Author’s response to reviews

Title: UTERINE AND PLACENTAL BLOOD FLOW INDEXES AND ANTINUCLEAR AUTOANTIBODIES IN UNEXPLAINED RECURRENT PREGNANCY LOSS: SHOULD THEY BE INVESTIGATED IN PREGNANCY AS CORRELATED POTENTIAL FACTORS? A RETROSPECTIVE STUDY

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PRCH-D-19-01104
UTERINE AND PLACENTAL BLOOD FLOW INDEXES AND ANTINUCLEAR AUTOANTIBODIES IN UNEXPLAINED RECURRENT PREGNANCY LOSS: SHOULD THEY BE INVESTIGATED IN PREGNANCY AS CORRELATED POTENTIAL FACTORS? A RETROSPECTIVE STUDY.

Valentina Bruno; Carlo Ticconi; Federica Martelli; Marzia Nuccetelli; Maria Vittoria Capogna; Roberto Sorge; Emilio Piccione; Adalgisa Pietropolli
BMC Pregnancy and Childbirth

Editor Comments:

Please also address the following essential point:

1. Please clarify your study design. If patients were prospectively assigned to different groups to receive the intervention your study would constitute a clinical trial and would therefore require registration in a suitable repository and to be reported in line with the CONSORT guidelines. Please see:
Patients were not prospectively assigned to different groups to receive the intervention, because of medical-legal and ethical issues. The study has a retrospective design.

Reviewer reports:

Jean Christophe Gris (Reviewer 1): This is a retrospective study which claimed aim is to analyse, in pregnant women, the impact of positive antinuclear auto-antibodies (ANA), but also of low-molecular weight heparin (LMWH) treatment, on various indices derived from ultrasonic explorations performed in order to evaluate the uterine and placental vascularisation. The clinical targeted population is composed of women with unexplained miscarriages, compared with women with at least 2 normal pregnancies at term, apparently matched on age and body mass index values. The underlying clinical question being on the association between ANA and abnormalities in placental perfusion which may favour miscarriages; also on any impact of LMWH treatment in that setting.

My main concerns are the following:

1- the number of women included in that study is very low. And ever more that low when considering the subgroups, categorised according to the presence/absence of positive ANA and the presence/absence of LMWH treatment during pregnancy. These subgroups being submitted to comparisons in order to clarify the purpose. It is impossible to hope for clear answers in such a situation, due to strongly limited statistical powers.

We are aware that the numbers of patients enrolled is low and this could be a limitation in our analysis and we have pointed out this issue in the discussion section. Nevertheless, we consider it as a pilot study, as a first step to further investigate this issue in a randomized clinical trials with a larger cohort. Regarding statistical power please refer to the answer to question 4.

2- LMWH treatment was given to women in the first part of the study, then not given in the second and last part of the study, without any blind, randomised allocation. This introduces a strong bias in the results, nobody can be sure that treated and non-treated women are really similar.

All the three groups have low numbers, since we were really strict in order to have homogenous groups to avoid bias, as much as possible. Unfortunately, we couldn’t perform at that time point a randomized, double blindered trial, because of some medical-legal and ethical issue, since LMWH was largely used empirically in clinical practice in Italy and only after ESHRE guidelines of November 2017, things were starting to change. This could represent a bias, but it offered us a way to have a first glance in
LMWH-ANA potential correlation. Next step will be to confirm our results in a randomized clinical trials with a larger cohort, since, as pointed out before, LMWH use is became less extensive also in our country.

3- Comparisons between groups are performed using parametric statistic tests. The recurrent miscarriage group contains, as a whole, 20 women, i.e. far from 30, and related subgroups between 10 and 16 women. It is thus strongly necessary to describe results using not mean and SD (!!!) values but median and range values, which will better match with the chosen representation of the data based on box and whiskers plots, and to perform comparisons using only non-parametric test, i.e. the Mann-Whitney test to compare 2 groups, and the Kruskall-Wallis test to compare more than 2 groups.

The reviewer points out the low number of patients per each groups and suggests that a non-parametric statistic should be avoided. However, our data submitted to the Komogorov-Smirnov test show that they adapt to Gaussian distributions with the consequent assumption that their description must be considered as mean and standard deviation. This involves that a parametrical statistic and in particular Anova tests should be performed, which is unquestionably the most "robust" and efficient test to be done, more than any non-parametric test! Furthermore, we used the Bonferroni's test, the more "restrictive" post-hoc test, rather than the Tukey’s one. For the sake of consistency, we do not present the "whiskers plots" graphs but the so-called "error bar" graphs.

4- The way the data were analysed, mainly through multiple focused comparisons, is archaic and cannot allow convincing. The possible interaction between ANA status and LMWH status in each woman opens the door to generate significant confounding factors leading to false, artificial apparent results. It cannot identify real risk factors of abnormal uterine/placenta vascularisation really independent from the other studied ones. A multivariate logistic regression analysis is needed: is positivity for ANA a real risk factor of an abnormal uterine/placenta vascularisation index independently from LMWH treatment, age, BMI,...?

The reviewer's suggestion could only be conducted on variable VI, which proved to be significantly different, but not up to the assessment of the risk of abnormal uterine/placental vascularization due to the low number of our groups. Indeed, the study wanted to demonstrate that VI can only be lowered for the ANA- group with sensitivity and specificity demonstrated by the Roc curve presented in the work. Due to the low sample representation, the logistic regression that we would have liked to present is not adequate for the suggested purpose...! Moreover, in order to have a plausible answer regarding the above mentioned risk, maybe it was necessary to evaluate also other competing parameters, most of which are still unkwn and which will be not possible to be considered and included in only one work. However, it should be borne in mind that the anagraphic-anamnestic characters of the groups (age, BMI, etc.) are similar in the treatment groups (homogeneity of the groups) demonstrating their unnecessary presence in a possible regression.

We have matched the three groups according to clinical characteristics and since no differences were found by comparing age and BMI in the three groups, and also in numbers of miscarriage and gestational week in which the miscarriages occurred, in the two RPL groups, we can exclude that these parameters could influence the results.

Regarding the influence of LMWH, its used leads the group of treated RPL patients to the same favorable conditions of controls, in terms of VI. For that reason, we consider the multivariate logistic regression analysis, not strictly indicated in this case, since we already excluded other factors because
of homogeneity of all groups. Therefore, we also considered to run the ROC curve, since it may be more convincing in reporting our results, because of information in term of sensitivity and specificity of vascularization parameters included in the study.

Furthermore, we are also aware, as a limitations of our study, that there are several factors which can influenced placental vascularization, most of which are also not really known. In that line, ANA could be not all the truth in this issue and they cannot explain all the abnormalities shown in RPL patient group.

5- The paper is very difficult to read due to the accumulation of abbreviations. We have now changed the text, in order to avoid as much abbreviations as possible.

6- The authors should give the state of the art between the ultrasonic explorations they used and their derivated parameters they studied on the risk of miscarriage. They should also tell us if early, embryonic-type miscarriages, before 10 weeks, can be studied in the same way than fetus deaths, from the 10th week.

There are no studies in the literature comparing the reliability of these methods between embryonic-type miscarriages, before 10 weeks and miscarriage after this gestational age: it could be a very interesting point to arise in order to further investigate this potential implication.

Mikiya Nakatsuka (Reviewer 2): Title: UTERINE AND PLACENTAL BLOOD FLOW INDEXES AND ANTINUCLEAR AUTOANTIBODIES IN UNEXPLAINED RECURRENT PREGNANCY LOSS: SHOULD THEY BE INVESTIGATED IN PREGNANCY AS CORRELATED POTENTIAL FACTORS?

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Authors have shown that ANA could be a factor involved in the impairment of placental blood flow in women with unexplained RPL and that LMWH could exert a potential beneficial effect. I have some comments as below.

Results

1. Please show birth weight and gestational week of the delivery of each group in Table 1. We add this information in table 1 and in the text.

2. Please show blood pressure of each group in Table 1 or 2. We add this information in table 1 and in the text.

3. Fig 2-5. Please show data of 6 groups in one figure and compare each other. We reduce the numbers of figure to reach a more clear design to help reader to follow our study results.
Discussion

1. Please describe the possible reasons why ANA causes impaired placental blood flow. We have changed our discussion, according to results coming up from the revised statistical analysis requested from Reviewer 1: please refer to discussion section in the revised manuscript.