Author's response to reviews

Title: Establishing normal ranges for fetal electrocardiogram values for the healthy fetus of 18-22 weeks of gestation: a prospective cohort study

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Author's response to reviews: see over

To: Editor BMC Pregnancy & Childbirth

From: Kim M.J. Verdurmen
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Veldhoven, 25th of January 2016

Dear editor,

Thank you for your response on our manuscript MS: 2066045883168059 entitled: "Normal ranges for fetal electrocardiogram values for the healthy fetus of 18-24 weeks of gestation: a prospective cohort study".

We are very pleased with the comments of both reviewers. With pleasure we would like to answer your questions.

1. Ethical and Funding Approval Documentation:
Copies of all ethical approval, including an English version of the relevant parts, were sent as email attachment to BMCSeriesEditorial@biomedcentral.com on the 11th of November 2015. No additional changes were made since.

2. Funding:
No external or commercial funding.

3. Study status:
This study is still ongoing: we are still collecting data and we have not started with analysing the data.

4. Related articles:
There are no publications containing the results of this study that have been published or submitted to any journal.

We have read the comments of the reviewers carefully, and we have adjusted the manuscript accordingly. We hope that the adjustments make this paper suitable for publication in BMC Pregnancy & Childbirth, and we are looking forward to your decision. We hope that this additional information is sufficient in answering your questions.

Yours sincerely, on behalf of all the authors,

Kim Verdurmen, MD
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Reviewers' Comments to Author:

Reviewer: 1

The study protocol "Establishing normal ranges for fetal electrocardiogram values for the healthy fetus of 18-22 weeks of gestation: a prospective cohort study" describes an interesting preliminary work about the investigation of fetal electrocardiography for screening of congenital heart disease.
The study design is simple but clear and adequate to test the hypothesis (reference values in the 2nd trimester).
Information about related works is provided, writing is acceptable and standard format for publication is adhered.
Some discretionary revisions are added to the PDF.
I´m looking forward to see the results.

We would like to thank the reviewer for this positive feedback.

Title: 18-24 or 18-22 weeks of gestation?

We agree this was not consistent in the title and the rest of the manuscript. We included patients until 24 weeks of gestational age. Therefore, we adjusted this in the title (line 3).

Abstract and Methods/Design:
Ten to twelve weeks postpartum, we will evaluate if a newborn is healthy through a questionnaire. Minor heart defect might not be detected- an echocardiography would be better.

We agree that not all minor heart defects will be detected. However, the goal of prenatal screening is to detect severe diseases, where early diagnosis will lead to an adequate treatment plan. We believe a questionnaire will be sufficient to evaluate if any severe CHD was present at birth, because a severe CHD has hemodynamic consequences which present early in neonatal life. In addition, it was not feasible to perform a postpartum ultrasound in all included patients, since this is not standard care and

can only be performed by specialized personnel. Our study is not funded; therefore we were not able to include this extra ultrasound in the study protocol.
Not all minor defects may be detected, but these CHDs are not likely to have major influence on pre- or postnatal care. In addition, until now only major CHDs have been described to cause changes in fetal ECG parameters. Therefore, it is important to detect all major CHDs and exclude them from our analysis.

Methods/Design; Study parameters
If possible with this method I would add the signal loss in %. Very important for further data calculation.

We agree signal loss is an important factor when performing fetal ECG measurements. In this study, the recording must contain a minimum of 200 ECG complexes that were assessed to have good signal quality and that were recorded within a time frame of maximally 5 minutes. The percentage of total patients in which the recording contains the required amount of data to perform the analysis is indeed important for future studies and data calculation. Therefore, we added this to our study parameters in het Methods/Design section (line 307-308).

Methods/Design; Statistical analysis
Bad signal quality (defined as a % of interpolated data); in my experience > 50% = exclude.

We agree this was not clear in the manuscript. Therefore, we included a description explaining when data is classified as erroneous detection in the Methods/Design section (lines 258-299).

Reviewer: 2

Review:
Paper by Kim M.J. Verdurmen et al. Establishing normal ranges for fetal electrocardiogram values for the healthy fetus of 18-22 weeks of gestation: a prospective cohort study
Authors describe a prospective study with aim to determine normal values in fetal ECG (amplitudes and important intervals) in healthy fetuses during gestation (18-22 weeks).
The protocol for study is clearly written and very well presented. The results of the proposed study will be useful in the field as fetal ECG characteristics from 18-22 weeks are not known.

We would like to thank the reviewer for this positive feedback.

Major comments:

1) The authors often use term "normal range" but this is never defined. What does normal range stands for? mean +- std or mean (95% confidence intervals)?

We agree the term “normal range” is not well-defined in our study protocol. Normal range stands for the mean values with a 95% confidence interval. This definition can be found in the Methods/Design section (lines 151-152 and 178-180).

2) It is not clear if the authors aim to determine normal ranges by per-week basis, i.e for 18, 19, ..., 23, 24 separately or if they are planning to group these weeks and consider it as one category? Natural approach is to consider these weeks separately as during weeks 18-24 there will be changes in fetal ECG as fetal heart grows and mature. This is discussed at P9L214-L217 for P-wave and QRS complex.

We agree it is not clear how we will group the weeks of gestation. Initially, we will consider all included patients as one group. Thereafter, we will perform a subanalysis for every group per week of gestational age. This is described in the Methods/Design section (lines 318-326).

3) Calculation of required sample size is not clear. The value of 200 cases is stated but never explained. Regarding my previous comments, is 200 cases enough for per-week analysis?

As reviewer 2 states above, the fetal ECG characteristics from 18-22 weeks are not known. Therefore, it was not possible to perform a sample size calculation. In order to obtain reliable results from this study, we used reference [22] (Altman DG: *Practical Statistics for Medical Research.* 1990, Chapman&Hall/CRC, London. Page 422-423) to define the required sample size. Here it is stated that a sample size of preferably at least 200 observations is required in order to reduce the uncertainty in width of the 95% confidence interval. The sample size and related reference are mentioned in the Methods/Design section (lines 177-181).
Our primary analysis will concern all included 200 patients. In the subanalysis (groups per week of gestational age), the 95% confidence intervals will be larger due to the reduced number of included patients.

4) Authors also need to consider: i) loss of some patients, ii) bad quality of some records and inability to determine e.g. P-wave. This needs to be discussed and taken into account for the required sample size.

We agree loss to follow-up and insufficient data quality need to be taken into account while performing fetal ECG measurements. Therefore, we will include 300 patients in our initial cohort,

anticipating on 1/3 possible drop-out of the study due to several reasons (including those mentioned by the reviewer and based on our experience with prior fetal ECG measurements). This is mentioned in the Methods/Design section (lines 180-181).

5) More information should be given about fetal ECG device and about algorithm for semi-automatic analysis. As no automatic algorithm is 100% reliable I would consider to involve experienced cardiologist to verify the automatic analysis. This would make the results of study more reliable.

We agree that the fetal ECG device and the algorithm required a more extensive description in the Methods/Design section (lines 224-256).

6) There is no need to complicate the study by anomaly ultrasound examination by multiple sonographist. The final and correct identification of CHD will be performed postpartum. This would mean ex-post exclusion of only 2-3 cases since the prevalence of CHD is only 6-12/1000.

We agree with reviewer 2 that final identification of CHD will be postpartum. In order to trace the included children with CHD diagnosed postpartum, we will perform follow-up three months postpartum through a questionnaire.
The sentence “The fetal anomaly ultrasounds are performed by multiple sonographists, all certified to perform these ultrasounds and with multiple years of experience” is prone to be misinterpreted. In our study every patient received only one fetal anomaly ultrasound (following the normal ultrasound standards in the Netherlands), but we did not select on sonographists. Therefore, we changed this sentence; “The fetal anomaly ultrasound is performed by a certified and experienced sonographist” in the Methods/Design section (line 164-165).

7) I do not understand the normalization procedure using vectorcardiogram.

We agree that the normalisation procedure required a more extensive description in the Methods/Design section (lines 241-256).

Minor comments:
- I suggest to delete the word "Establishing" in the paper title. Thus title would read: Normal ranges for fetal electrocardiogram .....

We agree with reviewer 2 and adjusted the title (line 2).

- Specify for how long ECG will be recorded?

The fetal ECG will be recorded for 30 minutes. In those 30 minutes, we determine the fetal position four times by ultrasound. The recording time is added to the Methods/Design section (line 219-220).

- Please be consistent with active/passive voice throughout the paper

We agree and adjusted the following lines in the paper: 70, 155, 160, 163, 169, 174, 209, 212, 214, 220, 221, 251, 252, 302, 316, 317.

- Authors will need informed consent from women

Patients will only be included in the study after written informed consent. This is described in het Methods/Design section (line 169).

- P5L86-92 - Authors discuss new ultrasound methods such 3D and 4D and how these aid sonographist to visualize heart anatomy. Having better visualization of anatomy I would expect also discussion how these methods help to reduce inter and intra observer variability in identification of CHD?

It is described in literature that 3D/4D and STIC enhance the accuracy of diagnosing CHD (Rogers L, Li J, Liu L et al. Advances in fetal echocardiography: early imaging, three/four dimensional imaging, and role of fetal echocardiography in guiding early postnatal management of congenital heart disease. Echocardiography 2013;30(4):428-438). However, it is also stated in this study that this better sensitivity and specificity applies to centers with expertise in 3D/4D fetal echocardiography. As we described in our Background information (line 107-108), “disadvantages of these ultrasound modalities are that they are extremely expensive and only applicable in centres with experienced personnel”.

- P9L199: What is heart rate irregularity and how it is computed

With “heart rate irregularity” we mean “fetal arrhythmia”, for example premature beats (extrasystoles) and atrioventriculair blocks. We replaced “heart rate irregularity” with “heart rate arrhythmia” in the Methods/Design section (line 303).
Heart rate arrhythmia is defined based on heuristic rules that dictate that during normal rhythm subsequent heartbeat intervals cannot differ more than 20%. Any rhythm not complying with this rule, and assessed to not be caused by erroneous detection of heartbeats, e.g. as a result of poor signal

quality, is labelled as a fetal arrhythmia. We added this definition to the Methods/Design section (lines 310-313).

- P9L208: quote: "defined as a % of interpolated data". What data are interpolated and when?

When analysing the fetal ECG, no data are interpolated (in contrast to fetal heart rate variability analysis). Therefore, we removed this sentence from the Methods/Design section.

- P10L230: quote: "the fetal ECG is evaluated by semi-computerized algorithms, taking away the performer-dependent variability" this is not true. As it is semi-automatic some observer variability will still be present.

We agree with reviewer 2 that in a semi-automatic analysis there will still be some observer variability, although it will be considerably less compared to prior analysis methods. We adjusted the sentence; "…, taking away some of the performer-dependent variability in diagnostic value" in the Discussion (line 347).

Minor issues: Awkward phrases, typographical errors, suggestions:
P2L28: "65 to 81 per cent" -> 65 to 81%?

We agree and changed this in the Abstract (line 30).

P2L29: "making this" -> making it

We agree and changed this in the Abstract (line 31)

Abstract: passive vs active voice

We agree and changes the following lines; 41, 43, 44.

P2L40: Awkward phrase: "Directly following, the fetal electrocardiogram will be conducted through dedicated signal processing methods"

We agree and changes this phrase in the Abstract (line 43-44).

P4L59,60,62: During pregnancy, during the course of pregnancy, during this examination -- consider rephrase and simplify

We agree and rephrased and simplified these lines in the Background information (line 70-72).

P4L61: "will be" -> are

This sentence was removed following simplifying and rephrasing.

P4L61: "will be" -> is

This sentence was removed following simplifying and rephrasing.

P5L106: delete "of the signal"

We agree and removed the words “of the signal” from this sentence in the Background information (line 122).

P5L110: "fetal hear rate: this is" -> fetal heart rate, which is

We agree and replaced this in the Backround information (line 126).

P8L179: Figure 2 provides no additional value to readers.

We believe that Figure 2 does provide additional value to the readers; it gives the readers a better understanding of how the real-time bedside monitoring system looks and what can be extracted from it. Therefore, we prefer to include this Figure in the manuscript.