topic" and "Prospective studies with large number of patients with endometriosis or prospective
After reading the reviewers’ comments, which we found very valuable for this discussion, we intended to make an additional effort to improve the manuscript by adding sample size calculation for future trial(s). We extensively searched the literature on sample size calculation, particularly concerning trials in IVF and reproductive medicine. Unfortunately, our effort to additionally improve the manuscript through the addition of sample size calculation for future trial(s) was without success after we analyzed the available literature on the issue. Sample size calculation for future studies was found to be highly dependent on too many unknowns (variables) which could be determined only by the decisions made by future investigators and circumstances related to future investigators, such as:

1) Decision about the primary endpoint of future studies
2) Estimated IVF outcomes in the centre(s) involved in study and decision on clinically Minimally Important Difference (MID)
3) A decision on the key elements needed for a proper sample size calculation (the effect size, variance, preset significance level, statistical power, the choice of a one or a two-tailed statistical analysis).

ad 1)

The decision about an outcome for trials of IVF is complex, owing to the multistage nature of the treatment. Different outcomes can be depicted as the primary endpoint, such as live birth outcomes, pregnancy outcomes (biochemical, clinical or ongoing pregnancy rates), stimulation outcomes (total gonadotrophin dose, stimulation duration, number of oocytes, number of mature oocytes, cycle cancellation...), fertilization outcomes (fertilization rates, number of good quality embryos, total number of embryos, number of frozen embryos...), transfer outcomes (number of transferred embryos, implantation rates). Consequently, endpoints could be dichotomous, ordered categorical and continuous and sample size will be different for each type of endpoint [Campbell MJ, Julious SA, Altman DG: Sample sizes for dichotomous, ordered categorical and continuous outcomes in two group comparisons. Br Med J 1995, 311:1145–1148.]. The recent review of outcome measures reported in 142 IVF trials demonstrated that no single outcome measure appeared in a majority of studies [Wilkinson J, Roberts SA, Showell M, Brison DR, Vail A. No common denominator: a review of outcome measures in IVF RCTs. Human Reproduction 2016; (31): 12: 2714–22.]. The situation is still complex even if we consider the different features of the two most commonly used primary endpoints, clinical pregnancy rate or live birth rates, which vary considerably [Braakhekke M, Kamphuis EI, Van Rumste MM, Mol
F, Van Der Veen F, Mol BW. How are neonatal and maternal outcomes reported in randomized controlled trials (RCTs) in reproductive medicine? Hum Reprod 2014b; 29:1211–1217.

[Dapuzzo L, Seitz FE, Dodson WC, Stetter C, Kunselman AR, Legro RS. Incomplete and inconsistent reporting of maternal and fetal outcomes in infertility treatment trials. Fertil Steril 2011; 95:2527–2530.] For example, in one fertility center, clinical pregnancy rate per embryo transfer is 66%, while live birth rate per embryo transfer is 58% in patients younger than 36 years of age [http://www.advancedfertility.com/ivf-success-rates.htm]. Therefore, in same study population sample size will be different for different endpoints; especially in cases where prevalences of different study subgroups differ considerably, which is the case for BMI subgroups of women with endometriosis undergoing IVF.

Sample size calculation in IVF trials is based on IVF cycle outcome, on the observed differences in IVF outcomes between study groups and on the judgment on what constitutes as a clinically Minimally Important Difference (MID) [Tarek El-Toukhy, Rudi Campo, Sesh Kamal Sunkara, Yacoub Khalaf and Arri Coomarasamy. A multi-centre randomized controlled study of pre-IVF outpatient hysteroscopy in women with recurrent IVF implantation failure: Trial of Outpatient Hysteroscopy - [TROPHY] in IVF. Reproductive Health 2009; 6:20.] Therefore, IVF outcomes and decision on MID are (again) dependent on the decision of future study design and circumstances related to future investigators.

In conclusion, according to data from preliminary studies, future investigators could calculate sample size according to their study design (for example, according to planned primary study endpoints and estimations of pregnancy or live birth rates that they expect to achieve). Furthermore, on those grounds, future investigators could appraise the time needed to enroll the target number of trial participants and the overall duration of the trial according to the number of IVF cycles they perform during year (Please see lines 260-264).

Response to Jesus Salvador Jiménez López, Ph.D, M.D. (Reviewer 1): We appreciate the reviewer's positive evaluation of our work.

Comment 1: "The way of exposing the justifications that lead to the hypothesis of the present article is somewhat confused. We would recommend revising the wording of this section (lines 27-34)."

Response: We deeply appreciate the reviewer's suggestion. Therefore, we have revised the wording of the manuscript's Abstract (Please see lines 27-34).
Comment 2: "Table 2 only shows the degree of endometriosis (they have not calculated percentages in patient with normo and overweight) and whether or not there were endometriomas."

Response: Firstly, we apologize for the missing percentages of the degree of endometriosis in normal weight and overweight patients. Secondly, we deeply appreciate the reviewer's suggestion about the information about existence of endometriomas. According to the reviewer's comment, we provided more comprehensive table (Please see Table 2, lines 194-195).

Comment 3: "It is not specified whether the time of infertility elapsed prior to surgery or its temporal relation to the intervention."

Response: We sincerely appreciate the reviewer's suggestion about clarification of this matter. Therefore, we rewrote the sentence in the Methods section of the revised manuscript as the following: "… duration of infertility prior to IVF procedure …" (Please see line 130).

Comment 4: "It is also not known whether they had undergone previous cycles of IVF before confirming endometriosis. It is assumed in the same way that the diagnosis of endometriosis was established in the context of infertility, and that therefore they did not receive medical treatment for this pathology, although this data does not appear either."

Response: It is stated in the first sentence of Methods section "We investigated the influence of BMI on outcome of first, fresh, autologous IVF cycles in non-obese patients with endometriosis (BMI<30)…". Therefore, patients in our study had not undergone IVF before confirmation of endometriosis. However, we express regret for some statements that we made were more ambiguous than intended, and we have adjusted the text to be clearer. We sincerely appreciate the reviewer's suggestion about clarification of this matter. Therefore, we rewrote the sentence in the revised manuscript as the following: "We investigated the influence of BMI on outcome of first, fresh, autologous IVF cycles in non-obese patients (BMI<30) with previously diagnosed endometriosis…” (Please see lines 86-90).

Comment 5: "Being aware of the compromise involved in compromising the ovarian reserve with the surgical manipulation of the appendages, exclude those patients who were submitted to it for reasons beyond the control of endometriosis. However, a cystostomy in the context of an endometriosis would hypothetically react in the same way as for another cause. We therefore consider that it could be a bias. Determining prior to and following the same parameters such as baseline levels of E2 and FSH, antimullerian hormone and count of antral follicles could throw information on this and support his theory exposed in lines 382-384."

Response: We agree with the reviewer's comment. However, considerable number of women with endometriosis, especially those with moderate and severe grades of the disease, has endometriomas which require to be treated with cystotomy. Since the assessment of ovarian reserve by antral follicle count, serum level measurements of E2, FSH and/or AMH is not part of
routine patient evaluation prior to laparoscopy at our clinic, this kind of the evaluation is not possible due to retrospective nature of our preliminary study. However, this appraisal should be strongly considered in future prospective studies.

Comment 6: "Regarding the IVF cycle: There are no grounds for using GnRH agonists or antagonists (lines227-228)."

Response: Thank you for your comment. Grounds for using GnRH agonists or antagonists were added in Methods section (Please see lines 141-145).

Comment 7: "The quality of the transferred embryos is not known, a fact that clearly influences the results of the cycle."

Response: We sincerely appreciate the reviewer's suggestion. Therefore, information about the quality of the embryos among study groups is presented in table number 3 (Please see line 151 and Table 3, lines 211-213).

Comment 8: "It is important to make an appreciation about the clinical utility of the same. Based on the hypothesis of their hypothesis, they should demonstrate that changes in BMI lead to changes in the severity of endometriosis and therefore in the results of the assisted reproduction technique; The objective of the analysis should be directed to the normopeso leads to a greater success of the same, in order to advise the patient, and not to do so through overweight."

Response: We appreciate the reviewer comment. According to this valuable comment we have added further information to the manuscript (Please see lines 357-374).

Response to Edgardo Somigliana (Reviewer 2):

Comment 1: "The rational for the investigation is weak"

Response: We have revised our present paper in the light of your useful comment. According to this comment we have added further information to the manuscript (Please see lines 27-34 and 357-374).

Comment 2: "The Materials and methods section is too long. It can be halved without losing relevant information."

Response: We sincerely appreciate the reviewer's suggestion about the length of Materials and methods section. Therefore, we have revised this section according to the reviewer's recommendation (Please see lines 87-171). Number of words in Materials section is 1050 in revised version, compared to 1438 in previously submitted version.

Comment 3: "The discussion is also exceedingly long. Surprisingly, the authors dedicate most of the discussion to discuss their results within the available literature but only few lines to the important limitations of their paper."
Response: We completely appreciate this remark of the reviewer and therefore discussion was modified to the extent that we hope will fulfill the requirements of the reviewer and your esteemed journal (Please see lines 228-402). Number of words in Materials section is 2106 in revised version (together with added text according to reviewers comments), compared to 2347 in previously submitted version.

We hope that the revisions in the manuscript and our accompanying responses will be sufficient to make our manuscript suitable for publication in BMC Women's Health.

Sincerely,

Milan Perovic

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