PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Assessment of cervical SofTening and the Prediction of Preterm birth (STIPP): protocol for a prospective cohort study
AUTHORS	Breuking, Sofie; Oudijk, Martijn; van Eekelen, Rik; de Boer, Marjon; Pajkrt, Eva; Hermans, Frederik

VERSION 1 – REVIEW

REVIEWER	Care, Angharad University of Liverpool, Centre for Women's Health Research
REVIEW RETURNED	16-Mar-2023

GENERAL COMMENTS	A very well designed study. Nicely presented. My questions to the authors: I note that you have not outlined any inclusion or exclusion criteria here but they are available to view on ClinicalTrials.gov It would be useful to either include here or signpost to your registered protocol. Will previous cervical surgery affect the CSI compared to women without surgery/scar tissue? Is it appropriate to include them? Could you explain how you came to the decision to combine singleton and multiple pregnancies? Would these populations not have a very different expected prevalence of the primary outcome potentially affecting your sample size calculation?

REVIEWER	Suhag, Anju NYU Langone Health
REVIEW RETURNED	21-Jul-2023

GENERAL COMMENTS	I commend Authors on this amazing study question that is very relevant in reduction of preterm birth in singleton and multiple gestations. Here a couple of questions and comments in regards to the study 1. Consider adding available evidence on using shear wave elasticity imaging to measure cervical softness in the background. And how does it compare to assessing softness of cervix using aspiration devices. Any safety concerns with either technique (bleeding on contact with aspiration device). 2. In terms of inclusion criteria, the authors included both singleton and multiple gestations in 2 different cohorts- first cohort includes asymptomatic patient with history of spontaneous preterm birth

	before 34 weeks and 2nd is the symptomatic cohort presenting with symptoms of threatened preterm labor between 24 and 34 weeks. My question is in regards to the power calculation that the authors presented in this draft. I am trying to understand how the authors calculate sample size for the primary outcome for A-STIPP cohort as twin gestations would likely be at increased risk of preterm delivery (as monochorionic diamniotic twins would be routinely delivered at 36 weeks, therefore increase the outcome if the cohort has higher mono-di twins or even didi twins with singletons). Please review the power calculation from a statistician to address above concern and double check the power calculation that justifies inclusion of both singleton and multiple gestation. 3. Is the technique of CSI assessment in woman with cervical cerclage and/or progesterone any different than those were not done any progesterone supplementation or have a mechanical support to the cervix? 4. In terms of demographics data, please make sure daughters collect the common prematurity related risk factors including previous diagnosis of primary cervical insufficiency, previous cervical surgery (cervical biopsy, LEEP or cone biopsy, prior h/o cerclage), previous second trimester loss and vaginal bleeding during pregnancy. Other factors to look at could be symptomatic vaginitis or STI and mullerian anomaly. 5. For S-STIPP cohort, please collect antenatal corticosteroids, tocolytics or magnesium sulfate for neuroprotection. 6. Also add chorioamnionitis, PPROM and composite neonatal outcomes (as secondary outcomes). 7. References (and background): I would advise the authors to look at this recent publication in a job MFM in April 2023 on use of aspiration based device in measurement of cervical os without softness before cerclage placement Stone J, House M. Measurement of cervical softness before cerclage placement with an aspiration-based device. Am J Obstet Gynecol MFM. 2023 Apr;5(4):100881. doi: 10.1016/j.ajogmf.2023.100881. Epub 2023 Jan 29. PMID: 36724813. 8. Please include the limitations of the study including Limited external validity and generalized debility due to inclusion criteria and a single site study.

VERSION 1 – AUTHOR RESPONSE

Reviewer#1 Comment 2

A. Reviewer Comment	Will previous cervical surgery affect the CSI compared to women without surgery/scar tissue? Is it appropriate to include them?
B. Response	We currently lack a comprehensive understanding of the impact of cervical surgery on the Cervical stiffness index. We hypothesize the CSI will be higher in women with previous cervical surgery due to scar tissue. However, considering that cervical surgery is a recognized significant factor contributing to premature birth, we would like to investigate and incorporate this aspect into the scope of this study.

	Furthermore, we intend to conduct subgroup analyses in women with cervical surgery versus women with no cervical surgery. We will add this in our protocol and make sure these subgroups are stated at Clinicaltrials.gov
C. Changes made	Added a subheading of subgroup analysis
D. Location of changes	Line 307

Reviewer#1 Comment 3

A. Reviewer Comment	Could you explain how you came to the decision to combine singleton and multiple pregnancies? Would these populations not have a very different expected prevalence of the primary outcome potentially affecting your sample size calculation?
B. Response	This study focuses on regular, natural pregnancies within a high-risk population. It is acknowledged that there are additional subgroups within this population with even higher risk, such as multiple pregnancies. We incorporate this consideration into our estimation of the expected number of preterm births (PTBs) within the overall cohort. Our research constitutes predictive investigation, where the count of PTB events drives the sample size calculations. Given the relatively low likelihood of multiple pregnancies, their occurrence is unlikely to substantially affect the prevalence of preterm birth in the entire cohort. However, we appreciate the point raised by the reviewers in this regard and concur that ignoring the differences in singletons and multiples is not justified. Consequently, we have incorporated subgroup analyses to examine the differences in CSI between singleton and multiple pregnancies.
C. Changes made	Added singletons and multiples in subgroup analyses to analyse the impact of these differences for the CSI
D. Location of changes	Line 307

Reviewer 2

Reviewer#2 Comment 1

A. Reviewer Comment	Consider adding available evidence on using shear wave elasticity imaging to measure cervical softness in the background. And how does it compare to assessing softness of cervix using aspiration devices. Any safety concerns with either technique (bleeding on contact with aspiration device).
B. Response	Thank you for your valuable suggestion. We have added a paragraph about elastography methods. Nevertheless, we have chosen not to assess any safety concerns, due to the word count and that we extensively look at safety issues in our study. Later on in the protocol we discuss this broadly.
C. Changes made	Added an paragraph in the introduction
D. Location of changes	Line 115

Reviewer#2 Comment 2

A. Reviewer Comment	In terms of inclusion criteria, the authors included both singleton and multiple gestations in 2 different cohorts- first cohort includes asymptomatic patient with history of spontaneous preterm birth before 34 weeks and 2nd is the symptomatic cohort presenting with

	symptoms of threatened preterm labor between 24 and 34 weeks. My question is in regards to the power calculation that the authors presented in this draft. I am trying to understand how the authors calculate sample size for the primary outcome for A-STIPP cohort as twin gestations would likely be at increased risk of preterm delivery (as monochorionic diamniotic twins would be routinely delivered at 36 weeks, therefore increase the outcome if the cohort has higher mono-di twins or even didi twins with singletons). Please review the power calculation from a statistician to address above concern and double check the power calculation that justifies inclusion of both singleton and multiple gestation.
B. Response	Thank you very much for your critical analysis. We have discussed this again with our statistician. As stated above (in comment 3 of reviewer 1), This study focuses on regular, natural pregnancies within a high-risk population. It is acknowledged that there are additional subgroups within this population with even higher risk, such as multiple pregnancies. We incorporate this consideration into our estimation of the expected number of preterm births (PTBs) within the overall cohort. Our research constitutes predictive investigation, where the count of PTB events drives the sample size calculations. Moreover, since our primary outcomes are (spontaneous) PTB<34 weeks for the A-STIPP cohort and delivery within 7 days after inclusion for the S-STIPP cohort, we are looking at outcomes that are not regular for either twin of singleton pregnancies. However, we appreciate the point raised by the reviewers in this regard and concur that ignoring the differences in singletons and multiples is not justified. Consequently, we have incorporated subgroup analyses to examine the differences in CSI between singleton and multiple pregnancies.
C. Changes made	Added singletons and multiples in subgroup analyses to analyse the impact of these differences for the CSI
D. Location of changes	Line 307

Reviewer#2 Comment 3

A. Reviewer Comment	Is the technique of CSI assessment in woman with cervical cerclage and/or progesterone any different than those were not done any progesterone supplementation or have a mechanical support to the cervix?
B. Response	No, the technique remains the same. However, we don't know what the impact of the cerclage or progesterone is on the CSI. Therefore, we will do multiple subgroup analyses
C. Changes made	Added the subgroup analyses in the manuscript
D. Location of changes	Line 307

Reviewer#2 Comment 4

A. Reviewer Comment	In terms of demographics data, please make sure daughters collect the common prematurity related risk factors including previous diagnosis of primary cervical insufficiency, previous cervical surgery (cervical biopsy, LEEP or cone biopsy, prior h/o cerclage), previous second trimester loss and vaginal bleeding during pregnancy. Other factors to look at could be symptomatic vaginitis or STI and mullerian anomaly.
B. Response	Thank you for your suggestions. In the questionnaire, we inquire about all these elements. Additionally, we gather patients' medical history and review their medical records to ensure no pertinent information is overlooked.

C. Changes made	We have clarified more detailed what questions we ask in the questionnaire
D. Location of changes	Line 214

Reviewer#2 Comment 5

A. Reviewer Comment	For S-STIPP cohort, please collect antenatal corticosteroids, tocolytics or magnesium sulfate for neuroprotection
B. Response	Thank you very much for your suggestion. We indeed already collect this information. We follow the participants until delivery and gather all information of the participants regarding pregnancy outcomes, maternal and neonatal outcomes. So this include hospital admittance for threatened preterm birth as well.
C. Changes made	To clarify above, we have added a subheadings 'follow-up'
D. Location of changes	Line 232

Reviewer#2 Comment 6

A. Reviewer Comment	Also add chorioamnionitis, PPROM and composite neonatal outcomes (as secondary outcomes).
B. Response	Thank you very much, we have added PPROM in the secondary outcomes. Since this is a study that examines at the maternal level and our initial aim is to purely assess the utility of the measurement and the prediction of preterm birth, we have not included composite or neonatal outcomes for now. If the measurement appears to be useful and its application leads to treatment implications, the next step would be to consider incorporating these aspects.
C. Changes made	See B
D. Location of changes	Line 252

Reviewer#2 Comment 7

A. Reviewer Comment	References (and background): I would advise the authors to look at this recent publication in a job MFM in April 2023 on use of aspiration based device in measurement of cervical os without softness before cerclage placement Stone J, House M. Measurement of cervical softness before cerclage placement with an aspiration-based device. Am J Obstet Gynecol MFM. 2023 Apr;5(4):100881. doi: 10.1016/j.ajogmf.2023.100881. Epub 2023 Jan 29. PMID: 36724813.
B. Response	Thank you very much for the reference! We have added this study in the background and references
C. Changes made	Added this study in the background
D. Location of changes	Linde 132

Reviewer#2 Comment 8

A. Reviewer Comment	Please include the limitations of the study including Limited external validity and generalized debility due to inclusion criteria and a single site study.

B. Response	We already stated the limitation of the study is the single centre design potentially limiting external validity. We think the reviewer means ‘generalizability’ in stead of generalized debility. We have added this in the limitations
C. Changes made	See line 63
D. Location of changes	Line 63

VERSION 2 – REVIEW

REVIEWER	Care, Angharad University of Liverpool, Centre for Women's Health Research
REVIEW RETURNED	15-Sep-2023

GENERAL COMMENTS	You have considered and adjusted for the reviewers comments appropriately. In this field it is difficult to practically recruit these numbers and this a well designed study that has walked the line between statistical consideration and what is practically achievable at a single centre site. Good luck with recruitment. My only (very minor) comment on the protocol is that I couldn't see that you have described how many measurements from the cervix (apologies if I missed it!). Figure 1 gives a nice illustration of the technique and appendix 1 has a flow diagram of the study. However I imagine there will be an error rate with the machine. Do you just take a single measurement on the cervix each time? Best of 3? Average of 3? What do you do if you get a completely erroneous reading or the machine fails to take a reading? Would be useful to have a sentence in the methods for transparency.

REVIEWER	Suhag, Anju NYU Langone Health
REVIEW RETURNED	12-Oct-2023

GENERAL COMMENTS	Thanks for submitting the revisions.

VERSION 2 – AUTHOR RESPONSE

Reviewer 1

Reviewer#1 Comment 1

A. Reviewer Comment	My only (very minor) comment on the protocol is that I couldn't see that you have described how many measurements from the cervix (apologies if I missed it!). Figure 1 gives a nice illustration of the technique and appendix 1 has a flow diagram of the study. However I imagine there will be an error rate with the machine. Do you just take a single measurement on the cervix each time? Best of 3? Average of 3? What do you do if you get a completely erroneous reading or the machine fails to take a reading? Would be useful to have a sentence in the methods for transparency.

B. Response	Thank you very much for suggestion. We clarified your questions in the manuscript
C. Changes made	See manuscript
D. Location of changes	Line number 201 Line number 295