BMJ Open

Assessment of cervical SofTening and the Prediction of Preterm birth: study protocol of the STIPP trial. A prospective cohort study.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-071597
Article Type:	Protocol
Date Submitted by the Author:	03-Jan-2023
Complete List of Authors:	Breuking, Sofie; Amsterdam UMC Locatie AMC, Obstetrics and gynaecology; Amsterdam Reproduction and Development Research Institute Oudijk, Martijn; Amsterdam UMC Locatie VUmc, Obstetrics; Amsterdam Reproduction and Development Research Institute van Eekelen, Rik; Amsterdam Reproduction and Development Research Institute, Obstetrics and Gynaecology de Boer, Marjon; Amsterdam UMC Locatie VUmc, Department of Obstetrics and Gynaecology; Amsterdam Reproduction and Development Research Institute Pajkrt, Eva; Amsterdam UMC Location AMC, Obstetrics and Gynaecology; Amsterdam Reproduction and Development Research Institute Hermans, Frederik; Amsterdam UMC Locatie AMC, Obstetrics and Gynaecology; Amsterdam Reproduction and Development Research Institute
Keywords:	Maternal medicine < OBSTETRICS, OBSTETRICS, Prenatal diagnosis < OBSTETRICS

Assessment of cervical SofTening and the Prediction of Preterm birth: study protocol of the STIPP trial. A prospective cohort study.

Sofie. H. Breuking, MD[1, 2, *] Martijn A. Oudijk, MD, PhD[2,3], R. van Eekelen[4] ,M.A. de Boer, MD, PhD[2,3] Eva. Pajkrt, MD, PhD[1, 2], Frederik J. R. Hermans, MD PhD[1,2]

1. Amsterdam UMC location AMC, University of Amsterdam, Obstetrics and Gynaecology, Meibergdreef 9, Amsterdam, The Netherlands

2. Amsterdam Reproduction and Development Research Institute, The Netherlands

3. Amsterdam UMC location Vrije Universiteit Amsterdam, Obstetrics and Gynaecology, Boelelaan 1117, Amsterdam, The Netherlands

4. Obstetrics and Gynaecology, Centre for Reproductive Medicine, Amsterdam Reproduction and Development Research Institute, Amsterdam UMC Location AMC, Amsterdam, The Netherlands

* corresponding author

Correspondence to: s.h.breuking@amsterdamumc.nl

Abstract

Introduction

Preterm birth (PTB) is amongst the leading causes of perinatal and childhood morbidity and mortality. Therefore, accurate identification of pregnant women at high risk of PTB is key to enable obstetric healthcare professionals to apply interventions to improve perinatal and childhood outcomes.

Serial transvaginal cervical length measurement is used to screen asymptomatic pregnant women with a history of PTB. In women presenting with symptoms of threatened PTB cervical length, fetal fibronectin test or a combination of both can be used to identify women at high risk of PTB. The predictive capacity of these can be improved.

Cervical softening is precursor of cervical shortening, effacement, and dilatation and could be a new marker to identify women a high risk of preterm birth. However, predictive value of cervical softening to predict spontaneous PTB still need to be determined.

Methods/design

This is a single centre cohort study. Cervical stiffness will be investigated with an available, non-invasive CE-marked device called the Pregnolia System®. This device has been developed to evaluate consistency of the cervix based on tissue elasticity.

Two different groups will be investigated.

1. Women with a history of spontaneous PTB <34 weeks. These women undergo biweekly measurement between 14 and 24 weeks of gestation.
2. Women with symptoms of threatened PTB. These women will be tested once on presentation between 24 and 34 weeks of gestation

Primary outcome is spontaneous PTB before 34 weeks for women with a history of PTB and delivery within seven days for women with threatened PTB cohort.

Ethics and dissemination

The study is approved by the Medical Ethics Committee of Amsterdam UMC (METC2022.0226). Results will be disseminated through peer-reviewed journals. This protocol is published before analysis of results is done.

Trial registration

Trial is registered at ClinicalTrials.gov, identifier NCT05477381, date of registration: 27th July 2022.

Strengths and limitations of the study

- Since cervical softening is a precursor of cervical shortening, effacement and dilatation, cervical softening and could be a promising new marker for preterm birth. This study will be the first to evaluate the relation with cervical stiffness, measured with an aspiration technique, and the risk of preterm birth.
- This study will provide evidence on the value of the cervical stiffness as a single clinical marker and in combination with other clinical markers such as cervical length to predict the risk of spontaneous preterm birth in groups of pregnant women with an increased risk of preterm birth.
- A limitation of the study is the single centre design potentially limiting external validity.

Background and rationale

Spontaneous preterm birth (PTB), defined as delivery before 37 weeks of gestation, is the

leading cause of perinatal and neonatal morbidity and mortality(1). Rates of spontaneous PTB appears to be increasing. Annually, 15 million children are born preterm annually and is directly responsible for the death of one million neonates(2, 3). Neonates who survive PTB are at increased risk for long-term neurologic sequelae and developmental disabilities(4, 5). Identifying pregnant women at risk is important to be able to take precautionary measures, however this is a challenge for obstetric healthcare professionals.

Important obstetric and gynaecological risk factors for PTB are, midtrimester short cervical length, prior cervical surgery and previous spontaneous PTB. (6-9)

Women with a history of spontaneous PTB before 34 weeks of gestation are at a 5-fold increased risk of a spontaneous PTB in a subsequent pregnancy compared to women with a previous term birth(10). In addition to vaginal progesterone administration, biweekly cervical length screening is recommended in these women to identify women at high risk of a recurrent PTB based on short cervical length that benefit from a vaginal cerclage. However, in women with a previous PTB, the positive predictive value (PPV) of a short cervical length is 34%. (11) Therefore this approach only identifies a proportion of women who will have a recurrent PTB. This calls for additional measurements to identify the group more adequately at risk for recurrent PTB.

Another group of pregnant women at risk of delivering PTB are women presenting with symptoms of preterm labour in the current pregnancy. These women can be triaged with transvaginal cervical length measurement and foetal fibronectin(fFN) to identify women with an increased risk of delivery within seven days. Women with a high risk of PTB at less than 32 weeks of gestation are admitted to a centre with NICU facilities and treated with antenatal corticosteroids and tocolysis for 48 hours to improve perinatal outcome.(12) This combination of markers are characterized by a high negative predictive value (NPV) but a poor PPV. This results in overtreatment and unnecessary healthcare costs in women with a positive fFN test.

A large proportion of women with symptoms of threatened PTB will not deliver within seven days due to the low PPV, however these women remain at risk for PTB later in pregnancy. (13-15)

More adequate techniques to assess women at high risk of recurrent PTB or at high risk of delivering in a short time frame when presenting with symptoms of threatened preterm birth

are urgently needed. Therefore, objective measurement of the cervical consistency is a promising technique.

To maintain pregnancy and deliver at term an appropriate function of the cervix is required. Delivery is preceded by softening and shortening of the cervix. (16) Changes in cervical consistency can be detected from fertilization until delivery. Throughout pregnancy consistency of the cervix changes and will soften when approaching delivery(17, 18). Softening of the cervix precedes shortening and therefore could be a promising marker to identify an upcoming delivery at an earlier stage. Parra-Saavedra *et al.*(19) investigated this phenomenon with transvaginal ultrasound. The cervical consistency was measured by measuring the difference of the anteroposterior cervical diameter before (AP) and after (AP^1) application of pressure on the cervix with the transvaginal probe. The cervical consistency was then calculated with the following formula: ((AP^1/AP) * 100) = Cervical consistency index. Cervical consistency had an inverse linear relationship with gestational age. This means that cervical consistency declines, thus becomes softer, during progression of pregnancy, and that this phenomenon can be detected in pregnancy. Secondly, it demonstrated also that pregnant women with a more progressive decline in cervical consistency are more likely to have a spontaneous PTB compared to women with physiological decline in cervical consistency.

Recently, a non-invasive technique has been developed to evaluate consistency of the cervix based on tissue elasticity. The Pregnolia®-system is a market available CE-marked device designed to measure cervical softening. This system provides quantitative measurements of the cervical consistency. This procedure is performed by placing a sterile probe with a diameter of 12 mm on the cervix. The probe is connected to a control unit which creates a vacuum between the tip of the probe and the cervix. The probe measures the aspiration pressure that is needed to displace the cervical tissue 4 mm inside the probe. The cervical consistency is expressed as cervical stiffness index (CSI), a pressure value (mbar) on a continuous scale from 0 (soft) to 100 (firm).

A prototype has been tested and measurements were carried out in 50 non-pregnant and 50 pregnant women(18). The results were in line with the study by Parra-Saavedra *et al.*(19) and showed that with progressing of pregnancy, the cervix softens and starts before shortening. By measuring the CSI, delivery could be detected earlier compared to conventional shortening of the cervix measured with transvaginal ultrasound.

Since cervical softening is a precursor of cervical shortening, this could be a novel marker to predict spontaneous PTB and contribute to better identification of women with an increased risk of PTB. Also, the predictive value of cervical softening in combination with cervical length could be promising to improve prediction of PTB. However, these hypotheses still must be examined.

Therefore, the aim of this cohort study is to evaluate the predictive value of the cervical stiffness index to predict the risk of spontaneous PTB in pregnant women with an increased risk of PTB.

Methods and analysis

Study design

This study is an investigator initiated, single centre cohort study and will be performed at the Amsterdam UMC in the Netherlands. Two cohorts will be investigated:

- Pregnant women with a history of spontaneous PTB before 34 weeks of gestation (Cohort A-STIPP).
- Pregnant women presenting with symptoms of threatened PTB between 24 and 34 weeks (Cohort S-STIPP).

The measurements of cervical stiffness will be performed in addition to standard care(Appendix 1), using the aspiration technique-based device named the Pregnolia system device.

Participants

A-STIPP cohort

Pregnant women, singleton or multiple gestation, with an increased risk of PTB based on a medical history of spontaneous PTB before 34 weeks of gestation will be included.

S-STIPP cohort

Pregnant women, singleton or multiple gestation, with a gestational age between 24 and 34 weeks presenting with symptoms of threatened PTB, such as abdominal pain, vaginal blood loss, contractions or other complaints suggestive for threatened PTB, will be included.

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Under 18 years of age.
- Signs of intrauterine infection.
- Obstetric indication for immediate delivery (advanced labour, cord prolapse, abruption, signs of foetal distress).
- Confirmed foetal abnormality.
- Confirmed preterm rupture of membranes.
- Confirmed vasa / placenta praevia.
- Severe vaginal bleeding and light bleeding that cannot be stopped.
- Signs of imminent labour such as advanced dilatation making it impossible to measure the cervix.

Measurements

Cervical stiffness measurement

The Cervical stiffness index (CSI) will be measured following measurement of the cervical length. The Pregnolia System is composed of two components: an active, reusable device and a disposable single-use sterile probe.

- The control unit is an active device with a power supply, foot switch, connector cable and an integrated pump that generates vacuum.
- The single-use sterile probe is connected to the control unit console through a connector cable. Air filters on the probe prevent microbiological contamination of the control unit. This probe is designed to minimise the contact interaction between the user and the patients during the measurement. The probe tip diameter is 12mm. Each single-use, disposable probe is packed in a sterile pouch.

To perform the measurement, the cervix is visualized with a speculum. The disposable probe is placed on the anterior lip of the cervix (12 o'clock position). The control will create a weak vacuum inside the probe that pulls the cervical tissue, very gently and slowly, into the probe tip by a fixed distance of 4mm. The negative aspiration pressure needed to deform the tissue is the outcome of the measurement. A high-pressure value corresponds with stiff tissue and a low pressure corresponds with soft tissue. An overview of the measurement is displayed in figure 1.

Secondary study parameters (predictors)

- Cervical length (1)
- Fetal Fibronectin# (1)
- Twin gestation (1)
- History of spontaneous PTB (4)
- Cervical surgery (1)
- Inter-pregnancy interval (1)
- Presence of infection (1)
- Family history (1)
- Social economic status (3)
- Smoking (1)
- BMI (1)

S-STIPP only (X = number of input variables)

Sonographic measurement

Cervical length measurement with transvaginal ultrasound is routine care in both cohorts. The cervical length will be determined as the linear distance between internal and external cervical os, excluding the endocervical funnel as described by Kagan et al. (2015) (20).

In the A-STIPP cohort, transvaginal ultrasound will be done biweekly from 14 until 24 weeks of gestation. In case a short cervix is detected at less than 25 mm, a cerclage, or a pessary in study context, is placed. Afterwards measurement of CSI will not be continued.

In the S-STIPP cohort, the transvaginal ultrasound will be performed when a participant presents with any symptom of threatened PTB, between 24 and 34 weeks of gestation. Threatened PTB is defined as premature contractions, lower back pain, vaginal blood loss or abdominal pain.

Questionnaire

Women will be asked to fill out a structured questionnaire to screen for additional risk factors of PTB. The questionnaire contains questions about the current pregnancy, previous pregnancies, family history of PTB and cervical surgery. Baseline characteristics such as height and weight and smoking as well as information about relevant medical history will be collected. The questionnaire will be checked with the patient's electronic file.

Blinding

For the A-STIPP cohort, clinicians and participants are blinded for the results of the CSI measurement.

In the S-STIPP cohort, the clinician working at the emergency department performs the measurement and therefore making it impossible to blind the treating clinician. The participant however is blinded for the results.

Primary outcome

- The primary outcome for the A-STIPP cohort is spontaneous PTB before 34 weeks of gestation.
- The primary outcome for the S-STIPP cohort is delivery within 7 days.

Secondary outcomes

- Spontaneous PTB before 37 weeks of gestation
- Spontaneous PTB before 34 weeks of gestation#
- Spontaneous PTB before 32 weeks of gestation
- Spontaneous PTB before 28 weeks of gestation
- Latency time (time between inclusion and delivery)
- Delivery within 48 hours#

S-STIPP only

Other outcomes

Safety of the use of the Pregnolia system (as defined in Appendix 2) will be investigated.

Also, patient discomfort of the measurement will be evaluated by a general questionnaire.

Power analysis

We used contemporary sample size calculations described by *Riley et al.(26)* for developing prediction models, based on three criteria that each provide a sample size to satisfy that criterion, then picking the highest sample size out of the three. The following input parameters are used to calculate the required number of inclusions; 1) expected prevalence of the primary

outcome, 2) expected amount of explained variance by the prediction model, and 3) number of predictors (input variables).

For the A-STIPP cohort the prevalence [0.18] was derived from the QUIPP-study (23, 24). (25) The standard level of variance [0.15] was used to calculate sample size.

For the S-STIPP cohort, the prevalence [0.12] and variance [0.45] were derived from the Apostel I study(21, 22). Both studies have comparable patients as the A-STIPP and S-STIPP study.

To analyse CSI for the primary outcome the minimum number of inclusions are 227 and 163 for A-STIPP and S-STIPP cohort, respectively.

To investigate additional input parameters with sufficient power, an increase in sample size is needed. When inclusion of participants continues and the second threshold is reached, another input parameter is added, until the next threshold and so on. The baseline predictors used in the first step will be the CSI measurement combined with cervical length measurement in the A-STIPP cohort, and cervical length with fFN in S-STIPP cohort.

See table 1 and table 2 for the steps and the threshold sample sizes. In both calculations the number of predictors was gradually increased. Continuous variables count as a single input variable, as well as dichotomous input variables. Categorical variables are counted as C-1, thus the number of input variables is the number categories minus one. The additional predictor variables are summarized in table 3.

Sample size calculations were performed using R (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/) with the use of the *pmsampsize* package *(26).* Table 1 and table 2 indicates the number of participants needed.

Statistical Analysis

Baseline characteristics will be calculated used descriptive statistics. Continuous variables will be reported as mean with standard deviation or median with inter quartile range. Categorical variables will be reported as proportions.

To incorporate repeated measures of CSI from the A-STIPP cohort, a logistic generalized mixed model will be used. As CSI is a continuous outcome, linear and non-linear functions will be

compared using restricted cubic splines. A lower Akaike's Information Criterion (AIC) or overall p-value will determine which functional form is chosen.(26) If there is censoring (i.e. loss-to-follow-up), a Cox proportional hazards model for time to delivery including a time-varying covariate for CSI will be used. As a sensitivity analysis, a comparison of either of these models with a joint survival model will be done (combining a Cox model for time to delivery with a linear mixed model for CSI measurements).

For the S-STIPP cohort, a logistic regression will be used to determine the relationship between input variables and a dichotomous outcome.

Monitoring and safety

An independent Data and Safety Monitoring Board (DSMB) is assigned to safeguard the safety of the trial participants and provide recommendations.

Since the measurement with the Pregnolia-system is minimally invasive, the risk of adverse events related to the measurement is small. However, any Adverse Events (AEs) and Serious Adverse Events (SAEs) will be reported. If evaluation by the DSMB demonstrates increased safety risks within the trial, the DSMB can always advice to stop the trial.

Data management

Data will be collected using an accredited electronic data capture system (Castor). To protect the privacy of the participant, personal data is encrypted. Data cannot be traced back participants in reports and publications about the study. All personal data is protected according to the General Data Protection Regulation (GDPR and Dutch privacy regulation (AVG)).

All agreements regarding data sharing are defined in a signed Clinical Trial Agreement (CTA). General Data Protection Regulation (GDPR) are applicable to this agreement.

Clinical impact

This STIPP study will provide evidence on the value of the cervical stiffness as a single clinical marker and in combination with other clinical markers such as cervical length to predict the risk of spontaneous PTB in groups of pregnant women with an increased risk of PTB.

Patient and public involvement

The patient organisation care4Neo was consulted to address the view of patients.

Ethics and dissemination

The Medical Ethics Committee of the Amsterdam UMC has given approval for this research (Number 2022.0226). All patients will give written and oral informed consent prior to entry to the study and will be made aware participation is completely voluntary.

The outcomes of the study will be published in a peer-reviewed journal.

References

1. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, Adler A, Vera Garcia C, Rohde S, Say L, Lawn JE. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012;379(9832):2162-72.
2. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, Rudan I, Campbell H, Cibulskis R, Li M, Mathers C, Black RE, Child Health Epidemiology Reference Group of WHO, Unicef. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet. 2012;379(9832):2151-61.
3. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, Cousens S, Mathers C, Black RE. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet. 2015;385(9966):430-40.
4. Marlow N, Wolke D, Bracewell MA, Samara M, Group EPS. Neurologic and developmental disability at six years of age after extremely preterm birth. N Engl J Med. 2005;352(1):9-19.
5. Mwaniki MK, Atieno M, Lawn JE, Newton CR. Long-term neurodevelopmental outcomes after intrauterine and neonatal insults: a systematic review. Lancet. 2012;379(9814):445-52.
6. van Zijl MD, Koullali B, Mol BW, Pajkrt E, Oudijk MA. Prevention of preterm delivery: current challenges and future prospects. Int J Womens Health. 2016;8:633-45.
7. van der Ven J, van Os MA, Kazemier BM, Kleinrouweler E, Verhoeven CJ, de Miranda E, van Wassenaer-Leemhuis AG, Kuiper PN, Porath M, Willekes C, Woiski MD, Sikkema MJ, Roumen FJ, Bossuyt PM, Haak MC, de Groot CJ, Mol BW, Pajkrt E. The capacity of mid-pregnancy cervical length to predict preterm birth in low-risk women: a national cohort study. Acta Obstet Gynecol Scand. 2015;94(11):1223-34.
8. Iams JD, Berghella V. Care for women with prior preterm birth. Am J Obstet Gynecol. 2010;203(2):89-100.
9. Conner SN, Frey HA, Cahill AG, Macones GA, Colditz GA, Tuuli MG. Loop electrosurgical excision procedure and risk of preterm birth: a systematic review and meta-analysis. Obstet Gynecol. 2014;123(4):752-61.
10. Kazemier BM, Buijs PE, Mignini L, Limpens J, de Groot CJ, Mol BW, Ebm C. Impact of obstetric history on the risk of spontaneous preterm birth in singleton and multiple pregnancies: a systematic review. BJOG. 2014;121(10):1197-208; discussion 209.
11. Hughes K, Kane SC, Araujo Junior E, Da Silva Costa F, Sheehan PM. Cervical length as a predictor for spontaneous preterm birth in high-risk singleton pregnancy: current knowledge. Ultrasound Obstet Gynecol. 2016;48(1):7-15.
12. Vis JY, van Baaren GJ, Wilms FF, Oudijk MA, Kwee A, Porath MM, Scheepers HC, Spaanderman ME, Bloemenkamp KW, van Lith JM, Bolte AC, Bax CJ, Cornette J, Duvekot JJ, Nij Bijvank SW, van Eyck

J, Franssen MT, Sollie KM, Woiski M, Vandenbussche FP, van der Post JA, Bossuyt PM, Opmeer BC, Mol BW. Randomized comparison of nifedipine and placebo in fibronectin-negative women with symptoms of preterm labor and a short cervix (APOSTEL-I Trial). Am J Perinatol. 2015;32(5):451-60.
13. Wilms FF, Vis JY, de Wit-Zuurendonk L, Porath MM, Mol BW. What is the risk of preterm delivery after arrested preterm labor? Am J Perinatol. 2015;32(1):63-70.
14. Cornelissen LGH, van Oostrum NHM, van der Woude DAA, Rolf C, Porath MM, Oei SG, van Laar J. The diagnostic value of fetal fibronectin testing in clinical practice. J Obstet Gynaecol Res. 2020;46(3):405-12.
15. Berghella V, Saccone G. Fetal fibronectin testing for reducing the risk of preterm birth. Cochrane Database Syst Rev. 2019;7:CD006843.
16. Mazza E, Parra-Saavedra M, Bajka M, Gratacos E, Nicolaides K, Deprest J. In vivo assessment of the biomechanical properties of the uterine cervix in pregnancy. Prenat Diagn. 2014;34(1):33-41.
17. Badir S, Bajka M, Mazza E. A novel procedure for the mechanical characterization of the uterine cervix during pregnancy. J Mech Behav Biomed Mater. 2013;27:143-53.
18. Badir S, Mazza E, Zimmermann R, Bajka M. Cervical softening occurs early in pregnancy: characterization of cervical stiffness in 100 healthy women using the aspiration technique. Prenat Diagn. 2013;33(8):737-41.
19. Parra-Saavedra M, Gomez L, Barrero A, Parra G, Vergara F, Navarro E. Prediction of preterm birth using the cervical consistency index. Ultrasound Obstet Gynecol. 2011;38(1):44-51.
20. Kagan KO, Sonek J. How to measure cervical length. Ultrasound Obstet Gynecol. 2015;45(3):358-62.
21. Vis JY, Van Baaren GJ, Wilms FF, Oudijk MA, Kwee A, Porath MM, Scheepers HC, Spaanderman ME, Bloemenkamp KW, Van Lith JM, Bolte AC, Bax CJ, Cornette J, Duvekot JJ, Nij Bijvank SW, Van Eyck J, Franssen MT, Sollie KM, Woiski M, Vandenbussche FP, Van Der Post JA, Bossuyt PM, Opmeer BC, Mol BW. Randomized comparison of nifedipine and placebo in fibronectin-negative women with symptoms of preterm labor and a short cervix (APOSTEL-I Trial). American Journal of Perinatology. 2015;32(5):451-9.
22. van Baaren GJ, Vis JY, Wilms FF, Oudijk MA, Kwee A, Porath MM, Scheepers HCJ, Spaanderman MEA, Bloemenkamp KWM, Haak MC, Bax CJ, Cornette JMJ, Duvekot JJ, Nij Bijvanck BWA, van Eyck J, Franssen MTM, Sollie KM, Vandenbussche F, Woiski M, Bolte AC, van der Post JAM, Bossuyt PMM, Opmeer BC, Mol BWJ. Cost-effectiveness of diagnostic testing strategies including cervical-length measurement and fibronectin testing in women with symptoms of preterm labor. Ultrasound Obstet Gynecol. 2018;51(5):596-603.
23. Watson HA, Carter J, Seed PT, Tribe RM, Shennan AH. The QUiPP App: a safe alternative to a treat-all strategy for threatened preterm labor. Ultrasound Obstet Gynecol. 2017;50(3):342-6.
24. Watson HA, Seed PT, Carter J, Hezelgrave NL, Kuhrt K, Tribe RM, Shennan AH. Development and validation of predictive models for QUiPP App v.2: tool for predicting preterm birth in asymptomatic high-risk women. Ultrasound Obstet Gynecol. 2020;55(3):348-56.
25. Goodfellow L, Care A, Sharp A, Ivandic J, Poljak B, Roberts D, Alfirevic Z. Effect of QUiPP prediction algorithm on treatment decisions in women with a previous preterm birth: a prospective cohort study. BJOG. 2019;126(13):1569-75.
26. Riley RD, Ensor J, Snell KIE, Harrell FE, Jr., Martin GP, Reitsma JB, Moons KGM, Collins G, van Smeden M. Calculating the sample size required for developing a clinical prediction model. BMJ. 2020;368:m441.

Authors contributions

SB wrote the proposal and the manuscript. FH initiated the research, designed the study, and revised the proposal and manuscript. MO, RE, MB and EP critically revised the proposal and manuscript. All authors read and approved the final manuscript.

Funding statement

This work was supported and funded by the Pregnolia institute. They also provided the system and the probes. The funders had no role in design and conduct of the study, collection, management, analysis and interpretation of the data. Also, the funder had no role in preparation, reviewing or approval of the manuscript.

Funding was not provided by the trial sponsor.

Competing interests

None declared

Word count

2549 words

Table 1:

Sample size A-STIPP cohort

Number of predictors (n)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Minimum required sample size (n)	227	240	360	480	600	720	840	960	1080	1200	1320	1440	1560	1680	1799

Table 2:

Sample size S-STIPP cohort

Number of predictors (n)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Minimum required sample size (n)	163	163	163	163	188	225	263	300	338	375	413	450	488	525	563

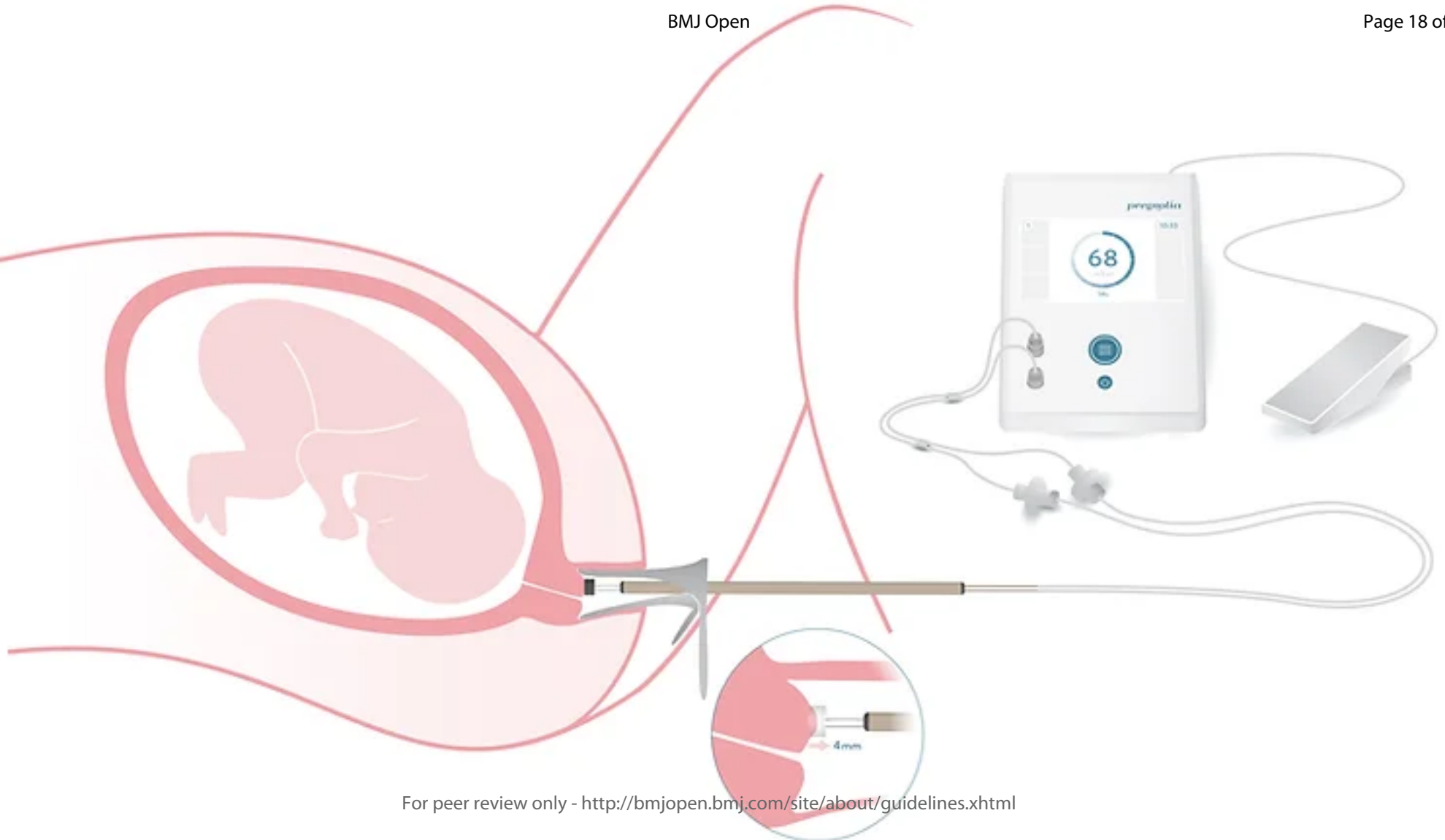
Table 3

Predictor variable

	Predictor variable
1	Cervical length
2	Fetal Fibronectin[#]
3	Twin gestation
4	History of spontaneous preterm birth
5	Cervical Surgery
6	Interpregnancy interval
7	Presence of infection
8	Family history
9	Social economic status
10	Smoking
11	BMI

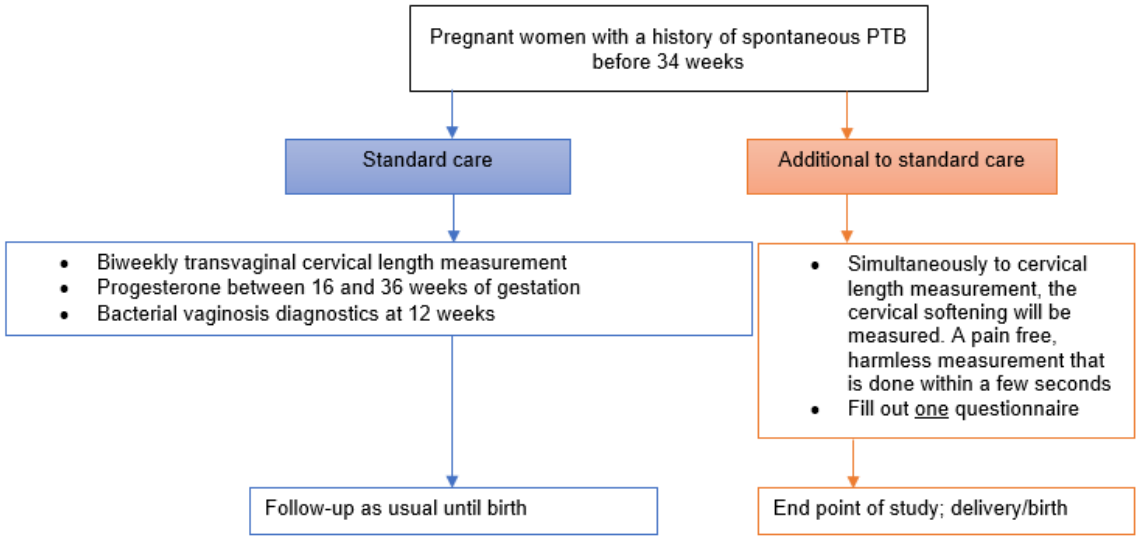
S-STIPP only

Figure 1: Illustration of the cervical weakness measurement

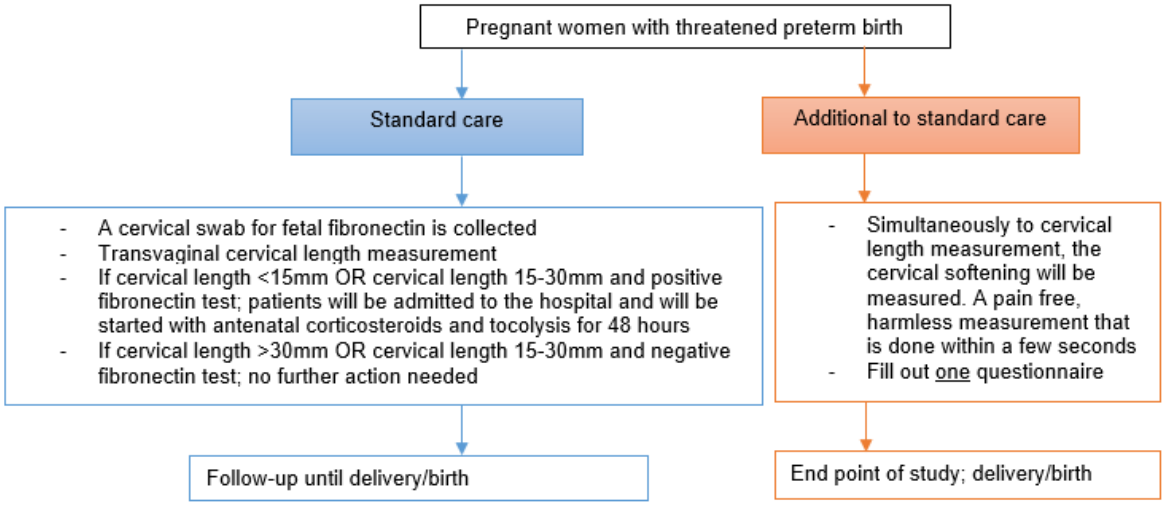


Appendix 1: Timeline of study procedures

Timeline A-STIPP cohort



Timeline S-STIPP cohort



Appendix 2: safety parameters of the Pregnolia System

- Vaginal or cervical blood loss (directly after measurement)
- Patient discomfort or dissatisfcaction during Pregnolia® measurement(scaled 0 to 10)
- Preterm Prelabour Rupture Of Membranes (PPROM) (directly after measurement)
- Preterm labour (directly after measurement)
- Infections within seven days of measurement (Urinary Tract Infections, Vaginal infections, Intra-uterine infections)
- Irritation and sensitization of mucosal tissue
- Infection of the vaginal or mucosal tissue
- Tissue abrasion and vaginal discharge
- Spotting, light bleeding
- Superficial lacerations or minor tissue abrasions

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	Abstract/ protocol
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	13
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	13
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and	NA

		other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5

Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	NA
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	NA
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8

Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Appendix 1
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	NA
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	NA
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque,	NA

		sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	NA
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	NA
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7 and 10

Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	NA
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	NA
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and	10

		competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	10
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	11
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	NA

Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	11
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	11
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	13
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	11
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	11

Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	NA
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Appendix 3
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

BMJ Open

Assessment of cervical SofTening and the Prediction of Preterm birth: study protocol of the STIPP study. A prospective cohort study.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-071597.R1
Article Type:	Protocol
Date Submitted by the Author:	30-Aug-2023
Complete List of Authors:	Breuking, Sofie; Amsterdam UMC Locatie AMC, Obstetrics and gynaecology; Amsterdam Reproduction and Development Research Institute Oudijk, Martijn; Amsterdam UMC Locatie VUmc, Obstetrics; Amsterdam Reproduction and Development Research Institute van Eekelen, Rik; Amsterdam Reproduction and Development Research Institute, Obstetrics and Gynaecology de Boer, Marjon; Amsterdam UMC Locatie VUmc, Department of Obstetrics and Gynaecology; Amsterdam Reproduction and Development Research Institute Pajkrt, Eva; Amsterdam UMC Location AMC, Obstetrics and Gynaecology; Amsterdam Reproduction and Development Research Institute Hermans, Frederik; Amsterdam UMC Locatie AMC, Obstetrics and Gynaecology; Amsterdam Reproduction and Development Research Institute
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Obstetrics and gynaecology
Keywords:	Maternal medicine < OBSTETRICS, OBSTETRICS, Prenatal diagnosis < OBSTETRICS

Assessment of cervical SofTening and the Prediction of Preterm birth: study protocol of the STIPP study. A prospective cohort study.

Sofie. H. Breuking, MD[1, 2, *] Martijn A. Oudijk, MD, PhD[2,3], R. van Eekelen[4] ,M.A. de Boer, MD, PhD[2,3] Eva. Pajkrt, MD, PhD[1, 2], Frederik J. R. Hermans, MD PhD[1,2]

1. Amsterdam UMC location AMC, University of Amsterdam, Obstetrics and Gynaecology, Meibergdreef 9, Amsterdam, The Netherlands

2. Amsterdam Reproduction and Development Research Institute, The Netherlands

3. Amsterdam UMC location Vrije Universiteit Amsterdam, Obstetrics and Gynaecology, Boelelaan 1117, Amsterdam, The Netherlands

4. Obstetrics and Gynaecology, Centre for Reproductive Medicine, Amsterdam Reproduction and Development Research Institute, Amsterdam UMC Location AMC, Amsterdam, The Netherlands

* corresponding author

Correspondence to: s.h.breuking@amsterdamumc.nl

Abstract

Introduction

Preterm birth (PTB) is amongst the leading causes of perinatal and childhood morbidity and mortality. Therefore, accurate identification of pregnant women at high risk of PTB is key to enable obstetric healthcare professionals to apply interventions to improve perinatal and childhood outcomes. Serial transvaginal cervical length measurement is used to screen asymptomatic pregnant women with a history of PTB. Cervical length measurement, fetal fibronectin test or a combination of both can be used to identify women at high risk of PTB in women presenting with symptoms of threatened PTB. The predictive capacity of these can be improved.

Cervical softening is precursor of cervical shortening, effacement and dilatation and could be a new marker to identify women a high risk of PTB. However, the predictive value of cervical softening to predict spontaneous PTB still need to be determined.

Methods and analysis

This is a single center, prospective cohort study, conducted at Amsterdam University Medical Center in the Netherlands. Cervical softening will be investigated with a non-invasive CE-marked device called the Pregnolia System®. This device has been developed to evaluate consistency of the cervix based on tissue elasticity. Two different cohorts will be investigated. The first cohort includes women with a history of spontaneous PTB <34 weeks. These women undergo biweekly measurement between 14 and 24 weeks of gestation. The second cohort includes women with symptoms of threatened PTB. These women will receive the measurement once on presentation between 24 and 34 weeks of gestation. Primary outcome is spontaneous PTB before 34 weeks for women with a history of PTB and delivery within seven days for women with threatened PTB. The minimum sample size to analyse the primary outcome is 227 women with a history of PTB, and 163 women with symptoms of threatened PTB. Once this number is achieved, the study will be continued to investigate secondary objectives.

Ethics and dissemination

The study is approved by the Medical Ethics Committee of Amsterdam UMC (METC2022.0226). All patients will give oral and written informed consent prior to entry of the study. Results will be disseminated through peer-reviewed journals. This protocol is published before analysis of results is done.

Study registration

Study is registered at ClinicalTrials.gov, identifier NCT05477381, date of registration: 27th July 2022.

Strengths and limitations of the study

- A strength of this study is that it is organized in a way with minimal interference in daily practice and therefore a high participation rate is expected.
- A notable strength of this study is its prospective cohort design, which includes women across a range of all cervical lengths, thereby establishing an internal control group of women with longer cervix lengths within the cohorts.
- This is the first study investigating the cervical stiffness index in a population of high risk women for preterm birth and combining the results with other important predictors for preterm birth.

- The is a prospectively cohort study, therefore, we expect less bias than in a retrospective cohort.
- A limitation of the study is the single centre design potentially limiting external validity and generalizability.

Introduction

Spontaneous preterm birth (PTB), defined as delivery before 37 weeks of gestation, is the leading cause of perinatal and neonatal morbidity and mortality(1). Rates of spontaneous PTB appears to be increasing. Annually, 15 million children are born preterm and is directly responsible for the death of one million neonates(2, 3). Neonates who survive PTB are at increased risk for long-term neurologic sequelae and developmental disabilities(4, 5). Identifying pregnant women at risk is important to be able to take precautionary measures, however this is a challenge for obstetric healthcare professionals.

Important obstetric and gynaecological risk factors for PTB are midtrimester short cervical length, prior cervical surgery and previous spontaneous PTB. (6-9) Women with a history of spontaneous PTB before 34 weeks of gestation are at a 5-fold increased risk of a spontaneous PTB in a subsequent pregnancy compared to women with a previous term birth(10). In addition to vaginal progesterone administration, biweekly cervical length screening is recommended in these women. This can identify women at high risk of a recurrent PTB based on short cervical length that benefit from a vaginal cerclage. However, in women with a previous PTB, the positive predictive value (PPV) of a short cervical length is 34%. (11) Therefore this approach only identifies a proportion of women who will have a recurrent PTB. This calls for additional measurements to identify the group more adequately at risk for recurrent PTB.

Another group of pregnant women at risk of delivering PTB are women presenting with symptoms of threatened PTB in their current pregnancy. These women can be triaged with transvaginal cervical length measurement and fetal fibronectin(fFN) to identify women with an increased risk of delivery within seven days. Women with a high risk of PTB at less than 32 weeks of gestation are admitted to a centre with NICU facilities and treated with antenatal corticosteroids and tocolysis for 48 hours to improve perinatal outcome.(12) This combination

of markers are characterized by a high negative predictive value (NPV) but a poor PPV. This results in overtreatment and unnecessary healthcare costs. A large proportion of women with symptoms of threatened PTB will not deliver within seven days due to the low PPV, however these women remain at risk for PTB later in pregnancy. (13-15)

More adequate techniques to assess women at high risk of recurrent PTB or at high risk of delivering in a short time frame when presenting with symptoms of threatened PTB are urgently needed. Therefore, objective measurement of the cervical consistency is a promising technique.

To maintain pregnancy and deliver at term an appropriate function of the cervix is required. Delivery is preceded by softening and shortening of the cervix.(16) Changes in cervical consistency can be detected from fertilization until delivery. Throughout pregnancy consistency of the cervix changes and will soften when approaching delivery.(17, 18) Softening of the cervix precedes shortening and therefore could be a promising marker to identify an upcoming delivery at an earlier stage.

Parra-Saavedra *et al.*(19) investigated this phenomenon with transvaginal ultrasound. The cervical consistency was measured by measuring the difference of the anteroposterior cervical diameter before (AP) and after (AP^1) application of pressure on the cervix with the transvaginal probe. The cervical consistency was then calculated with the following formula: ((AP^1/AP) * 100) = Cervical consistency index. Cervical consistency had an inverse linear relationship with gestational age. This means that cervical consistency declines, thus becomes softer, during progression of pregnancy and that this phenomenon can be detected in pregnancy. Secondly, it demonstrated also that pregnant women with a more progressive decline in cervical consistency are more likely to have a spontaneous PTB compared to women with physiological decline in cervical consistency.

Other techniques that show positive result in evaluating cervical softness is by using elastography methods, including strain elastography (SE) and shear wave elastography (SWE)(20). Nonetheless, there are technical considerations that first need to be resolved before elastography can be applied extensively. For example, the results of SE are affected by operator-applied pressure on the cervix, resulting in an inter-observer variability making it less objective and standardized (21, 22). Moreover, for SWE, safety concerns, such as the unknown

risk of fetal tissues(23), first must be addressed before elastography methods can be applied extensively.

Recently, a non-invasive technique has been developed to evaluate consistency of the cervix based on tissue elasticity. The Pregnolia®-system is a market available CE-marked device designed to measure cervical stiffness. This system provides quantitative measurements of the cervical consistency based on aspiration technique.

A prototype has been tested and measurements were carried out in 50 non-pregnant and 50 pregnant women(18). The results were in line with the study by Parra-Saavedra *et al.*(19) and showed that with progressing of pregnancy, the cervix softens and this process starts before shortening. Therefore, by measuring the CSI, delivery could be detected earlier compared to conventional shortening of the cervix measured with transvaginal ultrasound.

Also, a recent study by Stone *et al.(22)* investigated cervical softness before cerclage placement with the Pregnolia®-system. This study demonstrated patients with a ultra-sound indicated cerclage, had significantly softer cervices compared to normal controls. They also stated this aspiration technique is a promising technique for objective and quantitative measurement of cervical softness.

Since cervical softening is a precursor of cervical shortening, this could be a novel marker to predict spontaneous PTB and contribute to better identification of women with an increased risk of PTB. Also, the predictive value of cervical softening in combination with cervical length could be promising to improve prediction of PTB. However, these hypotheses still must be examined.

Therefore, the aim of this cohort study is to evaluate the predictive value of the CSI to predict the risk of spontaneous PTB in pregnant women with an increased risk of PTB.

Methods and analysis

Study design

This study is an investigator initiated, single centre prospective cohort study that will be performed at the Amsterdam UMC in the Netherlands. Recruitment started at 18th of august 2022. We expect a study duration of 3 years to investigate the primary objectives.

Two cohorts will be investigated:

- Pregnant women with a history of spontaneous PTB before 34 weeks of gestation (Cohort A-STIPP).
- Pregnant women presenting with symptoms of threatened PTB between 24 and 34 weeks of gestation (Cohort S-STIPP).

The measurements of cervical stiffness will be performed in addition to standard care(Appendix 1), using the aspiration technique-based device named the Pregnolia system device.

Participants

In order to be eligible to participate in this study, pregnant women must meet all of the following criteria:

- Age 18 years or above.
- Ability to understand Dutch or English (both spoken and written).
- Ultrasound-based gestational age determined by measurement of crown rump length (CRL), determined between 9 and 11 weeks of gestation.
- Singleton and twin pregnancies.

A-STIPP cohort

Pregnant women with an increased risk of PTB based on a medical history of spontaneous PTB before 34 weeks of gestation will be included.

S-STIPP cohort

Pregnant women, with a gestational age between 24 and 34 weeks presenting with symptoms of threatened PTB, such as abdominal pain, vaginal blood loss, contractions or other complaints suggestive for threatened PTB, will be included.

A potential subject who meets any of the following criteria will be excluded from participation:

- Signs of intrauterine infection.
- Obstetric indication for immediate delivery (advanced labour, cord prolapse, abruption, signs of foetal distress).
- Confirmed foetal abnormality.
- Confirmed preterm rupture of membranes.
- Confirmed vasa / placenta praevia.
- Severe vaginal bleeding and light bleeding that cannot be stopped.

- Signs of imminent labour such as advanced dilatation making it impossible to measure the cervix.

Measurements

Cervical stiffness measurement

The Cervical stiffness index (CSI) will be measured subsequent to measurement of the cervical length. The Pregnolia System is composed of two components: an active, reusable device and a disposable single-use sterile probe.

- The control unit is an active device with a power supply, foot switch, connector cable and an integrated pump that generates vacuum.
- The single-use sterile probe is connected to the control unit console through a connector cable. Air filters on the probe prevent microbiological contamination of the control unit. This probe is designed to minimise the contact interaction between the user and the patients during the measurement. The probe tip diameter is 12mm. Each single-use, disposable probe is packed in a sterile pouch.

To perform the measurement, the cervix is visualized with a speculum. The disposable probe is placed on the anterior lip of the cervix (12 o'clock position). The control will create a weak vacuum inside the probe that pulls the cervical tissue, very gently and slowly, into the probe tip by a fixed distance of 4mm. The negative aspiration pressure needed to deform the tissue is the outcome of the measurement. A high-pressure value corresponds with stiff tissue and a low pressure corresponds with soft tissue. An overview of the measurement is displayed in figure 1.

Sonographic measurement

Cervical length measurement with transvaginal ultrasound is routine care in both cohorts.

The cervical length will be determined as the linear distance between internal and external cervical os, excluding the endocervical funnel as described by Kagan *et al*. (24)

In the A-STIPP cohort, transvaginal ultrasound will be done biweekly from 14 until 24 weeks of gestation. In case a short cervix is detected at less than 25 mm, a cerclage, or a pessary in study context, is placed. Afterwards measurement of CSI will not be continued.

In the S-STIPP cohort, the transvaginal ultrasound will be performed when a women presents with any symptom of threatened PTB, between 24 and 34 weeks of gestation.

Questionnaire

Participants will be asked to fill out a structured questionnaire to screen for additional risk factors of PTB. The questionnaire contains questions about the current pregnancy and details about previous pregnancies, if applicable. Moreover, details on cervical surgery in the past and family history of PTB are requested. Baseline characteristics such as height, weight and smoking as well as information about medical history, including gynaecological history and uterus malformations, will be collected. The questionnaire will be checked with the patient's electronic file.

For the S-STIPP cohort, participants will be asked about the specific symptoms associated with threatened PTB.

Blinding

For the A-STIPP cohort, clinicians and participants are blinded for the results of the CSI measurement.

In the S-STIPP cohort, the clinician working at the emergency department performs the measurement and therefore making it impossible to blind the treating clinician. The participant however is blinded for the results.

Follow-up

Participants will be followed-up from inclusion until delivery. Detailed information regarding the pregnancy outcomes, including maternal and neonatal outcomes will be gathered.

Also, if applicable, detailed information about hospital admittance during pregnancy will be noted. Moreover, if a participant is admitted due to threatened PTB, received treatments like antenatal corticosteroids, tocolytic medicines, or magnesium sulfate for neonatal neuroprotection will be noted.

Primary outcome

- The primary outcome for the A-STIPP cohort is spontaneous PTB before 34 weeks of gestation.
- The primary outcome for the S-STIPP cohort is delivery within 7 days.

Secondary outcomes

- Spontaneous PTB before 37 weeks of gestation
- Spontaneous PTB before 34 weeks of gestation#
- Spontaneous PTB before 32 weeks of gestation
- Spontaneous PTB before 28 weeks of gestation
- Latency time (time between inclusion and delivery)
- Delivery within 48 hours#
- Preterm Premature rupture of Membranes (PPROM)

S-STIPP only

Other outcomes

Safety of the use of the Pregnolia system (as defined in Appendix 2) will be investigated.

Also, patient discomfort of the measurement will be evaluated by a general questionnaire.

Power analysis

We used contemporary sample size calculations described by *Riley et al.(25)* for developing prediction models, based on three criteria that each provide a sample size to satisfy that criterion, then picking the highest sample size out of the three. The following input parameters are used to calculate the required number of inclusions; 1) expected prevalence of the primary outcome, 2) expected amount of explained variance by the prediction model and 3) number of predictors (input variables).

For the A-STIPP cohort the prevalence [0.18] was derived from the QUIPP-study (26-28). The standard level of variance [0.15] was used to calculate sample size.

For the S-STIPP cohort, the prevalence [0.12] and variance [0.45] were derived from the Apostel I study(12, 29). Both studies have comparable patients as the A-STIPP and S-STIPP study.

To investigate additional input predictors with sufficient power, an increase in sample size is needed. When inclusion of participants continues and the second threshold is reached, another input parameter is added, until the next threshold and so on. The baseline predictors

used in the first step will be the CSI measurement combined with cervical length measurement in the A-STIPP cohort, and cervical length with fFN in S-STIPP cohort.

See table 1 and table 2 for the steps and the threshold sample sizes. In both calculations the number of predictors was gradually increased. Continuous variables count as a single input variable, as well as dichotomous input variables. Categorical variables are counted as C-1, thus the number of input variables is the number categories minus one. The additional predictor variables are summarized in table 3.

Sample size calculations were performed using R (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/) with the use of the *pmsampsize* package *(25).*

Table 1 and table 2 indicates the number of participants needed. For the A-STIPP, the minimum sample size of 227 patients is required to achieve the primary objective of this study. Once this number is achieved, the study will be continued to investigate secondary objectives. For the S-STIPP, the minimum sample size of 163 patients is required to achieve the primary objective of this study. Once this number is achieved, the study will be continued to investigate secondary objectives, by using the dynamical sample size as explained.

Statistical Analysis

Baseline characteristics will be calculated used descriptive statistics. Continuous variables will be reported as mean with standard deviation or median with inter quartile range. Categorical variables will be reported as proportions.

To incorporate repeated measures of CSI from the A-STIPP cohort, a logistic generalized mixed model will be used. As CSI is a continuous outcome, linear and non-linear functions will be compared using restricted cubic splines. A lower Akaike's Information Criterion (AIC) or overall p-value will determine which functional form is chosen.(25) If there is censoring (i.e. loss-to-follow-up), a Cox proportional hazards model for time to delivery including a time-varying covariate for CSI will be used. As a sensitivity analysis, a comparison of either of these models with a joint survival model will be done (combining a Cox model for time to delivery with a linear mixed model for CSI measurements).

For the S-STIPP cohort, a logistic regression will be used to determine the relationship between input variables and a dichotomous outcome.

Subgroup analysis

Subgroup analysis are planned for participants and treatments that may potentially effect cervical stiffness, in order to assess their impact on the CSI:

- Nulliparous versus multiparous women
- Singleton versus multiple pregnancies
- Women with previous cervical surgery versus women without
- Women with a (abdominal or cervical) cerclage in current pregnancy versus no cerclage
- Women treated with progesterone versus no treatment

A-STIPP cohort subgroup analysis

Subgroup of interest in asymptomatic participants are:

- Women with a short cervix (≤25mm) during screening vs. women with a long cervix (>25mm)
- Women who received additional treatment (pessary or cerclage) vs. no treatment.

S-STIPP cohort subgroup analysis

Subgroup of interest in symptomatic participants are performed in groups based on clinical risk stratification:

- Cervical length ≥30mm
- Cervical length ≥15 and <30mm with negative fFN
- Cervical length ≥15 and <30mm with positive fFN
- Cervical length < 15 mm

Monitoring and safety

An independent Data and Safety Monitoring Board (DSMB) is assigned to safeguard the safety of the study participants and provide recommendations.

Since the measurement with the Pregnolia-system is minimally invasive, the risk of adverse events(AEs) related to the measurement is small. However, any AEs and Serious Adverse Events (SAEs) will be reported. If evaluation by the DSMB demonstrates increased safety risks within the study, the DSMB can always advice to stop the study.

Data management

Data will be collected using an accredited electronic data capture system (Castor). To protect the privacy of the participant, personal data is encrypted. Data cannot be traced back to participants in reports and publications about the study. All personal data is protected according to the General Data Protection Regulation (GDPR and Dutch privacy regulation (AVG)).

All agreements regarding data sharing are defined in a signed Clinical Trial Agreement (CTA), GDPR are applicable to this agreement.

Clinical impact

This STIPP study will provide evidence on the value of the cervical stiffness as a single clinical marker and in combination with other clinical markers such as cervical length to predict the risk of spontaneous PTB in groups of pregnant women with an increased risk of PTB.

Patient and public involvement

The patient organisation care4Neo was informed about the study and was favourable about purpose of the study.

Ethics and dissemination

The Medical Ethics Committee of the Amsterdam UMC has given approval for this research (Number 2022.0226). All participants will give written and oral informed consent prior to entry to the study and will be made aware participation is completely voluntary. The outcomes of the study will be published in a peer-reviewed journal.

References

1. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, Adler A, Vera Garcia C, Rohde S, Say L, Lawn JE. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012;379(9832):2162-72.
2. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, Rudan I, Campbell H, Cibulskis R, Li M, Mathers C, Black RE, Child Health Epidemiology Reference Group of WHO, Unicef. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet. 2012;379(9832):2151-61.
3. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, Cousens S, Mathers C, Black RE. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet. 2015;385(9966):430-40.
4. Marlow N, Wolke D, Bracewell MA, Samara M, Group EPS. Neurologic and developmental disability at six years of age after extremely preterm birth. N Engl J Med. 2005;352(1):9-19.
5. Mwaniki MK, Atieno M, Lawn JE, Newton CR. Long-term neurodevelopmental outcomes after intrauterine and neonatal insults: a systematic review. Lancet. 2012;379(9814):445-52.
6. van Zijl MD, Koullali B, Mol BW, Pajkrt E, Oudijk MA. Prevention of preterm delivery: current challenges and future prospects. Int J Womens Health. 2016;8:633-45.
7. van der Ven J, van Os MA, Kazemier BM, Kleinrouweler E, Verhoeven CJ, de Miranda E, van Wassenaer-Leemhuis AG, Kuiper PN, Porath M, Willekes C, Woiski MD, Sikkema MJ, Roumen FJ, Bossuyt PM, Haak MC, de Groot CJ, Mol BW, Pajkrt E. The capacity of mid-pregnancy cervical length to predict preterm birth in low-risk women: a national cohort study. Acta Obstet Gynecol Scand. 2015;94(11):1223-34.
8. Iams JD, Berghella V. Care for women with prior preterm birth. Am J Obstet Gynecol. 2010;203(2):89-100.
9. Conner SN, Frey HA, Cahill AG, Macones GA, Colditz GA, Tuuli MG. Loop electrosurgical excision procedure and risk of preterm birth: a systematic review and meta-analysis. Obstet Gynecol. 2014;123(4):752-61.
10. Kazemier BM, Buijs PE, Mignini L, Limpens J, de Groot CJ, Mol BW, Ebm C. Impact of obstetric history on the risk of spontaneous preterm birth in singleton and multiple pregnancies: a systematic review. BJOG. 2014;121(10):1197-208; discussion 209.
11. Hughes K, Kane SC, Araujo Junior E, Da Silva Costa F, Sheehan PM. Cervical length as a predictor for spontaneous preterm birth in high-risk singleton pregnancy: current knowledge. Ultrasound Obstet Gynecol. 2016;48(1):7-15.
12. Vis JY, van Baaren GJ, Wilms FF, Oudijk MA, Kwee A, Porath MM, Scheepers HC, Spaanderman ME, Bloemenkamp KW, van Lith JM, Bolte AC, Bax CJ, Cornette J, Duvekot JJ, Nij Bijvank SW, van Eyck J, Franssen MT, Sollie KM, Woiski M, Vandenbussche FP, van der Post JA, Bossuyt PM, Opmeer BC, Mol BW. Randomized comparison of nifedipine and placebo in fibronectin-negative women with symptoms of preterm labor and a short cervix (APOSTEL-I Trial). Am J Perinatol. 2015;32(5):451-60.
13. Wilms FF, Vis JY, de Wit-Zuurendonk L, Porath MM, Mol BW. What is the risk of preterm delivery after arrested preterm labor? Am J Perinatol. 2015;32(1):63-70.
14. Cornelissen LGH, van Oostrum NHM, van der Woude DAA, Rolf C, Porath MM, Oei SG, van Laar J. The diagnostic value of fetal fibronectin testing in clinical practice. J Obstet Gynaecol Res. 2020;46(3):405-12.
15. Berghella V, Saccone G. Fetal fibronectin testing for reducing the risk of preterm birth. Cochrane Database Syst Rev. 2019;7:CD006843.
16. Mazza E, Parra-Saavedra M, Bajka M, Gratacos E, Nicolaides K, Deprest J. In vivo assessment of the biomechanical properties of the uterine cervix in pregnancy. Prenat Diagn. 2014;34(1):33-41.
17. Badir S, Bajka M, Mazza E. A novel procedure for the mechanical characterization of the uterine cervix during pregnancy. J Mech Behav Biomed Mater. 2013;27:143-53.

18. Badir S, Mazza E, Zimmermann R, Bajka M. Cervical softening occurs early in pregnancy: characterization of cervical stiffness in 100 healthy women using the aspiration technique. Prenat Diagn. 2013;33(8):737-41.
19. Parra-Saavedra M, Gomez L, Barrero A, Parra G, Vergara F, Navarro E. Prediction of preterm birth using the cervical consistency index. Ultrasound Obstet Gynecol. 2011;38(1):44-51.
20. Shiina T, Nightingale KR, Palmeri ML, Hall TJ, Bamber JC, Barr RG, Castera L, Choi BI, Chou YH, Cosgrove D, Dietrich CF, Ding H, Amy D, Farrokh A, Ferraioli G, Filice C, Friedrich-Rust M, Nakashima K, Schafer F, Sporea I, Suzuki S, Wilson S, Kudo M. WFUMB guidelines and recommendations for clinical use of ultrasound elastography: Part 1: basic principles and terminology. Ultrasound Med Biol. 2015;41(5):1126-47.
21. Ge W, Brooker G, Mogra R, Hyett J. Measured Hyperelastic Properties of Cervical Tissue with Shear-Wave Elastography. Sensors (Basel). 2021;22(1).
22. Stone J, House M. Measurement of cervical softness before cerclage placement with an aspiration-based device. Am J Obstet Gynecol MFM. 2023;5(4):100881.
23. Issaoui M, Debost-Legrand A, Skerl K, Chauveau B, Magnin B, Delabaere A, Boyer L, Sauvant-Rochat MP, Lemery D. Shear wave elastography safety in fetus: A quantitative health risk assessment. Diagn Interv Imaging. 2018;99(9):519-24.
24. Kagan KO, Sonek J. How to measure cervical length. Ultrasound Obstet Gynecol. 2015;45(3):358-62.
25. Riley RD, Ensor J, Snell KIE, Harrell FE, Jr., Martin GP, Reitsma JB, Moons KGM, Collins G, van Smeden M. Calculating the sample size required for developing a clinical prediction model. BMJ. 2020;368:m441.
26. Watson HA, Carter J, Seed PT, Tribe RM, Shennan AH. The QUiPP App: a safe alternative to a treat-all strategy for threatened preterm labor. Ultrasound Obstet Gynecol. 2017;50(3):342-6.
27. Watson HA, Seed PT, Carter J, Hezelgrave NL, Kuhrt K, Tribe RM, Shennan AH. Development and validation of predictive models for QUiPP App v.2: tool for predicting preterm birth in asymptomatic high-risk women. Ultrasound Obstet Gynecol. 2020;55(3):348-56.
28. Goodfellow L, Care A, Sharp A, Ivandic J, Poljak B, Roberts D, Alfirevic Z. Effect of QUiPP prediction algorithm on treatment decisions in women with a previous preterm birth: a prospective cohort study. BJOG. 2019;126(13):1569-75.
29. van Baaren GJ, Vis JY, Wilms FF, Oudijk MA, Kwee A, Porath MM, Scheepers HCJ, Spaanderman MEA, Bloemenkamp KWM, Haak MC, Bax CJ, Cornette JMJ, Duvekot JJ, Nij Bijvanck BWA, van Eyck J, Franssen MTM, Sollie KM, Vandenbussche F, Woiski M, Bolte AC, van der Post JAM, Bossuyt PMM, Opmeer BC, Mol BWJ. Cost-effectiveness of diagnostic testing strategies including cervical-length measurement and fibronectin testing in women with symptoms of preterm labor. Ultrasound Obstet Gynecol. 2018;51(5):596-603.

Authors contributions

FH initiated the research. FH and SB designed the study. SB wrote the proposal and the manuscript. FH revised the proposal and manuscript. MO, RE, MB and EP critically revised the proposal and manuscript. All authors read and approved the final manuscript.

Funding statement

This work was supported and funded by the Pregnolia institute. They also provided the system and the probes. The funders had no role in the study design, conduction of the study,

collection, management, analysis and interpretation of the data. Also, the funder had no role in preparation, reviewing or approval of the manuscript. Funding was not provided by the study sponsor.

Competing interests

None declared

Word count

2905 words

Table 1:

Sample size A-STIPP cohort

Number of predictors (n)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Minimum required sample size (n)	227	240	360	480	600	720	840	960	1080	1200	1320	1440	1560	1680	1799

Table 2:

Sample size S-STIPP cohort

Number of predictors (n)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Minimum required sample size (n)	163	163	163	163	188	225	263	300	338	375	413	450	488	525	563

Table 3

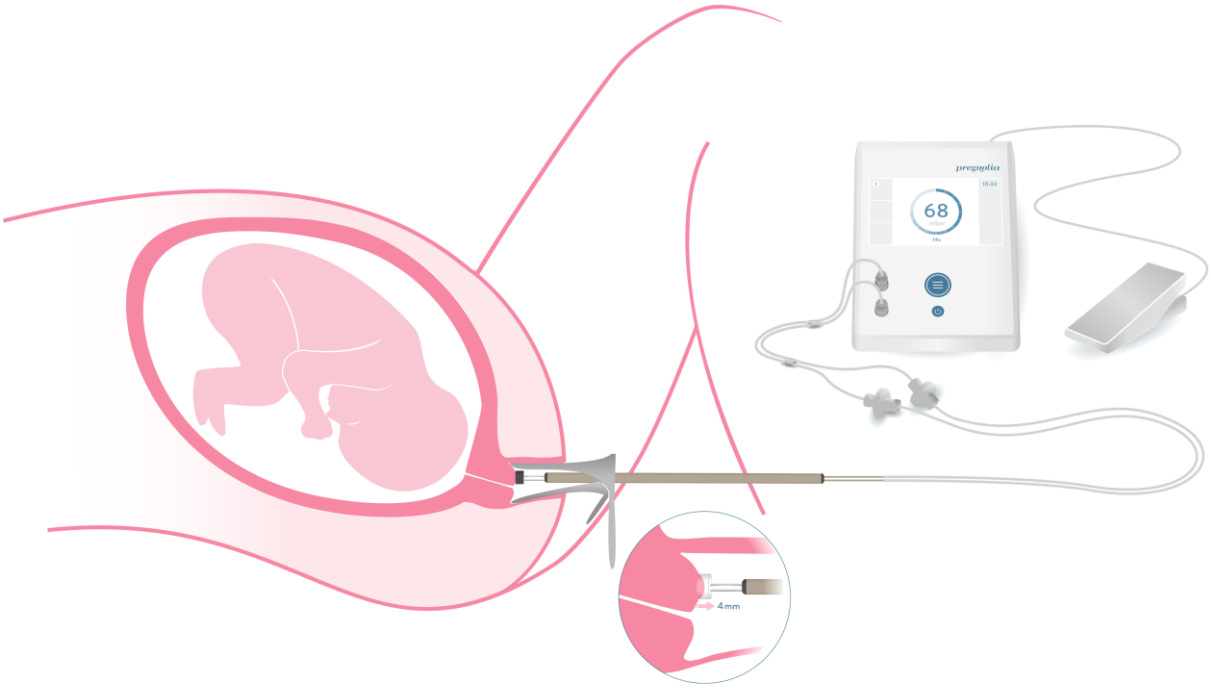
Predictor variable

	Predictor variable
1	Cervical length
2	Fetal Fibronectin[#]
3	Twin gestation
4	History of spontaneous preterm birth
5	Cervical Surgery
6	Interpregnancy interval
7	Presence of infection
8	Family history
9	Social economic status
10	Smoking
11	BMI

S-STIPP only

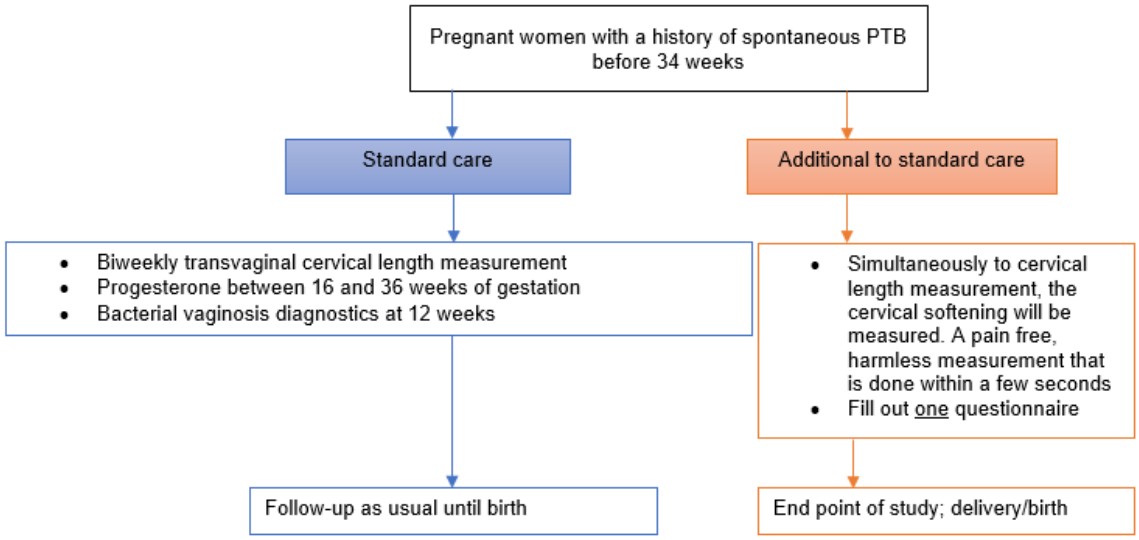
Figure 1: Illustration of the cervical softness measurement

Figure 1: Illustration of an overview of the measurement

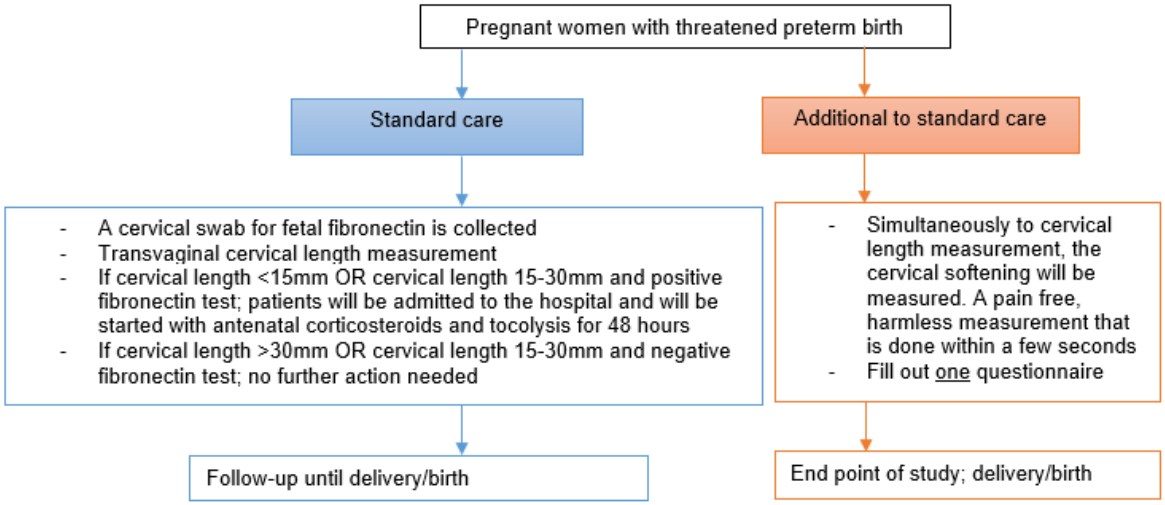


Appendix 1: Timeline of study procedures

Timeline A-STIPP cohort



Timeline S-STIPP cohort



Appendix 2: safety parameters of the Pregnolia System

- Vaginal or cervical blood loss (directly after measurement)
- Patient discomfort or dissatisfcaction during Pregnolia® measurement(scaled 0 to 10)
- Preterm Prelabour Rupture Of Membranes (PPROM) (directly after measurement)
- Preterm labour (directly after measurement)
- Infections within seven days of measurement (Urinary Tract Infections, Vaginal infections, Intra-uterine infections)
- Irritation and sensitization of mucosal tissue
- Infection of the vaginal or mucosal tissue
- Tissue abrasion and vaginal discharge
- Spotting, light bleeding
- Superficial lacerations or minor tissue abrasions

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	Abstract/ protocol
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	14
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and	NA

		other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5

Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	NA
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	NA
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8/9

Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Appendix 1
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	NA
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	NA
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque,	NA

		sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	NA
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7

Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	NA
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	NA
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and	12

		competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	12
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	NA

Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	11
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12

Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	NA
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Appendix 3
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

BMJ Open

Assessment of cervical SofTening and the Prediction of Preterm birth (STIPP): protocol for a prospective cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2023-071597.R2
Article Type:	Protocol
Date Submitted by the Author:	16-Oct-2023
Complete List of Authors:	Breuking, Sofie; Amsterdam UMC Locatie AMC, Obstetrics and gynaecology; Amsterdam Reproduction and Development Research Institute Oudijk, Martijn; Amsterdam UMC Locatie VUmc, Obstetrics; Amsterdam Reproduction and Development Research Institute van Eekelen, Rik; Amsterdam Reproduction and Development Research Institute, Obstetrics and Gynaecology de Boer, Marjon; Amsterdam UMC Locatie VUmc, Department of Obstetrics and Gynaecology; Amsterdam Reproduction and Development Research Institute Pajkrt, Eva; Amsterdam UMC Location AMC, Obstetrics and Gynaecology; Amsterdam Reproduction and Development Research Institute Hermans, Frederik; Amsterdam UMC Locatie AMC, Obstetrics and Gynaecology; Amsterdam Reproduction and Development Research Institute
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Obstetrics and gynaecology
Keywords:	Maternal medicine < OBSTETRICS, OBSTETRICS, Prenatal diagnosis < OBSTETRICS

Assessment of cervical SofTening and the Prediction of Preterm birth (STIPP): protocol for a prospective cohort study

Sofie. H. Breuking, MD[1, 2, *] Martijn A. Oudijk, MD, PhD[2,3], R. van Eekelen[4] ,M.A. de Boer, MD, PhD[2,3] Eva. Pajkrt, MD, PhD[1, 2], Frederik J. R. Hermans, MD PhD[1,2]

1. Amsterdam UMC location AMC, University of Amsterdam, Obstetrics and Gynaecology, Meibergdreef 9, Amsterdam, Netherlands

2. Amsterdam Reproduction and Development Research Institute, Netherlands

3. Amsterdam UMC location Vrije Universiteit Amsterdam, Obstetrics and Gynaecology, Boelelaan 1117, Amsterdam, Netherlands

4. Obstetrics and Gynaecology, Centre for Reproductive Medicine, Amsterdam Reproduction and Development Research Institute, Amsterdam UMC Location AMC, Amsterdam, Netherlands

*Correspondence to:

Sofie. H. Breuking

s.h.breuking@amsterdamumc.nl

Abstract

Introduction

Preterm birth (PTB) is amongst the leading causes of perinatal and childhood morbidity and mortality. Therefore, accurate identification of pregnant women at high risk of PTB is key to enable obstetric healthcare professionals to apply interventions that improve perinatal and childhood outcomes. Serial transvaginal cervical length measurement is used to screen asymptomatic pregnant women with a history of PTB and identify those at high risk for a recurrent PTB. Cervical length measurement, foetal fibronectin test or a combination of both can be used to identify women at high risk of PTB in women presenting with symptoms of threatened PTB. The predictive capacity of these methods can be improved. Cervical softening is precursor of cervical shortening, effacement and dilatation and could be a new marker to identify women a high risk of PTB. However, the predictive value of cervical softening to predict spontaneous PTB still needs to be determined.

Methods and analysis

This is a single-centre, prospective cohort study, being conducted at Amsterdam University Medical Center in the Netherlands. Cervical softening will be investigated with a non-invasive CE-marked device called the Pregnolia System. This device has been developed to evaluate consistency of the cervix based on tissue elasticity. Two different cohorts will be investigated. The first cohort includes women with a history of spontaneous PTB <34 weeks. These women undergo biweekly measurement between 14 and 24 weeks of gestation. The second cohort includes women with symptoms of threatened PTB. These women will receive the measurement once on presentation between 24 and 34 weeks of gestation. The primary outcome is spontaneous PTB before 34 weeks for women with a history of PTB and delivery within seven days for women with threatened PTB. The minimum sample size required to analyse the primary outcome is 227 women with a history of PTB and 163 women with symptoms of threatened PTB. Once this number is achieved, the study will be continued to investigate secondary objectives.

Ethics and dissemination

The study is approved by the Medical Ethics Committee of Amsterdam UMC (METC2022.0226). All patients will give oral and written informed consent prior to entry of the study. Results will be disseminated via a peer-reviewed journal.

Study registration

ClinicalTrials.gov, NCT05477381 (date of registration: 27th July 2022).

Strengths and limitations of this study

- A strength of this study is that it is organized in a way with minimal interference in daily practice and therefore a high participation rate is expected.
- Another notable strength of the study is its prospective cohort design, which includes women across a range of all cervical lengths, thereby establishing an internal control group of women with longer cervix lengths within the cohorts.
- This study investigates the cervical stiffness index in a population of high-risk women for preterm birth and combines the results with other important predictors for preterm birth.
- As a prospectively cohort study, we expect less bias than in a retrospective cohort.

- A limitation of the study is the single-centre setting, potentially limiting external validity and generalizability.

Introduction

Spontaneous preterm birth (PTB), defined as delivery before 37 weeks of gestation, is the leading cause of perinatal and neonatal morbidity and mortality(1). Rates of spontaneous PTB appears to be increasing. Annually, 15 million children are born preterm and this is directly responsible for the death of one million neonates(2, 3). Neonates who survive PTB are at increased risk for long-term neurologic sequelae and developmental disabilities(4, 5). Identifying pregnant women at risk is important to be able to take precautionary measures, however this is a challenge for obstetric healthcare professionals.

Important obstetric and gynaecological risk factors for PTB are midtrimester short cervical length, prior cervical surgery and previous spontaneous PTB. (6-9) Women with a history of spontaneous PTB before 34 weeks of gestation are at a 5-fold increased risk of a spontaneous PTB in a subsequent pregnancy compared to women with a previous term birth(10). In addition to vaginal progesterone administration, biweekly cervical length screening is recommended in these women. This can identify women at high risk of a recurrent PTB based on short cervical length that benefit from a vaginal cerclage. However, in women with a previous PTB, the positive predictive value (PPV) of a short cervical length is 34%. (11) Therefore, this approach only identifies a proportion of women who will have a recurrent PTB. This calls for additional measurements to identify the group more adequately at risk for recurrent PTB.

Another group of pregnant women at risk of PTB are women presenting with symptoms of threatened PTB in their current pregnancy. These women can be triaged with transvaginal cervical length measurement and foetal fibronectin (fFN) to identify women with an increased risk of delivery within seven days. Women with a high risk of PTB at less than 32 weeks of gestation are admitted to a centre with NICU facilities and treated with antenatal corticosteroids and tocolysis for 48 hours to improve perinatal outcome.(12) This combination of markers are characterized by a high negative predictive value (NPV) but a poor PPV. This results in overtreatment and unnecessary healthcare costs. A large proportion of women with

symptoms of threatened PTB will not deliver within seven days due to the low PPV, however these women remain at risk for PTB later in pregnancy. (13-15)

More adequate techniques to assess women at high risk of recurrent PTB or at high risk of delivering in a short time frame when presenting with symptoms of threatened PTB are urgently needed. Therefore, objective measurement of the cervical consistency is a promising technique.

To maintain pregnancy and deliver at term an appropriate function of the cervix is required. Delivery is preceded by softening and shortening of the cervix.(16) Changes in cervical consistency can be detected from fertilization until delivery. Throughout pregnancy consistency of the cervix changes and will soften when approaching delivery.(17, 18) Softening of the cervix precedes shortening and therefore could be a promising marker to identify an upcoming delivery at an earlier stage.

Parra-Saavedra *et al.*(19) investigated this phenomenon with transvaginal ultrasound. The cervical consistency was measured by measuring the difference of the anteroposterior cervical diameter before (AP) and after (AP^1) application of pressure on the cervix with the transvaginal probe. The cervical consistency was then calculated with the following formula: ((AP^1/AP) * 100) = Cervical consistency index. Cervical consistency had an inverse linear relationship with gestational age. This means that cervical consistency declines, thus becomes softer, during progression of pregnancy and this phenomenon can be detected during pregnancy. Secondly, it demonstrated that pregnant women with a more progressive decline in cervical consistency are more likely to have a spontaneous PTB compared to women with physiological decline in cervical consistency.

Other techniques that show positive result in evaluating cervical softness is by using elastography methods, including strain elastography (SE) and shear wave elastography (SWE)(20). Nonetheless, there are technical considerations that first need to be resolved before elastography can be applied extensively. For example, the results of SE are affected by operator-applied pressure on the cervix, resulting in an inter-observer variability making the technique less objective and standardized (21, 22). Moreover, for SWE, safety concerns, such as the unknown risk of foetal tissues(23), first must be addressed before elastography methods can be applied extensively.

Recently, a non-invasive technique has been developed to evaluate consistency of the cervix based on tissue elasticity. The Pregnolia System is a market-available, CE-marked device designed to measure cervical stiffness. This system provides quantitative measurements of the cervical consistency based on aspiration technique.

A prototype has been tested and measurements were carried out in 50 non-pregnant and 50 pregnant women(18). The results were in line with the study by Parra-Saavedra *et al.*(19) and showed that as pregnancy progresses, the cervix softens and this process starts before shortening. Therefore, by measuring the CSI, delivery could be detected earlier compared to conventional shortening of the cervix measured with transvaginal ultrasound.

Also, a recent study by Stone *et al.(22)* investigated cervical softness before cerclage placement with the Pregnolia System. This study demonstrated patients with a ultra-sound indicated cerclage, had significantly softer cervices compared to normal controls. They also stated this aspiration technique is a promising technique for objective and quantitative measurement of cervical softness.

Since cervical softening is a precursor of cervical shortening, this could be a novel marker to predict spontaneous PTB and contribute to better identification of women with an increased risk of PTB. Also, the predictive value of cervical softening in combination with cervical length could be promising to improve prediction of PTB. However, these hypotheses still must be examined.

Therefore, the aim of this cohort study is to evaluate the predictive value of the CSI to predict the risk of spontaneous PTB in pregnant women with an increased risk of PTB.

Methods and analysis

Study design

This study is an investigator-initiated, single-centre prospective cohort study being undertaken at the Amsterdam University Medical Center in the Netherlands. Recruitment started on 18th August 2022. We expect a study duration of 3 years to investigate the primary objectives.

Two cohorts will be investigated:

- Pregnant women with a history of spontaneous PTB before 34 weeks of gestation (Cohort A-STIPP).
- Pregnant women presenting with symptoms of threatened PTB between 24 and 34 weeks of gestation (Cohort S-STIPP).

The measurements of cervical stiffness will be performed in addition to standard care (Appendix 1), using the aspiration technique-based device named the Pregnolia System.

Participants

In order to be eligible to participate in this study, pregnant women must meet all of the following criteria:

- Age 18 years or above.
- Ability to understand Dutch or English (both spoken and written).
- Ultrasound-based gestational age determined by measurement of crown rump length (CRL), determined between 9 and 11 weeks of gestation.
- Singleton and twin pregnancies.

A-STIPP cohort

Pregnant women with an increased risk of PTB based on a medical history of spontaneous PTB before 34 weeks of gestation will be included.

S-STIPP cohort

Pregnant women, with a gestational age between 24 and 34 weeks presenting with symptoms of threatened PTB, such as abdominal pain, vaginal blood loss, contractions or other complaints suggestive for threatened PTB, will be included.

A potential subject who meets any of the following criteria will be excluded from participation:

- Signs of intrauterine infection.
- Obstetric indication for immediate delivery (e.g. advanced labour, cord prolapse, abruption, signs of foetal distress).
- Confirmed foetal abnormality.
- Confirmed preterm rupture of membranes.
- Confirmed vasa / placenta praevia.
- Severe vaginal bleeding and light bleeding that cannot be stopped.

- Signs of imminent labour such as advanced dilatation making it impossible to measure the cervix.

Measurements

Cervical stiffness measurement

The Cervical stiffness index (CSI) will be measured subsequent to measurement of the cervical length. The Pregnolia System is composed of two components: an active, reusable device and a disposable single-use sterile probe.

- The control unit is an active device with a power supply, foot switch, connector cable and an integrated pump that generates vacuum.
- The single-use sterile probe is connected to the control unit console through a connector cable. Air filters on the probe prevent microbiological contamination of the control unit. This probe is designed to minimise the contact interaction between the user and the patients during the measurement. The probe tip diameter is 12mm. Each single-use, disposable probe is packed in a sterile pouch.

To perform the measurement, the cervix is visualized with a speculum. The disposable probe is placed on the anterior lip of the cervix (12 o'clock position). The control will create a weak vacuum inside the probe that pulls the cervical tissue, very gently and slowly, into the probe tip by a fixed distance of 4mm. The negative aspiration pressure needed to deform the tissue is the outcome of the measurement. A high-pressure value corresponds with stiff tissue and a low pressure corresponds with soft tissue. The CSI assessment is performed in three consecutive measurements at the same location, without any time lag and without removing the probe from the cervix. For an overview of the measurement procedure, please refer to the diagram available at the Pregnolia website (https://en.pregnolia.com/fachpersonen2-1).

Sonographic measurement

Cervical length measurement with transvaginal ultrasound is routine care in both cohorts. The cervical length will be determined as the linear distance between internal and external cervical os, excluding the endocervical funnel as described by Kagan *et al*. (24)

In the A-STIPP cohort, transvaginal ultrasound will be done biweekly from 14 until 24 weeks of gestation. In case a short cervix is detected at less than 25 mm, a cerclage, or a pessary in study context, is placed. Afterwards, the measurement of CSI will not be continued.

In the S-STIPP cohort, the transvaginal ultrasound will be performed when a woman presents with any symptom of threatened PTB, between 24 and 34 weeks of gestation.

Questionnaire

Participants will be asked to fill out a structured questionnaire to screen for additional risk factors of PTB. The questionnaire contains questions about the current pregnancy and details about previous pregnancies, if applicable. Moreover, details on cervical surgery in the past and family history of PTB are requested. Baseline characteristics such as height, weight and smoking and medical history, including gynaecological history and uterus malformations, will be collected. The questionnaire will be checked with the patient's electronic file.

For the S-STIPP cohort, participants will be asked about the specific symptoms associated with threatened PTB.

Blinding

For the A-STIPP cohort, clinicians and participants are blinded for the results of the CSI measurement.

In the S-STIPP cohort, the clinician working at the emergency department performs the measurement and therefore making it impossible to blind the treating clinician. The participant however is blinded for the results.

Follow-up

Participants will be followed-up from inclusion until delivery. Detailed information regarding the pregnancy outcomes, including maternal and neonatal outcomes will be gathered.

Also, if applicable, detailed information about hospital admittance during pregnancy will be noted. Moreover, if a participant is admitted due to threatened PTB, received treatments such as antenatal corticosteroids, tocolytic medicines, or magnesium sulfate for neonatal neuroprotection will be documented.

Primary outcomes

- The primary outcome for the A-STIPP cohort is spontaneous PTB before 34 weeks of gestation.
- The primary outcome for the S-STIPP cohort is delivery within 7 days.

Secondary outcomes

- Spontaneous PTB before 37 weeks of gestation
- Spontaneous PTB before 34 weeks of gestation#
- Spontaneous PTB before 32 weeks of gestation
- Spontaneous PTB before 28 weeks of gestation
- Latency time (time between inclusion and delivery)
- Delivery within 48 hours#
- Preterm Premature rupture of Membranes (PPROM)

S-STIPP only

Other outcomes

Safety of the use of the Pregnolia System (as defined in Appendix 2) will be investigated.

Also, patient discomfort of the measurement will be evaluated by a general questionnaire.

Power analysis

We used contemporary sample size calculations described by *Riley et al.(25)* for developing prediction models, based on three criteria that each provide a sample size to satisfy that criterion, then picking the highest sample size out of the three. The following input parameters are used to calculate the required number of inclusions; 1) expected prevalence of the primary outcome, 2) expected amount of explained variance by the prediction model and 3) number of predictors (input variables).

For the A-STIPP cohort the prevalence [0.18] was derived from the QUIPP-study (26-28). The standard level of variance [0.15] was used to calculate sample size.

For the S-STIPP cohort, the prevalence [0.12] and variance [0.45] were derived from the Apostel I study(12, 29). Both studies have comparable patients as the A-STIPP and S-STIPP study.

To investigate additional input predictors with sufficient power, an increase in sample size is needed. When inclusion of participants continues and the second threshold is reached, another input parameter is added, until the next threshold and so on. The baseline predictors

used in the first step will be the CSI measurement combined with cervical length measurement in the A-STIPP cohort, and cervical length with fFN in S-STIPP cohort.

See table 1 and table 2 for the steps and the threshold sample sizes. In both calculations the number of predictors was gradually increased. Continuous variables count as a single-input variable, as well as dichotomous input variables. Categorical variables are counted as C-1, thus the number of input variables is the number categories minus one. The additional predictor variables are summarized in table 3.

Sample size calculations were performed using R (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/) with the use of the *pmsampsize* package *(25).*

Table 1 and table 2 indicates the number of participants needed. For the A-STIPP, the minimum sample size of 227 patients is required to achieve the primary objective of this study. Once this number is achieved, the study will be continued to investigate secondary objectives. For the S-STIPP, the minimum sample size of 163 patients is required to achieve the primary objective of this study. Once this number is achieved, the study will be continued to investigate secondary objectives, by using the dynamical sample size as explained.

Statistical analysis

Baseline characteristics will be calculated using descriptive statistics. Continuous variables will be reported as mean with standard deviation or median with interquartile range. Categorical variables will be reported as proportions.

Out of the three repetitive CSI measurements conducted, depending on which proves to be the best predictor, the first, the average or the lowest measurement values will be utilized.

To incorporate repeated measures of CSI from the A-STIPP cohort, a logistic generalized mixed model will be used. As CSI is a continuous outcome, linear and non-linear functions will be compared using restricted cubic splines. A lower Akaike's Information Criterion (AIC) or overall p-value will determine which functional form is chosen.(25) If there is censoring (i.e. loss-to-follow-up), a Cox proportional hazards model for time to delivery including a time-varying covariate for CSI will be used. As a sensitivity analysis, a comparison of either of these models with a joint

survival model will be done (combining a Cox model for time to delivery with a linear mixed model for CSI measurements).

For the S-STIPP cohort, a logistic regression will be used to determine the relationship between input variables and a dichotomous outcome.

Subgroup analysis

Subgroup analysis are planned for participants and treatments that may potentially effect cervical stiffness, in order to assess their impact on the CSI:

- Nulliparous versus multiparous women
- Singleton versus multiple pregnancies
- Women with previous cervical surgery versus women without
- Women with a (abdominal or cervical) cerclage in current pregnancy versus no cerclage
- Women treated with progesterone versus no treatment

A-STIPP cohort subgroup analysis

Subgroup of interest in asymptomatic participants are:

- Women with a short cervix (≤25mm) during screening vs. women with a long cervix (>25mm)
- Women who received additional treatment (pessary or cerclage) vs. no treatment.

S-STIPP cohort subgroup analysis

Subgroup of interest in symptomatic participants are performed in groups based on clinical risk stratification:

- Cervical length ≥30mm
- Cervical length ≥15 and <30mm with negative fFN
- Cervical length ≥15 and <30mm with positive fFN
- Cervical length < 15 mm

Monitoring and safety

An independent Data and Safety Monitoring Board (DSMB) is assigned to safeguard the safety of the study participants and provide recommendations.

Since the measurement with the Pregnolia System is minimally invasive, the risk of adverse events (AEs) related to the measurement is small. However, any AEs and serious adverse events (SAEs) will be reported. If evaluation by the DSMB demonstrates increased safety risks within the study, the DSMB can always advice to stop the study.

Data management

Data will be collected using an accredited electronic data capture system (Castor). To protect the privacy of the participant, personal data is encrypted. Data cannot be traced back to participants in reports and publications about the study. All personal data is protected according to the General Data Protection Regulation (GDPR and Dutch privacy regulation (AVG)).

All agreements regarding data sharing are defined in a signed Clinical Trial Agreement (CTA), GDPR are applicable to this agreement.

Clinical impact

This STIPP study will provide evidence on the value of the cervical stiffness as a single clinical marker and in combination with other clinical markers such as cervical length to predict the risk of spontaneous PTB in groups of pregnant women with an increased risk of PTB.

Patient and public involvement

The patient organisation care4Neo was informed about the study and was favourable about purpose of the study.

Ethics and dissemination

The Medical Ethics Committee of the Amsterdam UMC has given approval for this research (METC2022.0226). All participants will give written and oral informed consent prior to entry to the study and will be made aware participation is completely voluntary. The results of the study will be submitted for publication in a peer-reviewed journal.

Contributors

FH initiated the research. FH and SB designed the study. SB wrote the proposal and the manuscript. FH, MO, RE, MB and EP critically revised the proposal and manuscript. All authors read and approved the final manuscript.

Funding

This work was supported and funded by the Pregnolia Institute. They also provided the system and the probes. The funders had no role in the study design, conduction of the study, collection, management, analysis and interpretation of the data. Also, the funder had no role in preparation, reviewing or approval of the manuscript. Funding was not provided by the study sponsor.

Competing interests

None declared.

References

1. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, Adler A, Vera Garcia C, Rohde S, Say L, Lawn JE. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012;379(9832):2162-72.
2. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, Rudan I, Campbell H, Cibulskis R, Li M, Mathers C, Black RE, Child Health Epidemiology Reference Group of WHO, Unicef. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet. 2012;379(9832):2151-61.
3. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, Cousens S, Mathers C, Black RE. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet. 2015;385(9966):430-40.
4. Marlow N, Wolke D, Bracewell MA, Samara M, Group EPS. Neurologic and developmental disability at six years of age after extremely preterm birth. N Engl J Med. 2005;352(1):9-19.
5. Mwaniki MK, Atieno M, Lawn JE, Newton CR. Long-term neurodevelopmental outcomes after intrauterine and neonatal insults: a systematic review. Lancet. 2012;379(9814):445-52.
6. van Zijl MD, Koullali B, Mol BW, Pajkrt E, Oudijk MA. Prevention of preterm delivery: current challenges and future prospects. Int J Womens Health. 2016;8:633-45.
7. van der Ven J, van Os MA, Kazemier BM, Kleinrouweler E, Verhoeven CJ, de Miranda E, van Wassenaer-Leemhuis AG, Kuiper PN, Porath M, Willekes C, Woiski MD, Sikkema MJ, Roumen FJ, Bossuyt PM, Haak MC, de Groot CJ, Mol BW, Pajkrt E. The capacity of mid-pregnancy cervical length to predict preterm birth in low-risk women: a national cohort study. Acta Obstet Gynecol Scand. 2015;94(11):1223-34.
8. Iams JD, Berghella V. Care for women with prior preterm birth. Am J Obstet Gynecol. 2010;203(2):89-100.

9. Conner SN, Frey HA, Cahill AG, Macones GA, Colditz GA, Tuuli MG. Loop electrosurgical excision procedure and risk of preterm birth: a systematic review and meta-analysis. Obstet Gynecol. 2014;123(4):752-61.
10. Kazemier BM, Buijs PE, Mignini L, Limpens J, de Groot CJ, Mol BW, Ebm C. Impact of obstetric history on the risk of spontaneous preterm birth in singleton and multiple pregnancies: a systematic review. BJOG. 2014;121(10):1197-208; discussion 209.
11. Hughes K, Kane SC, Araujo Junior E, Da Silva Costa F, Sheehan PM. Cervical length as a predictor for spontaneous preterm birth in high-risk singleton pregnancy: current knowledge. Ultrasound Obstet Gynecol. 2016;48(1):7-15.
12. Vis JY, van Baaren GJ, Wilms FF, Oudijk MA, Kwee A, Porath MM, Scheepers HC, Spaanderman ME, Bloemenkamp KW, van Lith JM, Bolte AC, Bax CJ, Cornette J, Duvekot JJ, Nij Bijvank SW, van Eyck J, Franssen MT, Sollie KM, Woiski M, Vandenbussche FP, van der Post JA, Bossuyt PM, Opmeer BC, Mol BW. Randomized comparison of nifedipine and placebo in fibronectin-negative women with symptoms of preterm labor and a short cervix (APOSTEL-I Trial). Am J Perinatol. 2015;32(5):451-60.
13. Wilms FF, Vis JY, de Wit-Zuurendonk L, Porath MM, Mol BW. What is the risk of preterm delivery after arrested preterm labor? Am J Perinatol. 2015;32(1):63-70.
14. Cornelissen LGH, van Oostrum NHM, van der Woude DAA, Rolf C, Porath MM, Oei SG, van Laar J. The diagnostic value of fetal fibronectin testing in clinical practice. J Obstet Gynaecol Res. 2020;46(3):405-12.
15. Berghella V, Saccone G. Fetal fibronectin testing for reducing the risk of preterm birth. Cochrane Database Syst Rev. 2019;7:CD006843.
16. Mazza E, Parra-Saavedra M, Bajka M, Gratacos E, Nicolaides K, Deprest J. In vivo assessment of the biomechanical properties of the uterine cervix in pregnancy. Prenat Diagn. 2014;34(1):33-41.
17. Badir S, Bajka M, Mazza E. A novel procedure for the mechanical characterization of the uterine cervix during pregnancy. J Mech Behav Biomed Mater. 2013;27:143-53.
18. Badir S, Mazza E, Zimmermann R, Bajka M. Cervical softening occurs early in pregnancy: characterization of cervical stiffness in 100 healthy women using the aspiration technique. Prenat Diagn. 2013;33(8):737-41.
19. Parra-Saavedra M, Gomez L, Barrero A, Parra G, Vergara F, Navarro E. Prediction of preterm birth using the cervical consistency index. Ultrasound Obstet Gynecol. 2011;38(1):44-51.
20. Shiina T, Nightingale KR, Palmeri ML, Hall TJ, Bamber JC, Barr RG, Castera L, Choi BI, Chou YH, Cosgrove D, Dietrich CF, Ding H, Amy D, Farrokh A, Ferraioli G, Filice C, Friedrich-Rust M, Nakashima K, Schafer F, Sporea I, Suzuki S, Wilson S, Kudo M. WFUMB guidelines and recommendations for clinical use of ultrasound elastography: Part 1: basic principles and terminology. Ultrasound Med Biol. 2015;41(5):1126-47.
21. Ge W, Brooker G, Mogra R, Hyett J. Measured Hyperelastic Properties of Cervical Tissue with Shear-Wave Elastography. Sensors (Basel). 2021;22(1).
22. Stone J, House M. Measurement of cervical softness before cerclage placement with an aspiration-based device. Am J Obstet Gynecol MFM. 2023;5(4):100881.
23. Issaoui M, Debost-Legrand A, Skerl K, Chauveau B, Magnin B, Delabaere A, Boyer L, Sauvant-Rochat MP, Lemery D. Shear wave elastography safety in fetus: A quantitative health risk assessment. Diagn Interv Imaging. 2018;99(9):519-24.
24. Kagan KO, Sonek J. How to measure cervical length. Ultrasound Obstet Gynecol. 2015;45(3):358-62.
25. Riley RD, Ensor J, Snell KIE, Harrell FE, Jr., Martin GP, Reitsma JB, Moons KGM, Collins G, van Smeden M. Calculating the sample size required for developing a clinical prediction model. BMJ. 2020;368:m441.
26. Watson HA, Carter J, Seed PT, Tribe RM, Shennan AH. The QUiPP App: a safe alternative to a treat-all strategy for threatened preterm labor. Ultrasound Obstet Gynecol. 2017;50(3):342-6.

27. Watson HA, Seed PT, Carter J, Hezelgrave NL, Kuhrt K, Tribe RM, Shennan AH. Development and validation of predictive models for QUiPP App v.2: tool for predicting preterm birth in asymptomatic high-risk women. Ultrasound Obstet Gynecol. 2020;55(3):348-56.
28. Goodfellow L, Care A, Sharp A, Ivandic J, Poljak B, Roberts D, Alfirevic Z. Effect of QUiPP prediction algorithm on treatment decisions in women with a previous preterm birth: a prospective cohort study. BJOG. 2019;126(13):1569-75.
29. van Baaren GJ, Vis JY, Wilms FF, Oudijk MA, Kwee A, Porath MM, Scheepers HCJ, Spaanderman MEA, Bloemenkamp KWM, Haak MC, Bax CJ, Cornette JMJ, Duvekot JJ, Nij Bijvanck BWA, van Eyck J, Franssen MTM, Sollie KM, Vandenbussche F, Woiski M, Bolte AC, van der Post JAM, Bossuyt PMM, Opmeer BC, Mol BWJ. Cost-effectiveness of diagnostic testing strategies including cervical-length measurement and fibronectin testing in women with symptoms of preterm labor. Ultrasound Obstet Gynecol. 2018;51(5):596-603.

Table 1. Sample size: A-STIPP cohort

Number of predictors (n)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Minimum required sample size (n)	227	240	360	480	600	720	840	960	1080	1200	1320	1440	1560	1680	1799

Table 2. Sample size S-STIPP cohort

Number of predictors (n)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Minimum required sample size (n)	163	163	163	163	188	225	263	300	338	375	413	450	488	525	563

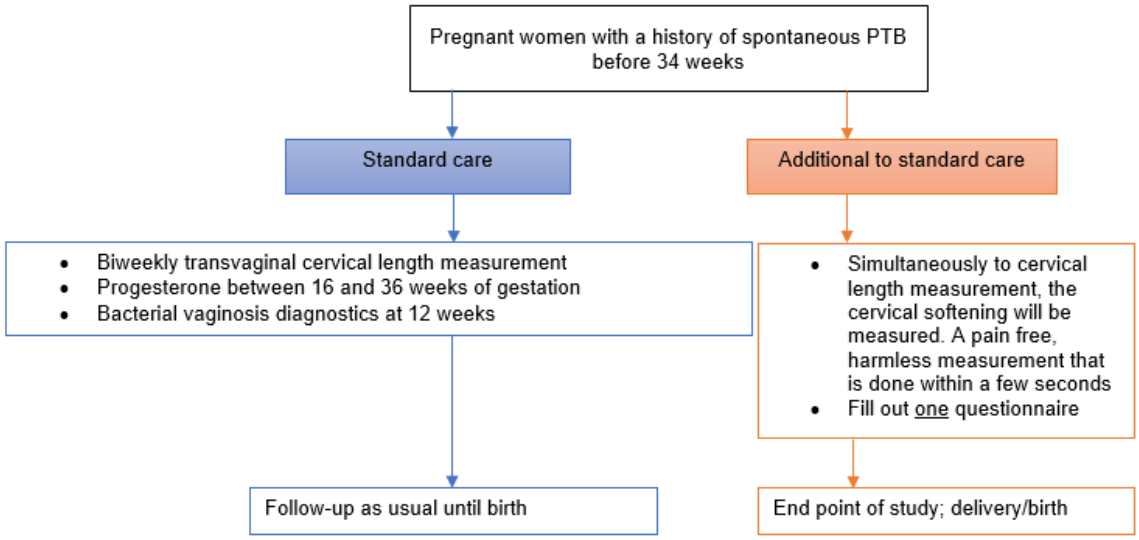
Table 3. Predictor variables

	Predictor variable
1	Cervical length
2	Foetal fibronectin[#]
3	Twin gestation
4	History of spontaneous preterm birth
5	Cervical surgery
6	Interpregnancy interval
7	Presence of infection
8	Family history
9	Social economic status
10	Smoking
11	BMI

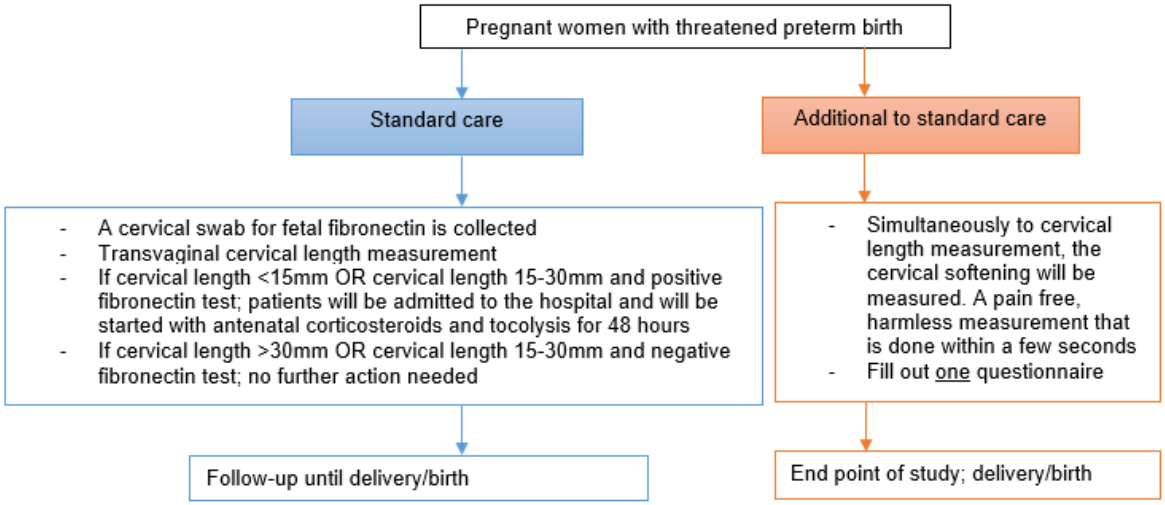
S-STIPP only.

Appendix 1: Timeline of study procedures

Timeline A-STIPP cohort



Timeline S-STIPP cohort



Appendix 2: safety parameters of the Pregnolia System

- Vaginal or cervical blood loss (directly after measurement)
- Patient discomfort or dissatisfcaction during Pregnolia® measurement(scaled 0 to 10)
- Preterm Prelabour Rupture Of Membranes (PPROM) (directly after measurement)
- Preterm labour (directly after measurement)
- Infections within seven days of measurement (Urinary Tract Infections, Vaginal infections, Intra-uterine infections)
- Irritation and sensitization of mucosal tissue
- Infection of the vaginal or mucosal tissue
- Tissue abrasion and vaginal discharge
- Spotting, light bleeding
- Superficial lacerations or minor tissue abrasions

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	Abstract/ protocol
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	14
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and	NA

		other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5

Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	NA
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	NA
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8/9

Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Appendix 1
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	NA
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	NA
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque,	NA

		sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	NA
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	7

Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	NA
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	NA
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and	12

		competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	12
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	12
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	NA

Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	11
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	12

Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	NA
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Appendix 3
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA