Author's response to reviews

Title: Foetal aortic flow velocity waveforms in healthy and hypertensive pregnant women

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Author's response to reviews: see over
Cover Letter

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Editor-in-Chief,
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We have modified the manuscript to directly address all comments of the reviewers. The revised version and the response to reviewer remarks is attached to this letter, after being approved by all authors. We look forward to hearing from you.

Yours sincerely,

[Signature]

Date
January 23, 2014

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• Major Compulsory Revisions

1) Ok.
2) The fact that BP was not measured at the moment of each Doppler study should be stated as a limitation. Anyway, blood pressure was measured, and, according to it, women were categorized into normotensive or hypertensive. I don't think it is acceptable to deal with such a subject without reporting any blood pressure value ever. As correctly stated on p. 6, lines 42-44, "mild to moderate hypertension in pregnancy corresponds QUITE CLOSELY to stage 1 hypertension", as the first includes women with diastolic BP 100-109 mmHg, which corresponds to stage 2. Therefore, the definition of the subjects as stage 1 hypertension should be abandoned all along the abstract and the manuscript - unless all of the women actually had diastolic BP under 100 mmHg (in the case, this should be clearly stated). Again, this highlights the need to report the BP of the analysed population.

We added a new section at the end of the Discussion section, where we report the main limitations of the study. The fact that BP was not measured at the moment of each Doppler study was stated as a limitation. Additionally, the definition of the subjects as stage 1 hypertension was abandoned all along the abstract and the manuscript. (lines 377-382, revised version)

3) It is still doubtful to me that three time points only could be treated as a continuous variable in the analyses (especially if you admit the intervals being "roughly" and "approximately" the same). Moreover, the fact that the model just fits better with a log-transformation of "time" does not seem to me a reason strong enough to log-transform it, especially when above you just said that the intervals between time points are the same (and we are still dealing with three time points only). And the graphs all have a linear scale. Part of the problem is probably just that the mathematical analyses are quite complex, while the general audience of the paper would mostly be composed of medical doctors. Should a simpler and more accessible explanation be included in the paper, leaving a detailed mathematical description as additional file?

Goodness of fit and model validation are amongst the most important issues in the identification of a coherent parametric model, given a dataset. There are several statistical methods that have been developed with that aim in mind, namely the well-known likelihood ratio test, the information criteria, normality tests, (...) among others. The conclusions of any parametric model may be inadequate (or even wrong) if model assumptions are not satisfied. In our situation, should we disregard log-transformations, normality and heterocedasticity would be compromised. The graphs are on the original scales for the sake of its interpretation, and clearly reading of the nonlinear model. Anyway, the linear model on the log-scales is equivalent to the nonlinear model on the original scales.

The mathematical description of the model is already reduced to a minimum as many mathematical details were left behind. However, we understand that essential issues as justification of the used statistical regression model (generalized least squares instead of mixed-effects models), clear description of the linear predictor of the obtained model and some information on its goodness of fit and validity ought to be included.

4) Ok. Should 95% CI be plotted in Figure 3 to show that they don't overlap?

We included the 95% confidence intervals for the mean prediction in Figure 3. As the figure is not totally clear, its legend keeps the information about the numerical values of the intervals at each value of the trimesters. Although for PI and at the 3rd trimester the intervals seem to touch each other, the upper limit of the lowest interval is below to the lower limit of the highest interval, up to 0.001.

5, 6, 7) Ok.

• Minor Essential Revisions

8-16) Ok.
17) The fact that all hypertensive women took ASA should be stated as a limitation, as this is actually a study comparing "normotensive" vs. "hypertensive treated with ASA", and we can't formally exclude a role of ASA in determining the results.

The fact that all hypertensive women took ASA was now stated as a limitation. (lines 381-382, revised version).

18-20) Ok.
21) Ok. So none of the women dropped out because of these criteria? This should be made explicit, whether true or not. 4 are reported to have quitted because of "fetal pathology" but foetal growth is not explicitly mentioned or excluded.

Thanks for your comment. We make this more explicit in the Results section (lines 252-256, revised version).

22-23) Ok.
24) The choice of using classes and explanatory variables instead of continuous variables seems arbitrary and I would not say that is more clearly understandable, as people are well used to see age and BMI as continuous. Moreover, chosen age cut-offs are totally arbitrary; those for BMI are more meaningful, but, as we are dealing with pregnant women, can hardly be considered to have the same value as for non-pregnant people. Continuous variables should be tested, and they will probably be excluded from the model as well. If classes are to be used, tertiles could be tested. Anyway, any choice other than using continuous variables should be clearly justified.

The plot of each index (PI or RI) values against a continuous age (or BMI), done in an explanatory initial statistical analysis, did not suggest any trend between the two variables. Age and BMI were therefore discretized and used only for descriptive purposes (as mentioned in the paper, their effect on the model was anyhow evaluated but failed to be statistically significant). The discretization of BMI was based on well-known and largely used guidelines (BMI cut-off values follow the WHO guidelines).

In the absence of similar guidelines for age cut-off, we elected the 35 years as it is an important time point in clinical obstetrics and prenatal diagnosis assessment. In fact, in North America and Western Europe, according to statistical data for maternal age at the time of birth, at least half of pregnant women older than 35 years, elect chorionic villus sampling or amniocentesis for foetal karyotyping. In addition, some investigators have shown an increased prevalence of congenital heart defects among children born to women 35 years and older.

25) Ok.
26, 31) There is no reason to not mark significant differences between HT and NT in Table 2.
We apologize but we totally disagree with this referee indication. Correct p-values for Table 2 would come from the fitting of a gls (generalized least squares) model having the index values as its response variable, and time, index identification and hypertensive status as explanatory variables. Except from parity, this is our final model, which is explored in the paper. The opinion of the authors is that Table 2 is in the paper for descriptive purposes only.

We remark that p-values computed independently for each row are meaningless; time effects have to be evaluated globally, in a model that considers all data at the same time, due to the proven existent correlations.

27) "puts impedance at a lower level" is still in the abstract. Differences of indexes between NT and HT should be reported in the Results section in the abstract.

The sentence ‘puts impedance at a lower level’ was removed and differences of indexes between NT and HT reported in the Results section in the Abstract. (lines 43-48, revised version)

28-30, 32) Ok.
33) See point 4.

95% CI are now represented in Figure 3.
• Discretionary Revisions
41) I do apologize for my wrong use of the term "histogram". I actually meant "bar graph". Box-and-whiskers would be preferable to bar graphs here, as they would show the actual distribution of the indexes, instead of just a mean and 95% CI. Statistical significance of mean comparisons could be shown above the box-and-whiskers exactly as they are shown now on the bar graphs.

We apologize again but we think the bar graphs are more appropriate in this situation. They directly indicate the sample mean and the correspondent 95% confidence interval. Although box-and-whisker plots do show the variable empirical distributions, for asymmetric distributions, they fail to give precise informations on the mean. The main purpose of this figure is to provide the reader with the information on the mean values evolution across gestational time and not on the distributions evolution.

Page 4, line 81: "as a means" -> "as a mean". It is anyway questionable that a circulation could be "a mean".
Line 92: "acid base" -> "acid-base".
Page 6, line 138: "chronic infections and measured" -> "chronic infections, and measured"

All suggested changes were made.