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

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Manuscripts

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3 **Assessment of cervical Softening and the Prediction of Preterm birth: study protocol of the**
4 **STIPP trial. A prospective cohort study.**
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32
33 **Abstract**
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35
36 **Introduction**

37 Preterm birth (PTB) is amongst the leading causes of perinatal and childhood morbidity and
38 mortality. Therefore, accurate identification of pregnant women at high risk of PTB is key to
39 enable obstetric healthcare professionals to apply interventions to improve perinatal and
40 childhood outcomes.
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43 Serial transvaginal cervical length measurement is used to screen asymptomatic pregnant
44 women with a history of PTB. In women presenting with symptoms of threatened PTB
45 cervical length, fetal fibronectin test or a combination of both can be used to identify
46 women at high risk of PTB. The predictive capacity of these can be improved.
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49 Cervical softening is precursor of cervical shortening, effacement, and dilatation and could be
50 a new marker to identify women a high risk of preterm birth. However, predictive value of
51 cervical softening to predict spontaneous PTB still need to be determined.
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57 **Methods/design**
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3 This is a single centre cohort study. Cervical stiffness will be investigated with an available,
4 non-invasive CE-marked device called the Pregnoia System®. This device has been developed
5 to evaluate consistency of the cervix based on tissue elasticity.
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8 Two different groups will be investigated.
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- 10 1. Women with a history of spontaneous PTB <34 weeks. These women undergo biweekly
11 measurement between 14 and 24 weeks of gestation.
12
- 13 2. Women with symptoms of threatened PTB. These women will be tested once on
14 presentation between 24 and 34 weeks of gestation
15

16 Primary outcome is spontaneous PTB before 34 weeks for women with a history of PTB and
17 delivery within seven days for women with threatened PTB cohort.
18

19 **Ethics and dissemination**

20 The study is approved by the Medical Ethics Committee of Amsterdam UMC
21 (METC2022.0226). Results will be disseminated through peer-reviewed journals. This protocol
22 is published before analysis of results is done.
23

24 **Trial registration**

25 Trial is registered at ClinicalTrials.gov, identifier NCT05477381, date of registration: 27th July
26 2022.
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29 **Strengths and limitations of the study**

- 30 - Since cervical softening is a precursor of cervical shortening, effacement and dilatation,
31 cervical softening and could be a promising new marker for preterm birth. This study will
32 be the first to evaluate the relation with cervical stiffness, measured with an aspiration
33 technique, and the risk of preterm birth.
34
- 35 - This study will provide evidence on the value of the cervical stiffness as a single clinical
36 marker and in combination with other clinical markers such as cervical length to
37 predict the risk of spontaneous preterm birth in groups of pregnant women with an
38 increased risk of preterm birth.
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- 40 - A limitation of the study is the single centre design potentially limiting external validity.
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55 **Background and rationale**

56 Spontaneous preterm birth (PTB), defined as delivery before 37 weeks of gestation, is the
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3 leading cause of perinatal and neonatal morbidity and mortality(1). Rates of spontaneous PTB
4 appears to be increasing. Annually, 15 million children are born preterm annually and is
5 directly responsible for the death of one million neonates(2, 3). Neonates who survive PTB are
6 at increased risk for long-term neurologic sequelae and developmental disabilities(4, 5).
7 Identifying pregnant women at risk is important to be able to take precautionary measures,
8 however this is a challenge for obstetric healthcare professionals.
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15 Important obstetric and gynaecological risk factors for PTB are, midtrimester short cervical
16 length, prior cervical surgery and previous spontaneous PTB. (6-9)

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19 Women with a history of spontaneous PTB before 34 weeks of gestation are at a 5-fold
20 increased risk of a spontaneous PTB in a subsequent pregnancy compared to women with a
21 previous term birth(10). In addition to vaginal progesterone administration, biweekly cervical
22 length screening is recommended in these women to identify women at high risk of a
23 recurrent PTB based on short cervical length that benefit from a vaginal cerclage. However, in
24 women with a previous PTB, the positive predictive value (PPV) of a short cervical length is
25 34%. (11) Therefore this approach only identifies a proportion of women who will have a
26 recurrent PTB. This calls for additional measurements to identify the group more adequately
27 at risk for recurrent PTB.
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36 Another group of pregnant women at risk of delivering PTB are women presenting with
37 symptoms of preterm labour in the current pregnancy. These women can be triaged with
38 transvaginal cervical length measurement and foetal fibronectin(ffn) to identify women with
39 an increased risk of delivery within seven days. Women with a high risk of PTB at less than 32
40 weeks of gestation are admitted to a centre with NICU facilities and treated with antenatal
41 corticosteroids and tocolysis for 48 hours to improve perinatal outcome.(12) This combination
42 of markers are characterized by a high negative predictive value (NPV) but a poor PPV. This
43 results in overtreatment and unnecessary healthcare costs in women with a positive ffn test.
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51 A large proportion of women with symptoms of threatened PTB will not deliver within seven
52 days due to the low PPV, however these women remain at risk for PTB later in pregnancy. (13-
53 15)
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57 More adequate techniques to assess women at high risk of recurrent PTB or at high risk of
58 delivering in a short time frame when presenting with symptoms of threatened preterm birth
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3 are urgently needed. Therefore, objective measurement of the cervical consistency is a
4 promising technique.
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7 To maintain pregnancy and deliver at term an appropriate function of the cervix is required.
8 Delivery is preceded by softening and shortening of the cervix. (16) Changes in cervical
9 consistency can be detected from fertilization until delivery. Throughout pregnancy
10 consistency of the cervix changes and will soften when approaching delivery(17, 18). Softening
11 of the cervix precedes shortening and therefore could be a promising marker to identify an
12 upcoming delivery at an earlier stage. Parra-Saavedra *et al.*(19) investigated this phenomenon
13 with transvaginal ultrasound. The cervical consistency was measured by measuring the
14 difference of the anteroposterior cervical diameter before (AP) and after (AP¹) application of
15 pressure on the cervix with the transvaginal probe. The cervical consistency was then
16 calculated with the following formula: $((AP^1/AP) * 100) = \text{Cervical consistency index}$. Cervical
17 consistency had an inverse linear relationship with gestational age. This means that cervical
18 consistency declines, thus becomes softer, during progression of pregnancy, and that this
19 phenomenon can be detected in pregnancy. Secondly, it demonstrated also that pregnant
20 women with a more progressive decline in cervical consistency are more likely to have a
21 spontaneous PTB compared to women with physiological decline in cervical consistency.
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25 Recently, a non-invasive technique has been developed to evaluate consistency of the cervix
26 based on tissue elasticity. The Pregnolia®-system is a market available CE-marked device
27 designed to measure cervical softening. This system provides quantitative measurements of
28 the cervical consistency. This procedure is performed by placing a sterile probe with a
29 diameter of 12 mm on the cervix. The probe is connected to a control unit which creates a
30 vacuum between the tip of the probe and the cervix. The probe measures the aspiration
31 pressure that is needed to displace the cervical tissue 4 mm inside the probe. The cervical
32 consistency is expressed as cervical stiffness index (CSI), a pressure value (mbar) on a
33 continuous scale from 0 (soft) to 100 (firm).
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37 A prototype has been tested and measurements were carried out in 50 non-pregnant and 50
38 pregnant women(18). The results were in line with the study by Parra-Saavedra *et al.*(19) and
39 showed that with progressing of pregnancy, the cervix softens and starts before shortening.
40 By measuring the CSI, delivery could be detected earlier compared to conventional shortening
41 of the cervix measured with transvaginal ultrasound.
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3 Since cervical softening is a precursor of cervical shortening, this could be a novel marker to
4 predict spontaneous PTB and contribute to better identification of women with an increased
5 risk of PTB. Also, the predictive value of cervical softening in combination with cervical length
6 could be promising to improve prediction of PTB. However, these hypotheses still must be
7 examined.
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13 Therefore, the aim of this cohort study is to evaluate the predictive value of the cervical
14 stiffness index to predict the risk of spontaneous PTB in pregnant women with an increased
15 risk of PTB.
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21 **Methods and analysis**

22 **Study design**

23 This study is an investigator initiated, single centre cohort study and will be performed at the
24 Amsterdam UMC in the Netherlands. Two cohorts will be investigated:
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- 28 - Pregnant women with a history of spontaneous PTB before 34 weeks of gestation
29 (Cohort A-STIPP).
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- 31 - Pregnant women presenting with symptoms of threatened PTB between 24 and 34
32 weeks (Cohort S-STIPP).
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37 The measurements of cervical stiffness will be performed in addition to standard care(Appendix
38 1), using the aspiration technique-based device named the Pregnolia system device.
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41 **Participants**

42 **A-STIPP cohort**

43 Pregnant women, singleton or multiple gestation, with an increased risk of PTB based on a
44 medical history of spontaneous PTB before 34 weeks of gestation will be included.
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48 **S-STIPP cohort**

49 Pregnant women, singleton or multiple gestation, with a gestational age between 24 and 34
50 weeks presenting with symptoms of threatened PTB, such as abdominal pain, vaginal blood
51 loss, contractions or other complaints suggestive for threatened PTB, will be included.
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57 A potential subject who meets any of the following criteria will be excluded from participation
58 in this study:
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- 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)

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- 3 - Cervical length (1)
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- 5 - Fetal Fibronectin# (1)
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- 7 - Twin gestation (1)
- 8
- 9 - History of spontaneous PTB (4)
- 10
- 11 - Cervical surgery (1)
- 12
- 13 - Inter-pregnancy interval (1)
- 14
- 15 - Presence of infection (1)
- 16
- 17 - Family history (1)
- 18
- 19 - Social economic status (3)
- 20
- 21 - Smoking (1)
- 22
- 23 - BMI (1)

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

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27 *Sonographic measurement*

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

29 The cervical length will be determined as the linear distance between internal and external
30 cervical os, excluding the endocervical funnel as described by Kagan et al. (2015) (20).

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

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

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42 *Questionnaire*

43 Women will be asked to fill out a structured questionnaire to screen for additional risk factors
44 of PTB. The questionnaire contains questions about the current pregnancy, previous
45 pregnancies, family history of PTB and cervical surgery. Baseline characteristics such as height
46 and weight and smoking as well as information about relevant medical history will be
47 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 Pregnotia 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

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

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6 For the A-STIPP cohort the prevalence [0.18] was derived from the QUIPP-study (23, 24). (25)

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8 The standard level of variance [0.15] was used to calculate sample size.

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10 For the S-STIPP cohort, the prevalence [0.12] and variance [0.45] were derived from the
11 Apostel I study(21, 22). Both studies have comparable patients as the A-STIPP and S-STIPP
12 study.
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15 To analyse CSI for the primary outcome the minimum number of inclusions are 227 and 163
16 for A-STIPP and S-STIPP cohort, respectively.
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19 To investigate additional input parameters with sufficient power, an increase in sample size is
20 needed. When inclusion of participants continues and the second threshold is reached,
21 another input parameter is added, until the next threshold and so on. The baseline predictors
22 used in the first step will be the CSI measurement combined with cervical length measurement
23 in the A-STIPP cohort, and cervical length with fFN in S-STIPP cohort.
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26 See table 1 and table 2 for the steps and the threshold sample sizes. In both calculations the
27 number of predictors was gradually increased. Continuous variables count as a single input
28 variable, as well as dichotomous input variables. Categorical variables are counted as C-1, thus
29 the number of input variables is the number categories minus one. The additional predictor
30 variables are summarized in table 3.
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40 Sample size calculations were performed using R (R: A language and environment for
41 statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
42 URL <https://www.R-project.org/>) with the use of the *pmsampsize* package (26). Table 1 and
43 table 2 indicates the number of participants needed.
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49 **Statistical Analysis**

50 Baseline characteristics will be calculated used descriptive statistics. Continuous variables will be
51 reported as mean with standard deviation or median with inter quartile range. Categorical
52 variables will be reported as proportions.
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56 To incorporate repeated measures of CSI from the A-STIPP cohort, a logistic generalized mixed
57 model will be used. As CSI is a continuous outcome, linear and non-linear functions will be
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3 compared using restricted cubic splines. A lower Akaike's Information Criterion (AIC) or overall p-
4 value will determine which functional form is chosen.(26) If there is censoring (i.e. loss-to-follow-
5 up), a Cox proportional hazards model for time to delivery including a time-varying covariate for
6 CSI will be used. As a sensitivity analysis, a comparison of either of these models with a joint
7 survival model will be done (combining a Cox model for time to delivery with a linear mixed model
8 for CSI measurements).

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11 For the S-STIPP cohort, a logistic regression will be used to determine the relationship between
12 input variables and a dichotomous outcome.
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15 16 17 18 19 20 21 **Monitoring and safety**

22 An independent Data and Safety Monitoring Board (DSMB) is assigned to safeguard the safety of
23 the trial participants and provide recommendations.
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26 Since the measurement with the Pregnonia-system is minimally invasive, the risk of adverse
27 events related to the measurement is small. However, any Adverse Events (AEs) and Serious
28 Adverse Events (SAEs) will be reported. If evaluation by the DSMB demonstrates increased
29 safety risks within the trial, the DSMB can always advice to stop the trial.
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34 35 36 **Data management**

37 Data will be collected using an accredited electronic data capture system (Castor). To protect the
38 privacy of the participant, personal data is encrypted. Data cannot be traced back participants in
39 reports and publications about the study. All personal data is protected according to the General
40 Data Protection Regulation (GDPR and Dutch privacy regulation (AVG)).
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45 All agreements regarding data sharing are defined in a signed Clinical Trial Agreement (CTA).
46 General Data Protection Regulation (GDPR) are applicable to this agreement.
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51 52 **Clinical impact**

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

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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.

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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

For peer review only

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

For peer review only

Table 3

Predictor variable

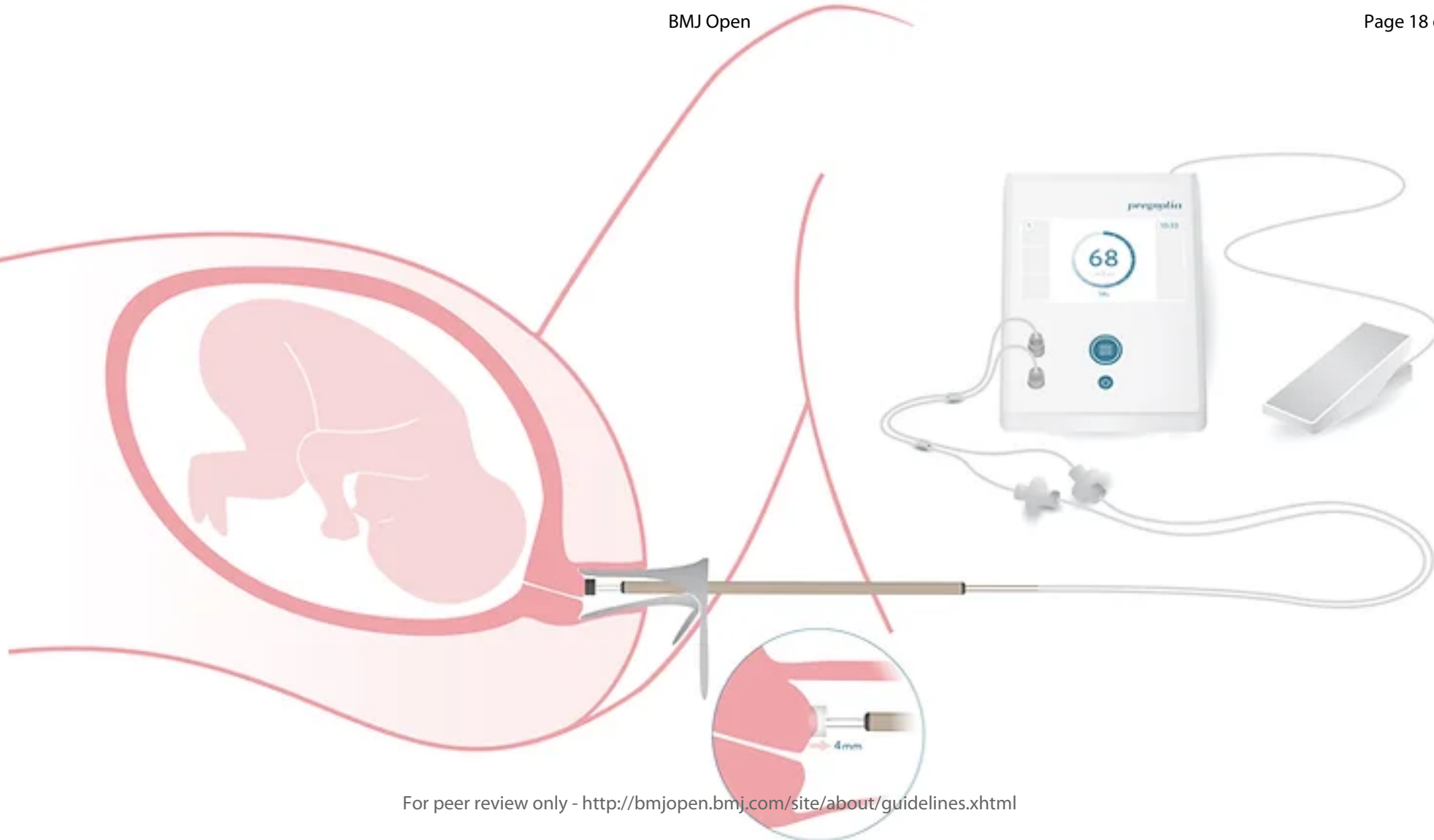
	Predictor variable
1	Cervical length
2	Fetal Fibronectin [#]
3	Twin gestation
4	History of spontaneous preterm birth
5	Cervical Surgery
6	Interpregnancy interval
7	Presence of infection
8	Family history
9	Social economic status
10	Smoking
11	BMI

S-STIPP only

Figure 1: Illustration of the cervical weakness measurement

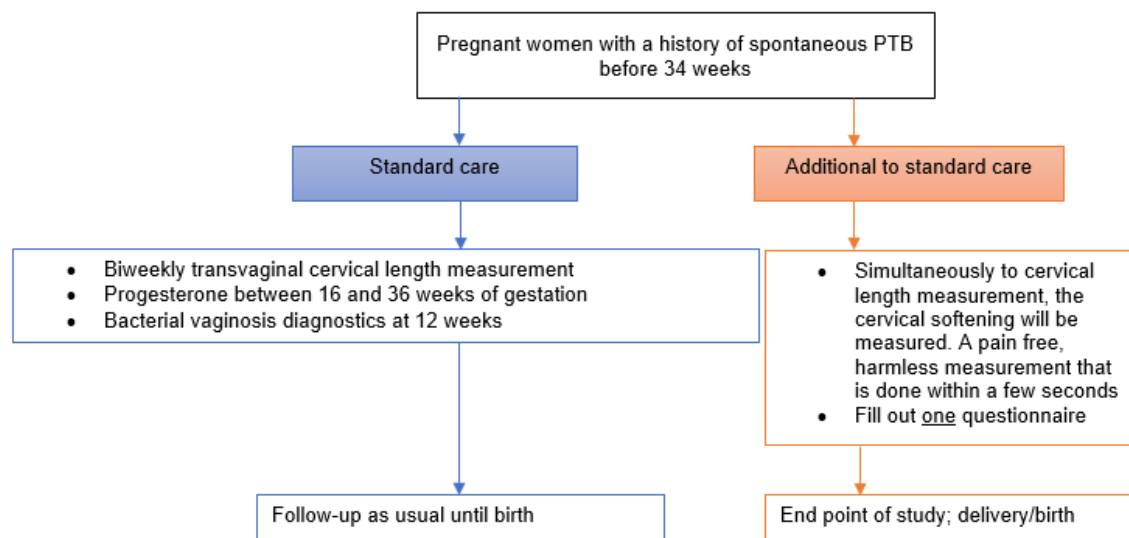
For peer review only

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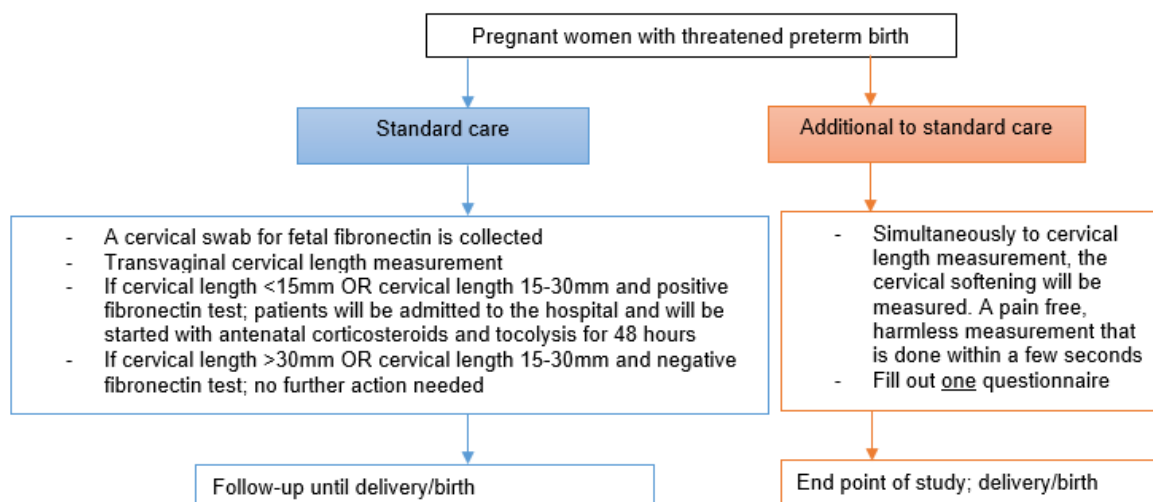


Appendix 1: Timeline of study procedures

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 dissatisfaction 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 SPIRIT reporting 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

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

1	Trial registration	#2a	Trial identifier and registry name. If not yet registered,	2
2			name of intended registry	
3				
4				
5				
6	Trial registration:	#2b	All items from the World Health Organization Trial	Abstract/
7	data set		Registration Data Set	protocol
8				
9				
10				
11	Protocol version	#3	Date and version identifier	2
12				
13				
14				
15	Funding	#4	Sources and types of financial, material, and other	13
16			support	
17				
18				
19				
20	Roles and	#5a	Names, affiliations, and roles of protocol contributors	1
21	responsibilities:			
22				
23	contributorship			
24				
25				
26				
27				
28	Roles and	#5b	Name and contact information for the trial sponsor	1
29	responsibilities:			
30				
31	sponsor contact			
32				
33	information			
34				
35				
36				
37				
38	Roles and	#5c	Role of study sponsor and funders, if any, in study	13
39	responsibilities:		design; collection, management, analysis, and	
40			interpretation of data; writing of the report; and the	
41	sponsor and funder		decision to submit the report for publication, including	
42			whether they will have ultimate authority over any of	
43			these activities	
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51				
52	Roles and	#5d	Composition, roles, and responsibilities of the	NA
53	responsibilities:		coordinating centre, steering committee, endpoint	
54			adjudication committee, data management team, and	
55	committees			
56				
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60				

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

1	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If	6
2				
3				
4			applicable, eligibility criteria for study centres and	
5				
6			individuals who will perform the interventions (eg,	
7				
8			surgeons, psychotherapists)	
9				
10				
11	Interventions:	#11a	Interventions for each group with sufficient detail to	6
12				
13	description		allow replication, including how and when they will be	
14				
15				
16			administered	
17				
18				
19	Interventions:	#11b	Criteria for discontinuing or modifying allocated	NA
20				
21	modifications		interventions for a given trial participant (eg, drug dose	
22				
23			change in response to harms, participant request, or	
24				
25			improving / worsening disease)	
26				
27				
28				
29	Interventions:	#11c	Strategies to improve adherence to intervention	NA
30				
31	adherence		protocols, and any procedures for monitoring adherence	
32				
33			(eg, drug tablet return; laboratory tests)	
34				
35				
36	Interventions:	#11d	Relevant concomitant care and interventions that are	7
37				
38	concomitant care		permitted or prohibited during the trial	
39				
40				
41				
42	Outcomes	#12	Primary, secondary, and other outcomes, including the	8
43				
44			specific measurement variable (eg, systolic blood	
45				
46			pressure), analysis metric (eg, change from baseline,	
47				
48			final value, time to event), method of aggregation (eg,	
49				
50			median, proportion), and time point for each outcome.	
51				
52				
53			Explanation of the clinical relevance of chosen efficacy	
54				
55			and harm outcomes is strongly recommended	
56				
57				
58				
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60				

1	Participant timeline	#13	Time schedule of enrolment, interventions (including any	Appendix
2			run-ins and washouts), assessments, and visits for	1
3			participants. A schematic diagram is highly	
4			recommended (see Figure)	
5				
6				
7				
8				
9				
10				
11	Sample size	#14	Estimated number of participants needed to achieve	9
12			study objectives and how it was determined, including	
13			clinical and statistical assumptions supporting any	
14			sample size calculations	
15				
16				
17				
18				
19				
20				
21	Recruitment	#15	Strategies for achieving adequate participant enrolment	NA
22			to reach target sample size	
23				
24				
25				
26	Methods:			
27				
28	Assignment of			
29	interventions (for			
30	controlled trials)			
31				
32				
33				
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35				
36	Allocation: sequence	#16a	Method of generating the allocation sequence (eg,	NA
37	generation		computer-generated random numbers), and list of any	
38			factors for stratification. To reduce predictability of a	
39			random sequence, details of any planned restriction (eg,	
40			blocking) should be provided in a separate document	
41			that is unavailable to those who enrol participants or	
42			assign interventions	
43				
44				
45				
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49				
50				
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52				
53	Allocation	#16b	Mechanism of implementing the allocation sequence	NA
54	concealment		(eg, central telephone; sequentially numbered, opaque,	
55	mechanism			
56				
57				
58				
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sealed envelopes), describing any steps to conceal the sequence until interventions are assigned

Allocation: [#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): [#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

1	Data collection plan:	#18b	Plans to promote participant retention and complete	NA
2				
3	retention		follow-up, including list of any outcome data to be	
4			collected for participants who discontinue or deviate	
5			from intervention protocols	
6				
7				
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9				
10				
11	Data management	#19	Plans for data entry, coding, security, and storage,	10
12			including any related processes to promote data quality	
13			(eg, double data entry; range checks for data values).	
14			Reference to where details of data management	
15			procedures can be found, if not in the protocol	
16				
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22				
23	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary	10
24			outcomes. Reference to where other details of the	
25			statistical analysis plan can be found, if not in the	
26			protocol	
27				
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33	Statistics: additional	#20b	Methods for any additional analyses (eg, subgroup and	10
34	analyses		adjusted analyses)	
35				
36				
37				
38				
39	Statistics: analysis	#20c	Definition of analysis population relating to protocol non-	NA
40	population and		adherence (eg, as randomised analysis), and any	
41	missing data		statistical methods to handle missing data (eg, multiple	
42			imputation)	
43				
44				
45				
46				
47				
48	Methods: Monitoring			
49				
50				
51	Data monitoring:	#21a	Composition of data monitoring committee (DMC);	10
52	formal committee		summary of its role and reporting structure; statement of	
53			whether it is independent from the sponsor and	
54				
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1 competing interests; and reference to where further
 2 details about its charter can be found, if not in the
 3 protocol. Alternatively, an explanation of why a DMC is
 4 not needed
 5
 6
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 9

10	Data monitoring:	#21b	Description of any interim analyses and stopping	NA
11	interim analysis		guidelines, including who will have access to these	
12			interim results and make the final decision to terminate	
13			the trial	
14				
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20	Harms	#22	Plans for collecting, assessing, reporting, and managing	10
21			solicited and spontaneously reported adverse events	
22			and other unintended effects of trial interventions or trial	
23			conduct	
24				
25				
26				
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30	Auditing	#23	Frequency and procedures for auditing trial conduct, if	NA
31			any, and whether the process will be independent from	
32			investigators and the sponsor	
33				
34				
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36				
37				
38	Ethics and			
39	dissemination			
40				
41				
42				
43	Research ethics	#24	Plans for seeking research ethics committee /	11
44	approval		institutional review board (REC / IRB) approval	
45				
46				
47				
48	Protocol	#25	Plans for communicating important protocol	NA
49	amendments		modifications (eg, changes to eligibility criteria,	
50			outcomes, analyses) to relevant parties (eg,	
51			investigators, REC / IRBs, trial participants, trial	
52			registries, journals, regulators)	
53				
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1	Consent or assent	#26a	Who will obtain informed consent or assent from	11
2				
3				
4			potential trial participants or authorised surrogates, and	
5				
6			how (see Item 32)	
7				
8				
9	Consent or assent:	#26b	Additional consent provisions for collection and use of	NA
10				
11	ancillary studies		participant data and biological specimens in ancillary	
12				
13			studies, if applicable	
14				
15				
16	Confidentiality	#27	How personal information about potential and enrolled	11
17				
18			participants will be collected, shared, and maintained in	
19				
20			order to protect confidentiality before, during, and after	
21				
22			the trial	
23				
24				
25				
26	Declaration of	#28	Financial and other competing interests for principal	13
27				
28	interests		investigators for the overall trial and each study site	
29				
30				
31				
32	Data access	#29	Statement of who will have access to the final trial	11
33				
34			dataset, and disclosure of contractual agreements that	
35				
36			limit such access for investigators	
37				
38				
39	Ancillary and post	#30	Provisions, if any, for ancillary and post-trial care, and	NA
40				
41	trial care		for compensation to those who suffer harm from trial	
42				
43			participation	
44				
45				
46				
47	Dissemination policy:	#31a	Plans for investigators and sponsor to communicate trial	11
48				
49	trial results		results to participants, healthcare professionals, the	
50				
51			public, and other relevant groups (eg, via publication,	
52				
53			reporting in results databases, or other data sharing	
54				
55			arrangements), including any publication restrictions	
56				
57				
58				
59				
60				

1 Dissemination policy: [#31b](#) Authorship eligibility guidelines and any intended use of NA
 2 authorship professional writers
 3
 4
 5

6 Dissemination policy: [#31c](#) Plans, if any, for granting public access to the full NA
 7 reproducible protocol, participant-level dataset, and statistical code
 8 research
 9
 10
 11
 12

13 Appendices

14
 15
 16
 17 Informed consent [#32](#) Model consent form and other related documentation Appendix
 18 materials given to participants and authorised surrogates 3
 19
 20
 21

22 Biological specimens [#33](#) Plans for collection, laboratory evaluation, and storage NA
 23 of biological specimens for genetic or molecular analysis
 24 in the current trial and for future use in ancillary studies,
 25 if applicable
 26
 27
 28
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 31

32
 33 None The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative
 34 Commons Attribution License CC-BY-NC. This checklist can be completed online using
 35 <https://www.goodreports.org/>, a tool made by the [EQUATOR Network](#) in collaboration with
 36 [Penelope.ai](#)
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BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2023-071597.R1
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Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Obstetrics and gynaecology
Keywords:	Maternal medicine < OBSTETRICS, OBSTETRICS, Prenatal diagnosis < OBSTETRICS

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Manuscripts

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2
3 1 **Assessment of cervical Softening and the Prediction of Preterm birth: study protocol of the**
4 **STIPP study. A prospective cohort study.**

5 2
6
7 3 Sofie. H. Breuking, MD^{1, 2, *} Martijn A. Oudijk, MD, PhD^{2,3}, R. van Eekelen⁴, M.A. de Boer, MD,
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24 15

25 16 **Abstract**

26 17 **Introduction**

27 18 Preterm birth (PTB) is amongst the leading causes of perinatal and childhood morbidity and
28
29 19 mortality. Therefore, accurate identification of pregnant women at high risk of PTB is key to
30
31 20 enable obstetric healthcare professionals to apply interventions to improve perinatal and
32
33 21 childhood outcomes. Serial transvaginal cervical length measurement is used to screen
34
35 22 asymptomatic pregnant women with a history of PTB. Cervical length measurement, fetal
36
37 23 fibronectin test or a combination of both can be used to identify women at high risk of PTB
38
39 24 in women presenting with symptoms of threatened PTB. The predictive capacity of these can
40
41 25 be improved.

42 26 Cervical softening is precursor of cervical shortening, effacement and dilatation and could be
43
44 27 a new marker to identify women a high risk of PTB. However, the predictive value of cervical
45
46 28 softening to predict spontaneous PTB still need to be determined.

47 29 **Methods and analysis**

1
2
3 30 This is a single center, prospective cohort study, conducted at Amsterdam University Medical
4
5 31 Center in the Netherlands. Cervical softening will be investigated with a non-invasive CE-
6
7 32 marked device called the Pregnolia System®. This device has been developed to evaluate
8
9 33 consistency of the cervix based on tissue elasticity. Two different cohorts will be investigated.
10
11 34 The first cohort includes women with a history of spontaneous PTB <34 weeks. These women
12
13 35 undergo biweekly measurement between 14 and 24 weeks of gestation. The second cohort
14
15 36 includes women with symptoms of threatened PTB. These women will receive the
16
17 37 measurement once on presentation between 24 and 34 weeks of gestation. Primary outcome
18
19 38 is spontaneous PTB before 34 weeks for women with a history of PTB and delivery within
20
21 39 seven days for women with threatened PTB. The minimum sample size to analyse the primary
22
23 40 outcome is 227 women with a history of PTB, and 163 women with symptoms of threatened
24
25 41 PTB. Once this number is achieved, the study will be continued to investigate secondary
26
27 42 objectives.

27 43 **Ethics and dissemination**

28
29 44 The study is approved by the Medical Ethics Committee of Amsterdam UMC
30
31 45 (METC2022.0226). All patients will give oral and written informed consent prior to entry of
32
33 46 the study. Results will be disseminated through peer-reviewed journals. This protocol is
34
35 47 published before analysis of results is done.

36 48 **Study registration**

37
38 49 Study is registered at ClinicalTrials.gov, identifier NCT05477381, date of registration: 27th July
39
40 50 2022.

43 52 **Strengths and limitations of the study**

- 44
45 53 - A strength of this study is that it is organized in a way with minimal interference in daily
46
47 54 practice and therefore a high participation rate is expected.
48
49 55 - A notable strength of this study is its prospective cohort design, which includes women
50
51 56 across a range of all cervical lengths, thereby establishing an internal control group of
52
53 57 women with longer cervix lengths within the cohorts.
54
55 58 - This is the first study investigating the cervical stiffness index in a population of high
56
57 59 risk women for preterm birth and combining the results with other important
58
59 60 predictors for preterm birth.

- 1
2
3 61 - The is a prospectively cohort study, therefore, we expect less bias than in a
4
5 62 retrospective cohort.
6
7 63 - A limitation of the study is the single centre design potentially limiting external validity
8
9 64 and generalizability.
10
11 65

12 13 66 **Introduction**

14
15 67 Spontaneous preterm birth (PTB), defined as delivery before 37 weeks of gestation, is the
16
17 68 leading cause of perinatal and neonatal morbidity and mortality(1). Rates of spontaneous PTB
18
19 69 appears to be increasing. Annually, 15 million children are born preterm and is directly
20
21 70 responsible for the death of one million neonates(2, 3). Neonates who survive PTB are at
22
23 71 increased risk for long-term neurologic sequelae and developmental disabilities(4, 5).
24
25 72 Identifying pregnant women at risk is important to be able to take precautionary measures,
26
27 73 however this is a challenge for obstetric healthcare professionals.
28

29 74 Important obstetric and gynaecological risk factors for PTB are midtrimester short cervical
30
31 75 length, prior cervical surgery and previous spontaneous PTB. (6-9) Women with a history of
32
33 76 spontaneous PTB before 34 weeks of gestation are at a 5-fold increased risk of a spontaneous
34
35 77 PTB in a subsequent pregnancy compared to women with a previous term birth(10). In
36
37 78 addition to vaginal progesterone administration, biweekly cervical length screening is
38
39 79 recommended in these women. This can identify women at high risk of a recurrent PTB based
40
41 80 on short cervical length that benefit from a vaginal cerclage. However, in women with a
42
43 81 previous PTB, the positive predictive value (PPV) of a short cervical length is 34%. (11)
44
45 82 Therefore this approach only identifies a proportion of women who will have a recurrent PTB.
46
47 83 This calls for additional measurements to identify the group more adequately at risk for
48
49 84 recurrent PTB.

50 85 Another group of pregnant women at risk of delivering PTB are women presenting with
51
52 86 symptoms of threatened PTB in their current pregnancy. These women can be triaged with
53
54 87 transvaginal cervical length measurement and fetal fibronectin(fFN) to identify women with
55
56 88 an increased risk of delivery within seven days. Women with a high risk of PTB at less than 32
57
58 89 weeks of gestation are admitted to a centre with NICU facilities and treated with antenatal
59
60 90 corticosteroids and tocolysis for 48 hours to improve perinatal outcome.(12) This combination

1
2
3 91 of markers are characterized by a high negative predictive value (NPV) but a poor PPV. This
4
5 92 results in overtreatment and unnecessary healthcare costs. A large proportion of women with
6
7 93 symptoms of threatened PTB will not deliver within seven days due to the low PPV, however
8
9 94 these women remain at risk for PTB later in pregnancy. (13-15)

10
11 95 More adequate techniques to assess women at high risk of recurrent PTB or at high risk of
12
13 96 delivering in a short time frame when presenting with symptoms of threatened PTB are
14
15 97 urgently needed. Therefore, objective measurement of the cervical consistency is a promising
16
17 98 technique.

18
19 99 To maintain pregnancy and deliver at term an appropriate function of the cervix is required.
20
21 100 Delivery is preceded by softening and shortening of the cervix.(16) Changes in cervical
22
23 101 consistency can be detected from fertilization until delivery. Throughout pregnancy
24
25 102 consistency of the cervix changes and will soften when approaching delivery.(17, 18) Softening
26
27 103 of the cervix precedes shortening and therefore could be a promising marker to identify an
28
29 104 upcoming delivery at an earlier stage.

30
31 105 Parra-Saavedra *et al.*(19) investigated this phenomenon with transvaginal ultrasound. The
32
33 106 cervical consistency was measured by measuring the difference of the anteroposterior cervical
34
35 107 diameter before (AP) and after (AP¹) application of pressure on the cervix with the transvaginal
36
37 108 probe. The cervical consistency was then calculated with the following formula: ((AP¹/AP) *
38
39 109 100) = Cervical consistency index. Cervical consistency had an inverse linear relationship with
40
41 110 gestational age. This means that cervical consistency declines, thus becomes softer, during
42
43 111 progression of pregnancy and that this phenomenon can be detected in pregnancy. Secondly,
44
45 112 it demonstrated also that pregnant women with a more progressive decline in cervical
46
47 113 consistency are more likely to have a spontaneous PTB compared to women with physiological
48
49 114 decline in cervical consistency.

50 115 Other techniques that show positive result in evaluating cervical softness is by using
51
52 116 elastography methods, including strain elastography (SE) and shear wave elastography
53
54 117 (SWE)(20). Nonetheless, there are technical considerations that first need to be resolved
55
56 118 before elastography can be applied extensively. For example, the results of SE are affected by
57
58 119 operator-applied pressure on the cervix, resulting in an inter-observer variability making it less
59
60 120 objective and standardized (21, 22). Moreover, for SWE, safety concerns, such as the unknown

1
2
3 121 risk of fetal tissues(23), first must be addressed before elastography methods can be applied
4
5 122 extensively.

6
7 123 Recently, a non-invasive technique has been developed to evaluate consistency of the cervix
8
9 124 based on tissue elasticity. The Pregnolia®-system is a market available CE-marked device
10
11 125 designed to measure cervical stiffness. This system provides quantitative measurements of
12
13 126 the cervical consistency based on aspiration technique.

14
15 127 A prototype has been tested and measurements were carried out in 50 non-pregnant and 50
16
17 128 pregnant women(18). The results were in line with the study by Parra-Saavedra *et al.*(19) and
18
19 129 showed that with progressing of pregnancy, the cervix softens and this process starts before
20
21 130 shortening. Therefore, by measuring the CSI, delivery could be detected earlier compared to
22
23 131 conventional shortening of the cervix measured with transvaginal ultrasound.

24
25 132 Also, a recent study by Stone *et al.*(22) investigated cervical softness before cerclage
26
27 133 placement with the Pregnolia®-system. This study demonstrated patients with a ultra-sound
28
29 134 indicated cerclage, had significantly softer cervices compared to normal controls. They also
30
31 135 stated this aspiration technique is a promising technique for objective and quantitative
32
33 136 measurement of cervical softness.

34
35 137 Since cervical softening is a precursor of cervical shortening, this could be a novel marker to
36
37 138 predict spontaneous PTB and contribute to better identification of women with an increased
38
39 139 risk of PTB. Also, the predictive value of cervical softening in combination with cervical length
40
41 140 could be promising to improve prediction of PTB. However, these hypotheses still must be
42
43 141 examined.

44
45 142 Therefore, the aim of this cohort study is to evaluate the predictive value of the CSI to predict
46
47 143 the risk of spontaneous PTB in pregnant women with an increased risk of PTB.

48
49 144

50 51 145 **Methods and analysis**

52 53 54 146 **Study design**

55
56 147 This study is an investigator initiated, single centre prospective cohort study that will be
57
58 148 performed at the Amsterdam UMC in the Netherlands. Recruitment started at 18th of august
59
60 149 2022. We expect a study duration of 3 years to investigate the primary objectives.

1
2
3 150 Two cohorts will be investigated:

- 4
5 151 - Pregnant women with a history of spontaneous PTB before 34 weeks of gestation
6
7 152 (Cohort A-STIPP).
8
9 153 - Pregnant women presenting with symptoms of threatened PTB between 24 and 34
10
11 154 weeks of gestation (Cohort S-STIPP).

12 155 The measurements of cervical stiffness will be performed in addition to standard care(Appendix
13
14 156 1), using the aspiration technique-based device named the Pregnolia system device.

157 **Participants**

18 158 In order to be eligible to participate in this study, pregnant women must meet all of the
19
20 159 following criteria:

- 21
22 160 - Age 18 years or above.
23
24 161 - Ability to understand Dutch or English (both spoken and written).
25
26 162 - Ultrasound-based gestational age determined by measurement of crown rump
27
28 163 length (CRL), determined between 9 and 11 weeks of gestation.
29
30 164 - Singleton and twin pregnancies.

31 A-STIPP cohort

32
33 166 Pregnant women with an increased risk of PTB based on a medical history of spontaneous PTB
34
35 167 before 34 weeks of gestation will be included.

36 S-STIPP cohort

37
38 169 Pregnant women, with a gestational age between 24 and 34 weeks presenting with symptoms
39
40 170 of threatened PTB, such as abdominal pain, vaginal blood loss, contractions or other
41
42 171 complaints suggestive for threatened PTB, will be included.
43

44 172

45
46 173 A potential subject who meets any of the following criteria will be excluded from participation:

- 47
48 174 - Signs of intrauterine infection.
49
50 175 - Obstetric indication for immediate delivery (advanced labour, cord prolapse,
51
52 176 abruption, signs of foetal distress).
53
54 177 - Confirmed foetal abnormality.
55
56 178 - Confirmed preterm rupture of membranes.
57
58 179 - Confirmed vasa / placenta praevia.
59
60 180 - Severe vaginal bleeding and light bleeding that cannot be stopped.

- 1
2
3 181 - Signs of imminent labour such as advanced dilatation making it impossible to
4
5 182 measure the cervix.
6
7 183

184 **Measurements**

185 *Cervical stiffness measurement*

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

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

196 To perform the measurement, the cervix is visualized with a speculum. The disposable probe
197 is placed on the anterior lip of the cervix (12 o'clock position). The control will create a weak
198 vacuum inside the probe that pulls the cervical tissue, very gently and slowly, into the probe
199 tip by a fixed distance of 4mm. The negative aspiration pressure needed to deform the tissue
200 is the outcome of the measurement. A high-pressure value corresponds with stiff tissue and a
201 low pressure corresponds with soft tissue. An overview of the measurement is displayed in
202 figure 1.
203

204 *Sonographic measurement*

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

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

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

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

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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.

1
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3 245 **Secondary outcomes**

- 4
5 246 - Spontaneous PTB before 37 weeks of gestation
6
7 247 - Spontaneous PTB before 34 weeks of gestation[#]
8
9 248 - Spontaneous PTB before 32 weeks of gestation
10
11 249 - Spontaneous PTB before 28 weeks of gestation
12
13 250 - Latency time (time between inclusion and delivery)
14
15 251 - Delivery within 48 hours[#]
16
17 252 - Preterm Premature rupture of Membranes (PPROM)

18 253 # S-STIPP only
19
20 254

21
22 255 **Other outcomes**

23
24
25 256 Safety of the use of the Pregnolia system (as defined in Appendix 2) will be investigated.

26
27 257 Also, patient discomfort of the measurement will be evaluated by a general questionnaire.
28
29

30 258

31 259 **Power analysis**

32
33 260 We used contemporary sample size calculations described by *Riley et al.*(25) for developing
34
35 261 prediction models, based on three criteria that each provide a sample size to satisfy that
36
37 262 criterion, then picking the highest sample size out of the three. The following input parameters
38
39 263 are used to calculate the required number of inclusions; 1) expected prevalence of the primary
40
41 264 outcome, 2) expected amount of explained variance by the prediction model and 3) number
42
43 265 of predictors (input variables).

44 266 For the A-STIPP cohort the prevalence [0.18] was derived from the QUIPP-study (26-28). The
45
46 267 standard level of variance [0.15] was used to calculate sample size.

47
48 268 For the S-STIPP cohort, the prevalence [0.12] and variance [0.45] were derived from the
49
50 269 Apostel I study(12, 29). Both studies have comparable patients as the A-STIPP and S-STIPP
51
52 270 study.

53 271 To investigate additional input predictors with sufficient power, an increase in sample size is
54
55 272 needed. When inclusion of participants continues and the second threshold is reached,
56
57 273 another input parameter is added, until the next threshold and so on. The baseline predictors
58
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3 274 used in the first step will be the CSI measurement combined with cervical length measurement
4
5 275 in the A-STIPP cohort, and cervical length with fFN in S-STIPP cohort.

6
7 276 See table 1 and table 2 for the steps and the threshold sample sizes. In both calculations the
8
9 277 number of predictors was gradually increased. Continuous variables count as a single input
10
11 278 variable, as well as dichotomous input variables. Categorical variables are counted as C-1, thus
12
13 279 the number of input variables is the number categories minus one. The additional predictor
14
15 280 variables are summarized in table 3.

16 281

17
18 282 Sample size calculations were performed using R (R: A language and environment for
19
20 283 statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
21
22 284 URL <https://www.R-project.org/>) with the use of the *pmsampsize* package (25).

23
24 285 Table 1 and table 2 indicates the number of participants needed. For the A-STIPP, the
25
26 286 minimum sample size of 227 patients is required to achieve the primary objective of this study.
27
28 287 Once this number is achieved, the study will be continued to investigate secondary objectives.
29
30 288 For the S-STIPP, the minimum sample size of 163 patients is required to achieve the primary
31
32 289 objective of this study. Once this number is achieved, the study will be continued to
33
34 290 investigate secondary objectives, by using the dynamical sample size as explained.

34 291

36 292 **Statistical Analysis**

37
38 293 Baseline characteristics will be calculated used descriptive statistics. Continuous variables will be
39
40 294 reported as mean with standard deviation or median with inter quartile range. Categorical
41
42 295 variables will be reported as proportions.

43
44 296 To incorporate repeated measures of CSI from the A-STIPP cohort, a logistic generalized mixed
45
46 297 model will be used. As CSI is a continuous outcome, linear and non-linear functions will be
47
48 298 compared using restricted cubic splines. A lower Akaike's Information Criterion (AIC) or overall p-
49
50 299 value will determine which functional form is chosen.(25) If there is censoring (i.e. loss-to-follow-
51
52 300 up), a Cox proportional hazards model for time to delivery including a time-varying covariate for
53
54 301 CSI will be used. As a sensitivity analysis, a comparison of either of these models with a joint
55
56 302 survival model will be done (combining a Cox model for time to delivery with a linear mixed model
57
58 303 for CSI measurements).

1
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3 304 For the S-STIPP cohort, a logistic regression will be used to determine the relationship between
4
5 305 input variables and a dichotomous outcome.
6
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8 306

9
10 307 **Subgroup analysis**

11
12 308 Subgroup analysis are planned for participants and treatments that may potentially effect cervical
13
14 309 stiffness, in order to assess their impact on the CSI:

- 15
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17 310 - Nulliparous versus multiparous women
18
19 311 - Singleton versus multiple pregnancies
20
21 312 - Women with previous cervical surgery versus women without
22
23 313 - Women with a (abdominal or cervical) cerclage in current pregnancy versus no cerclage
24
25 314 - Women treated with progesterone versus no treatment

26
27 315 *A-STIPP cohort subgroup analysis*

28
29 316 Subgroup of interest in asymptomatic participants are:

- 30
31 317 - Women with a short cervix ($\leq 25\text{mm}$) during screening vs. women with a long cervix
32
33 318 ($> 25\text{mm}$)
34
35
36 319 - Women who received additional treatment (pessary or cerclage) vs. no treatment.
37

38
39 320 *S-STIPP cohort subgroup analysis*

40
41 321 Subgroup of interest in symptomatic participants are performed in groups based on clinical risk
42
43 322 stratification:

- 44
45 323 - Cervical length $\geq 30\text{mm}$
46
47 324 - Cervical length ≥ 15 and $< 30\text{mm}$ with negative fFN
48
49 325 - Cervical length ≥ 15 and $< 30\text{mm}$ with positive fFN
50
51
52 326 - Cervical length $< 15\text{ mm}$
53
54

55 327

56
57 328 **Monitoring and safety**
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3 329 An independent Data and Safety Monitoring Board (DSMB) is assigned to safeguard the safety of
4
5 330 the study participants and provide recommendations.
6

7 331 Since the measurement with the PregnoIia-system is minimally invasive, the risk of adverse
8
9 332 events(AEs) related to the measurement is small. However, any AEs and Serious Adverse
10
11 333 Events (SAEs) will be reported. If evaluation by the DSMB demonstrates increased safety risks
12
13 334 within the study, the DSMB can always advice to stop the study.
14

15 335

16 336 **Data management**

17
18 337 Data will be collected using an accredited electronic data capture system (Castor). To protect the
19
20 338 privacy of the participant, personal data is encrypted. Data cannot be traced back to participants
21
22 339 in reports and publications about the study. All personal data is protected according to the General
23
24 340 Data Protection Regulation (GDPR and Dutch privacy regulation (AVG)).
25

26 341 All agreements regarding data sharing are defined in a signed Clinical Trial Agreement (CTA), GDPR
27
28 342 are applicable to this agreement.
29

30 343

31 344 **Clinical impact**

32
33 345 This STIPP study will provide evidence on the value of the cervical stiffness as a single clinical
34
35 346 marker and in combination with other clinical markers such as cervical length to predict the
36
37 347 risk of spontaneous PTB in groups of pregnant women with an increased risk of PTB.
38
39 348

40 349 **Patient and public involvement**

41
42 350 The patient organisation care4Neo was informed about the study and was favourable about
43
44 351 purpose of the study.
45

46 352

47 353 **Ethics and dissemination**

48
49 354 The Medical Ethics Committee of the Amsterdam UMC has given approval for this research
50
51 355 (Number 2022.0226). All participants will give written and oral informed consent prior to entry
52
53 356 to the study and will be made aware participation is completely voluntary. The outcomes of
54
55 357 the study will be published in a peer-reviewed journal.
56
57 358

58 359

59 360 **References**

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446

447 **Authors contributions**

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

451

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1
2
3 455 collection, management, analysis and interpretation of the data. Also, the funder had no role
4
5 456 in preparation, reviewing or approval of the manuscript. Funding was not provided by the
6
7 457 study sponsor.

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9 458

10 459 **Competing interests**

11
12 460 None declared

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16 462 **Word count**

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18 463 2905 words

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For peer review only

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

For peer review only

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

For peer review only

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

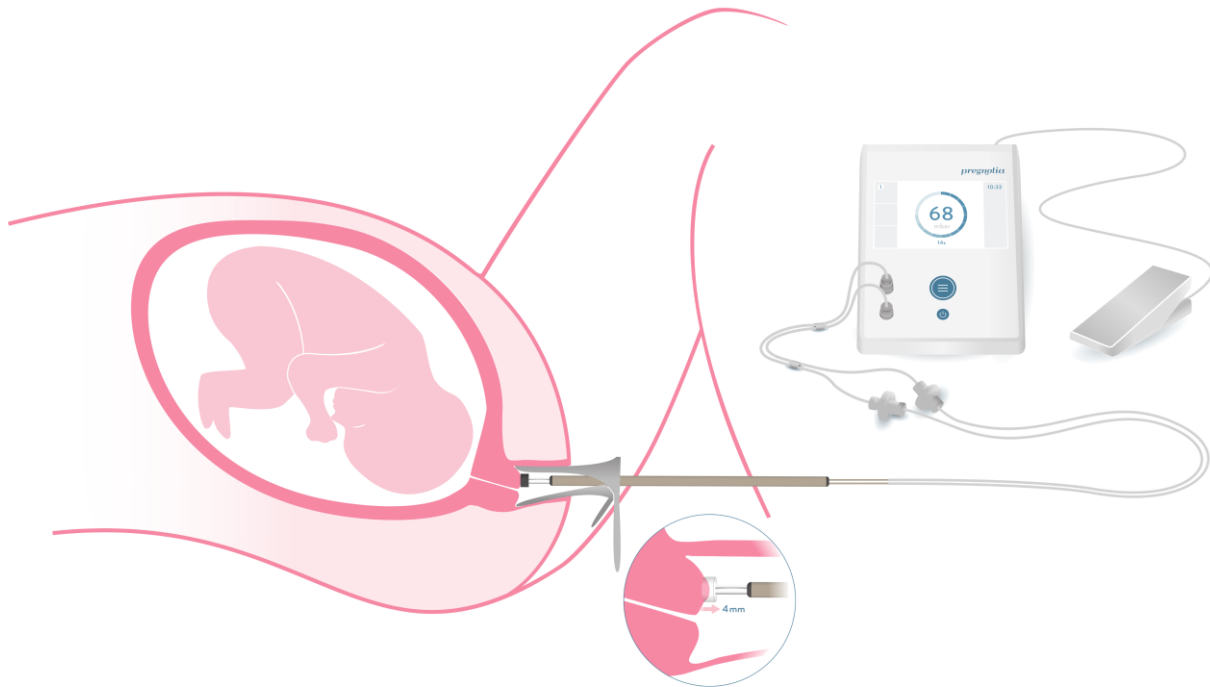
S-STIPP only

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Figure 1: Illustration of the cervical softness measurement

For peer review only

1
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3 **Figure 1:** Illustration of an overview of the measurement
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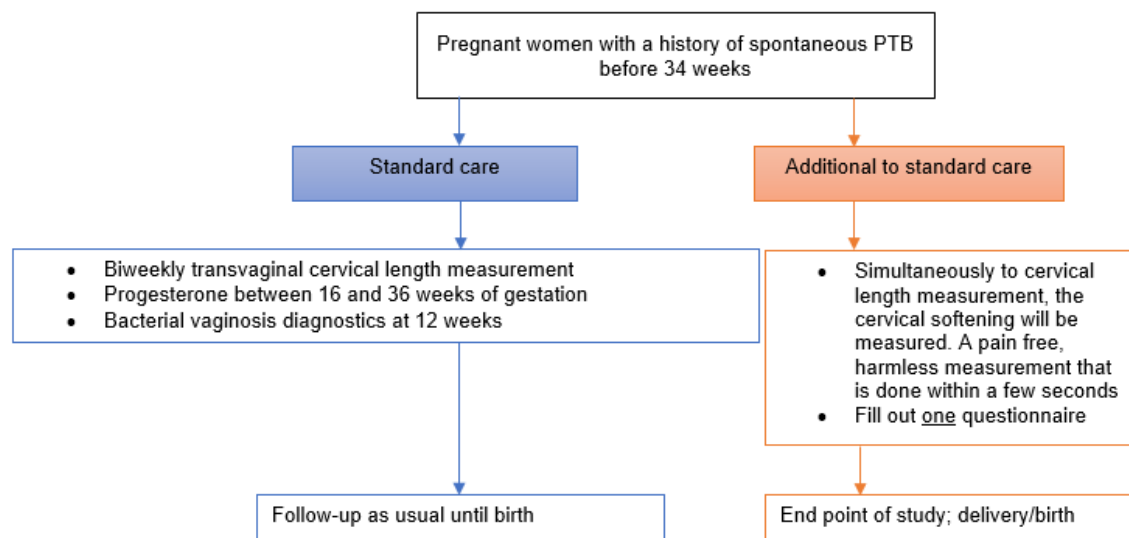


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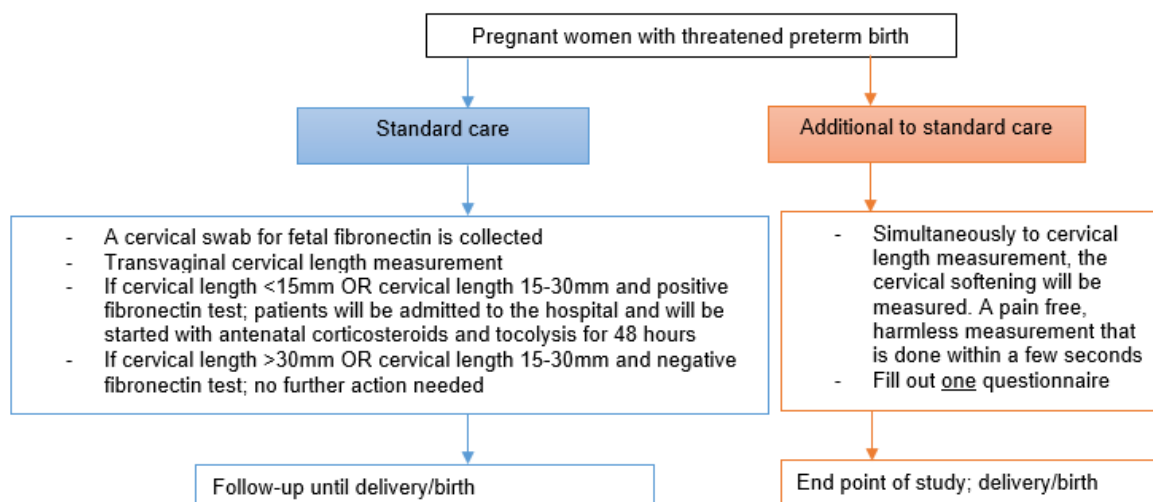
review only

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 dissatisfaction 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 SPIRIT reporting 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

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

1	Trial registration	#2a	Trial identifier and registry name. If not yet registered,	2
2			name of intended registry	
3				
4				
5				
6	Trial registration:	#2b	All items from the World Health Organization Trial	Abstract/
7	data set		Registration Data Set	protocol
8				
9				
10				
11	Protocol version	#3	Date and version identifier	2
12				
13				
14				
15	Funding	#4	Sources and types of financial, material, and other	14
16			support	
17				
18				
19				
20	Roles and	#5a	Names, affiliations, and roles of protocol contributors	1
21	responsibilities:			
22				
23	contributorship			
24				
25				
26				
27				
28	Roles and	#5b	Name and contact information for the trial sponsor	1
29	responsibilities:			
30				
31	sponsor contact			
32				
33	information			
34				
35				
36				
37				
38	Roles and	#5c	Role of study sponsor and funders, if any, in study	14
39	responsibilities:		design; collection, management, analysis, and	
40			interpretation of data; writing of the report; and the	
41	sponsor and funder		decision to submit the report for publication, including	
42			whether they will have ultimate authority over any of	
43			these activities	
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52	Roles and	#5d	Composition, roles, and responsibilities of the	NA
53	responsibilities:		coordinating centre, steering committee, endpoint	
54			adjudication committee, data management team, and	
55	committees			
56				
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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

1	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If	6
2				
3			applicable, eligibility criteria for study centres and	
4			individuals who will perform the interventions (eg,	
5			surgeons, psychotherapists)	
6				
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10				
11	Interventions:	#11a	Interventions for each group with sufficient detail to	7
12				
13	description		allow replication, including how and when they will be	
14			administered	
15				
16				
17				
18				
19	Interventions:	#11b	Criteria for discontinuing or modifying allocated	NA
20				
21	modifications		interventions for a given trial participant (eg, drug dose	
22			change in response to harms, participant request, or	
23			improving / worsening disease)	
24				
25				
26				
27				
28				
29	Interventions:	#11c	Strategies to improve adherence to intervention	NA
30				
31	adherence		protocols, and any procedures for monitoring adherence	
32			(eg, drug tablet return; laboratory tests)	
33				
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35				
36	Interventions:	#11d	Relevant concomitant care and interventions that are	7
37				
38	concomitant care		permitted or prohibited during the trial	
39				
40				
41				
42	Outcomes	#12	Primary, secondary, and other outcomes, including the	8/9
43			specific measurement variable (eg, systolic blood	
44			pressure), analysis metric (eg, change from baseline,	
45			final value, time to event), method of aggregation (eg,	
46			median, proportion), and time point for each outcome.	
47				
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52				
53			Explanation of the clinical relevance of chosen efficacy	
54			and harm outcomes is strongly recommended	
55				
56				
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1	Participant timeline	#13	Time schedule of enrolment, interventions (including any	Appendix 1
2			run-ins and washouts), assessments, and visits for	
3				
4			participants. A schematic diagram is highly	
5				
6			recommended (see Figure)	
7				
8				
9				
10	Sample size	#14	Estimated number of participants needed to achieve	9
11			study objectives and how it was determined, including	
12				
13			clinical and statistical assumptions supporting any	
14			sample size calculations	
15	Recruitment	#15	Strategies for achieving adequate participant enrolment	NA
16				
17			to reach target sample size	
18	Methods:			
19	Assignment of			
20	interventions (for			
21	controlled trials)			
22	Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg,	NA
23			computer-generated random numbers), and list of any	
24			factors for stratification. To reduce predictability of a	
25			random sequence, details of any planned restriction (eg,	
26			blocking) should be provided in a separate document	
27			that is unavailable to those who enrol participants or	
28			assign interventions	
29				
30				
31	Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence	NA
32			(eg, central telephone; sequentially numbered, opaque,	
33				

sealed envelopes), describing any steps to conceal the sequence until interventions are assigned

Allocation: [#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): [#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

1	Data collection plan:	#18b	Plans to promote participant retention and complete	NA
2				
3	retention		follow-up, including list of any outcome data to be	
4			collected for participants who discontinue or deviate	
5			from intervention protocols	
6				
7				
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11	Data management	#19	Plans for data entry, coding, security, and storage,	12
12			including any related processes to promote data quality	
13			(eg, double data entry; range checks for data values).	
14			Reference to where details of data management	
15			procedures can be found, if not in the protocol	
16				
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23	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary	10
24			outcomes. Reference to where other details of the	
25			statistical analysis plan can be found, if not in the	
26			protocol	
27				
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33	Statistics: additional	#20b	Methods for any additional analyses (eg, subgroup and	10
34	analyses		adjusted analyses)	
35				
36				
37				
38				
39	Statistics: analysis	#20c	Definition of analysis population relating to protocol non-	NA
40	population and		adherence (eg, as randomised analysis), and any	
41	missing data		statistical methods to handle missing data (eg, multiple	
42			imputation)	
43				
44				
45				
46				
47				
48	Methods: Monitoring			
49				
50				
51	Data monitoring:	#21a	Composition of data monitoring committee (DMC);	12
52	formal committee		summary of its role and reporting structure; statement of	
53			whether it is independent from the sponsor and	
54				
55				
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1 competing interests; and reference to where further
 2 details about its charter can be found, if not in the
 3 protocol. Alternatively, an explanation of why a DMC is
 4 not needed
 5
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10 11 12 13 14 15 16 17 18 19	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
20 21 22 23 24 25 26 27 28 29	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
30 31 32 33 34 35 36 37	Auditing	#23 Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
38 39 40 41 42	Ethics and dissemination		
43 44 45 46 47	Research ethics approval	#24 Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	12
48 49 50 51 52 53 54 55 56 57 58 59 60	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

1	Consent or assent	#26a	Who will obtain informed consent or assent from	11
2				
3				
4			potential trial participants or authorised surrogates, and	
5				
6			how (see Item 32)	
7				
8				
9	Consent or assent:	#26b	Additional consent provisions for collection and use of	NA
10				
11	ancillary studies		participant data and biological specimens in ancillary	
12				
13			studies, if applicable	
14				
15				
16	Confidentiality	#27	How personal information about potential and enrolled	12
17				
18			participants will be collected, shared, and maintained in	
19				
20			order to protect confidentiality before, during, and after	
21				
22			the trial	
23				
24				
25				
26	Declaration of	#28	Financial and other competing interests for principal	14
27				
28	interests		investigators for the overall trial and each study site	
29				
30				
31				
32	Data access	#29	Statement of who will have access to the final trial	12
33				
34			dataset, and disclosure of contractual agreements that	
35				
36			limit such access for investigators	
37				
38				
39	Ancillary and post	#30	Provisions, if any, for ancillary and post-trial care, and	NA
40				
41	trial care		for compensation to those who suffer harm from trial	
42				
43			participation	
44				
45				
46				
47	Dissemination policy:	#31a	Plans for investigators and sponsor to communicate trial	12
48				
49	trial results		results to participants, healthcare professionals, the	
50				
51			public, and other relevant groups (eg, via publication,	
52				
53			reporting in results databases, or other data sharing	
54				
55			arrangements), including any publication restrictions	
56				
57				
58				
59				
60				

1 Dissemination policy: [#31b](#) Authorship eligibility guidelines and any intended use of NA
 2 authorship professional writers
 3
 4
 5

6 Dissemination policy: [#31c](#) Plans, if any, for granting public access to the full NA
 7 reproducible protocol, participant-level dataset, and statistical code
 8 research
 9
 10
 11
 12

13 Appendices

14
 15
 16
 17 Informed consent [#32](#) Model consent form and other related documentation Appendix
 18 materials given to participants and authorised surrogates 3
 19
 20
 21

22 Biological specimens [#33](#) Plans for collection, laboratory evaluation, and storage NA
 23 of biological specimens for genetic or molecular analysis
 24 in the current trial and for future use in ancillary studies,
 25 if applicable
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32
 33 None The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative
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BMJ Open

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

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Keywords:	Maternal medicine < OBSTETRICS, OBSTETRICS, Prenatal diagnosis < OBSTETRICS

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Manuscripts

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3 1 **Assessment of cervical Softening and the Prediction of Preterm birth (STIPP): protocol for**
4 **a prospective cohort study**
5 2

6
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31
32 16
33 17 **Abstract**

34
35 18 **Introduction**

36
37 19 Preterm birth (PTB) is amongst the leading causes of perinatal and childhood morbidity and
38
39 20 mortality. Therefore, accurate identification of pregnant women at high risk of PTB is key to
40
41 21 enable obstetric healthcare professionals to apply interventions that improve perinatal and
42
43 22 childhood outcomes. Serial transvaginal cervical length measurement is used to screen
44
45 23 asymptomatic pregnant women with a history of PTB and identify those at high risk for a
46
47 24 recurrent PTB. Cervical length measurement, foetal fibronectin test or a combination of both
48
49 25 can be used to identify women at high risk of PTB in women presenting with symptoms of
50
51 26 threatened PTB. The predictive capacity of these methods can be improved. Cervical
52
53 27 softening is precursor of cervical shortening, effacement and dilatation and could be a new
54
55 28 marker to identify women a high risk of PTB. However, the predictive value of cervical
56
57 29 softening to predict spontaneous PTB still needs to be determined.

58
59 30 **Methods and analysis**
60

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3 31 This is a single-centre, prospective cohort study, being conducted at Amsterdam University
4 32 Medical Center in the Netherlands. Cervical softening will be investigated with a non-invasive
5 33 CE-marked device called the Pregnolia System. This device has been developed to evaluate
6 34 consistency of the cervix based on tissue elasticity. Two different cohorts will be investigated.
7 35 The first cohort includes women with a history of spontaneous PTB <34 weeks. These women
8 36 undergo biweekly measurement between 14 and 24 weeks of gestation. The second cohort
9 37 includes women with symptoms of threatened PTB. These women will receive the
10 38 measurement once on presentation between 24 and 34 weeks of gestation. The primary
11 39 outcome is spontaneous PTB before 34 weeks for women with a history of PTB and delivery
12 40 within seven days for women with threatened PTB. The minimum sample size required to
13 41 analyse the primary outcome is 227 women with a history of PTB and 163 women with
14 42 symptoms of threatened PTB. Once this number is achieved, the study will be continued to
15 43 investigate secondary objectives.

26 44 **Ethics and dissemination**

27 45 The study is approved by the Medical Ethics Committee of Amsterdam UMC
28 46 (METC2022.0226). All patients will give oral and written informed consent prior to entry of
29 47 the study. Results will be disseminated via a peer-reviewed journal.

30 48 **Study registration**

31 49 ClinicalTrials.gov, NCT05477381 (date of registration: 27th July 2022).
32 50

33 51 **Strengths and limitations of this study**

- 34 52 - A strength of this study is that it is organized in a way with minimal interference in daily
35 53 practice and therefore a high participation rate is expected.
- 36 54 - Another notable strength of the study is its prospective cohort design, which includes
37 55 women across a range of all cervical lengths, thereby establishing an internal control
38 56 group of women with longer cervix lengths within the cohorts.
- 39 57 - This study investigates the cervical stiffness index in a population of high-risk women
40 58 for preterm birth and combines the results with other important predictors for
41 59 preterm birth.
- 42 60 - As a prospectively cohort study, we expect less bias than in a retrospective cohort.

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3 61 - A limitation of the study is the single-centre setting, potentially limiting external
4 validity and generalizability.
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8
9 64 **Introduction**

10
11 65 Spontaneous preterm birth (PTB), defined as delivery before 37 weeks of gestation, is the
12 leading cause of perinatal and neonatal morbidity and mortality(1). Rates of spontaneous PTB
13 appears to be increasing. Annually, 15 million children are born preterm and this is directly
14 responsible for the death of one million neonates(2, 3). Neonates who survive PTB are at
15 increased risk for long-term neurologic sequelae and developmental disabilities(4, 5).
16 Identifying pregnant women at risk is important to be able to take precautionary measures,
17 however this is a challenge for obstetric healthcare professionals.
18

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25 72 Important obstetric and gynaecological risk factors for PTB are midtrimester short cervical
26 length, prior cervical surgery and previous spontaneous PTB. (6-9) Women with a history of
27 spontaneous PTB before 34 weeks of gestation are at a 5-fold increased risk of a spontaneous
28 PTB in a subsequent pregnancy compared to women with a previous term birth(10). In
29 addition to vaginal progesterone administration, biweekly cervical length screening is
30 recommended in these women. This can identify women at high risk of a recurrent PTB based
31 on short cervical length that benefit from a vaginal cerclage. However, in women with a
32 previous PTB, the positive predictive value (PPV) of a short cervical length is 34%. (11)
33 Therefore, this approach only identifies a proportion of women who will have a recurrent PTB.
34 This calls for additional measurements to identify the group more adequately at risk for
35 recurrent PTB.
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48 83 Another group of pregnant women at risk of PTB are women presenting with symptoms of
49 threatened PTB in their current pregnancy. These women can be triaged with transvaginal
50 cervical length measurement and foetal fibronectin (fFN) to identify women with an increased
51 risk of delivery within seven days. Women with a high risk of PTB at less than 32 weeks of
52 gestation are admitted to a centre with NICU facilities and treated with antenatal
53 corticosteroids and tocolysis for 48 hours to improve perinatal outcome.(12) This combination
54 of markers are characterized by a high negative predictive value (NPV) but a poor PPV. This
55 results in overtreatment and unnecessary healthcare costs. A large proportion of women with
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3 91 symptoms of threatened PTB will not deliver within seven days due to the low PPV, however
4
5 92 these women remain at risk for PTB later in pregnancy. (13-15)
6

7 93 More adequate techniques to assess women at high risk of recurrent PTB or at high risk of
8
9 94 delivering in a short time frame when presenting with symptoms of threatened PTB are
10
11 95 urgently needed. Therefore, objective measurement of the cervical consistency is a promising
12
13 96 technique.
14

15 97 To maintain pregnancy and deliver at term an appropriate function of the cervix is required.
16
17 98 Delivery is preceded by softening and shortening of the cervix.(16) Changes in cervical
18
19 99 consistency can be detected from fertilization until delivery. Throughout pregnancy
20
21 100 consistency of the cervix changes and will soften when approaching delivery.(17, 18) Softening
22
23 101 of the cervix precedes shortening and therefore could be a promising marker to identify an
24
25 102 upcoming delivery at an earlier stage.
26

27 103 Parra-Saavedra *et al.*(19) investigated this phenomenon with transvaginal ultrasound. The
28
29 104 cervical consistency was measured by measuring the difference of the anteroposterior cervical
30
31 105 diameter before (AP) and after (AP¹) application of pressure on the cervix with the transvaginal
32
33 106 probe. The cervical consistency was then calculated with the following formula: ((AP¹/AP) *
34
35 107 100) = Cervical consistency index. Cervical consistency had an inverse linear relationship with
36
37 108 gestational age. This means that cervical consistency declines, thus becomes softer, during
38
39 109 progression of pregnancy and this phenomenon can be detected during pregnancy. Secondly,
40
41 110 it demonstrated that pregnant women with a more progressive decline in cervical consistency
42
43 111 are more likely to have a spontaneous PTB compared to women with physiological decline in
44
45 112 cervical consistency.
46

47 113 Other techniques that show positive result in evaluating cervical softness is by using
48
49 114 elastography methods, including strain elastography (SE) and shear wave elastography
50
51 115 (SWE)(20). Nonetheless, there are technical considerations that first need to be resolved
52
53 116 before elastography can be applied extensively. For example, the results of SE are affected by
54
55 117 operator-applied pressure on the cervix, resulting in an inter-observer variability making the
56
57 118 technique less objective and standardized (21, 22). Moreover, for SWE, safety concerns, such
58
59 119 as the unknown risk of foetal tissues(23), first must be addressed before elastography
60
120 methods can be applied extensively.

1
2
3 121 Recently, a non-invasive technique has been developed to evaluate consistency of the cervix
4
5 122 based on tissue elasticity. The Pregnolia System is a market-available, CE-marked device
6
7 123 designed to measure cervical stiffness. This system provides quantitative measurements of
8
9 124 the cervical consistency based on aspiration technique.

10
11 125 A prototype has been tested and measurements were carried out in 50 non-pregnant and 50
12
13 126 pregnant women(18). The results were in line with the study by Parra-Saavedra *et al.*(19) and
14
15 127 showed that as pregnancy progresses, the cervix softens and this process starts before
16
17 128 shortening. Therefore, by measuring the CSI, delivery could be detected earlier compared to
18
19 129 conventional shortening of the cervix measured with transvaginal ultrasound.

20
21 130 Also, a recent study by Stone *et al.*(22) investigated cervical softness before cerclage
22
23 131 placement with the Pregnolia System. This study demonstrated patients with a ultra-sound
24
25 132 indicated cerclage, had significantly softer cervices compared to normal controls. They also
26
27 133 stated this aspiration technique is a promising technique for objective and quantitative
28
29 134 measurement of cervical softness.

30
31 135 Since cervical softening is a precursor of cervical shortening, this could be a novel marker to
32
33 136 predict spontaneous PTB and contribute to better identification of women with an increased
34
35 137 risk of PTB. Also, the predictive value of cervical softening in combination with cervical length
36
37 138 could be promising to improve prediction of PTB. However, these hypotheses still must be
38
39 139 examined.

40
41 140 Therefore, the aim of this cohort study is to evaluate the predictive value of the CSI to predict
42
43 141 the risk of spontaneous PTB in pregnant women with an increased risk of PTB.

44
45 142

46 47 143 **Methods and analysis**

48 49 144 **Study design**

50
51 145 This study is an investigator-initiated, single-centre prospective cohort study being
52
53 146 undertaken at the Amsterdam University Medical Center in the Netherlands. Recruitment
54
55 147 started on 18th August 2022. We expect a study duration of 3 years to investigate the primary
56
57 148 objectives.

58
59 149 Two cohorts will be investigated:
60

1
2
3 150 - Pregnant women with a history of spontaneous PTB before 34 weeks of gestation
4 151 (Cohort A-STIPP).

5
6
7 152 - Pregnant women presenting with symptoms of threatened PTB between 24 and 34
8 153 weeks of gestation (Cohort S-STIPP).

9
10 154 The measurements of cervical stiffness will be performed in addition to standard care (Appendix
11 155 1), using the aspiration technique-based device named the Pregnolia System.

156 **Participants**

16 157 In order to be eligible to participate in this study, pregnant women must meet all of the
17 158 following criteria:

- 18
19
20 159 - Age 18 years or above.
21
22 160 - Ability to understand Dutch or English (both spoken and written).
23
24 161 - Ultrasound-based gestational age determined by measurement of crown rump
25 162 length (CRL), determined between 9 and 11 weeks of gestation.
26
27 163 - Singleton and twin pregnancies.

28 164 A-STIPP cohort

29 165 Pregnant women with an increased risk of PTB based on a medical history of spontaneous PTB
30 166 before 34 weeks of gestation will be included.

31 167 S-STIPP cohort

32 168 Pregnant women, with a gestational age between 24 and 34 weeks presenting with symptoms
33 169 of threatened PTB, such as abdominal pain, vaginal blood loss, contractions or other
34 170 complaints suggestive for threatened PTB, will be included.

35 171
36 172 A potential subject who meets any of the following criteria will be excluded from participation:

- 37 173 - Signs of intrauterine infection.
38 174 - Obstetric indication for immediate delivery (e.g. advanced labour, cord prolapse,
39 175 abruption, signs of foetal distress).
40 176 - Confirmed foetal abnormality.
41 177 - Confirmed preterm rupture of membranes.
42 178 - Confirmed vasa / placenta praevia.
43 179 - Severe vaginal bleeding and light bleeding that cannot be stopped.

- 1
2
3 180 - Signs of imminent labour such as advanced dilatation making it impossible to
4
5 181 measure the cervix.
6
7 182

183 **Measurements**

184 *Cervical stiffness measurement*

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

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

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

205 *Sonographic measurement*

206 Cervical length measurement with transvaginal ultrasound is routine care in both cohorts.
207 The cervical length will be determined as the linear distance between internal and external
208 cervical os, excluding the endocervical funnel as described by Kagan *et al.* (24)
209 In the A-STIPP cohort, transvaginal ultrasound will be done biweekly from 14 until 24 weeks
210 of gestation. In case a short cervix is detected at less than 25 mm, a cerclage, or a pessary in
211 study context, is placed. Afterwards, the measurement of CSI will not be continued.

1
2
3 212 In the S-STIPP cohort, the transvaginal ultrasound will be performed when a woman presents
4
5 213 with any symptom of threatened PTB, between 24 and 34 weeks of gestation.
6

7 214

8 215 *Questionnaire*

9
10 216 Participants will be asked to fill out a structured questionnaire to screen for additional risk
11
12 217 factors of PTB. The questionnaire contains questions about the current pregnancy and details
13
14 218 about previous pregnancies, if applicable. Moreover, details on cervical surgery in the past
15
16 219 and family history of PTB are requested. Baseline characteristics such as height, weight and
17
18 220 smoking and medical history, including gynaecological history and uterus malformations, will
19
20 221 be collected. The questionnaire will be checked with the patient's electronic file.

21 222 For the S-STIPP cohort, participants will be asked about the specific symptoms associated with
22
23 223 threatened PTB.
24

25 224

26 225 **Blinding**

27
28 226 For the A-STIPP cohort, clinicians and participants are blinded for the results of the CSI
29
30 227 measurement.

31
32 228 In the S-STIPP cohort, the clinician working at the emergency department performs the
33
34 229 measurement and therefore making it impossible to blind the treating clinician. The
35
36 230 participant however is blinded for the results.
37

38 231

39 232 **Follow-up**

40
41 233 Participants will be followed-up from inclusion until delivery. Detailed information regarding
42
43 234 the pregnancy outcomes, including maternal and neonatal outcomes will be gathered.

44
45 235 Also, if applicable, detailed information about hospital admittance during pregnancy will be
46
47 236 noted. Moreover, if a participant is admitted due to threatened PTB, received treatments such
48
49 237 as antenatal corticosteroids, tocolytic medicines, or magnesium sulfate for neonatal
50
51 238 neuroprotection will be documented.
52

53 239

54 240 **Primary outcomes**

55
56 241 - The primary outcome for the A-STIPP cohort is spontaneous PTB before 34 weeks of
57
58 242 gestation.

59
60 243 - The primary outcome for the S-STIPP cohort is delivery within 7 days.

1
2
3 244 **Secondary outcomes**

- 4
5 245 - Spontaneous PTB before 37 weeks of gestation
6
7 246 - Spontaneous PTB before 34 weeks of gestation[#]
8
9 247 - Spontaneous PTB before 32 weeks of gestation
10
11 248 - Spontaneous PTB before 28 weeks of gestation
12
13 249 - Latency time (time between inclusion and delivery)
14
15 250 - Delivery within 48 hours[#]
16
17 251 - Preterm Premature rupture of Membranes (PPROM)

18 252 # S-STIPP only
19

20 253
21

22 254 **Other outcomes**

23
24
25 255 Safety of the use of the Pregnolia System (as defined in Appendix 2) will be investigated.

26
27 256 Also, patient discomfort of the measurement will be evaluated by a general questionnaire.
28
29

30 257

31 258 **Power analysis**

32
33 259 We used contemporary sample size calculations described by *Riley et al.*(25) for developing
34
35 260 prediction models, based on three criteria that each provide a sample size to satisfy that
36
37 261 criterion, then picking the highest sample size out of the three. The following input parameters
38
39 262 are used to calculate the required number of inclusions; 1) expected prevalence of the primary
40
41 263 outcome, 2) expected amount of explained variance by the prediction model and 3) number
42
43 264 of predictors (input variables).

44 265 For the A-STIPP cohort the prevalence [0.18] was derived from the QUIPP-study (26-28). The
45
46 266 standard level of variance [0.15] was used to calculate sample size.

47
48 267 For the S-STIPP cohort, the prevalence [0.12] and variance [0.45] were derived from the
49
50 268 Apostel I study(12, 29). Both studies have comparable patients as the A-STIPP and S-STIPP
51
52 269 study.

53 270 To investigate additional input predictors with sufficient power, an increase in sample size is
54
55 271 needed. When inclusion of participants continues and the second threshold is reached,
56
57 272 another input parameter is added, until the next threshold and so on. The baseline predictors
58
59
60

1
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3 273 used in the first step will be the CSI measurement combined with cervical length measurement
4
5 274 in the A-STIPP cohort, and cervical length with fFN in S-STIPP cohort.

6
7 275 See table 1 and table 2 for the steps and the threshold sample sizes. In both calculations the
8
9 276 number of predictors was gradually increased. Continuous variables count as a single-input
10
11 277 variable, as well as dichotomous input variables. Categorical variables are counted as C-1, thus
12
13 278 the number of input variables is the number categories minus one. The additional predictor
14
15 279 variables are summarized in table 3.

16 280

17
18 281 Sample size calculations were performed using R (R: A language and environment for
19
20 282 statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
21
22 283 URL <https://www.R-project.org/>) with the use of the *pmsampsize* package (25).

23
24 284 Table 1 and table 2 indicates the number of participants needed. For the A-STIPP, the
25
26 285 minimum sample size of 227 patients is required to achieve the primary objective of this study.
27
28 286 Once this number is achieved, the study will be continued to investigate secondary objectives.
29
30 287 For the S-STIPP, the minimum sample size of 163 patients is required to achieve the primary
31
32 288 objective of this study. Once this number is achieved, the study will be continued to
33
34 289 investigate secondary objectives, by using the dynamical sample size as explained.

35 290

36 291 **Statistical analysis**

37
38 292 Baseline characteristics will be calculated using descriptive statistics. Continuous variables will be
39
40 293 reported as mean with standard deviation or median with interquartile range. Categorical
41
42 294 variables will be reported as proportions.

43
44 295 Out of the three repetitive CSI measurements conducted, depending on which proves to be the
45
46 296 best predictor, the first, the average or the lowest measurement values will be utilized.

47
48 297 To incorporate repeated measures of CSI from the A-STIPP cohort, a logistic generalized mixed
49
50 298 model will be used. As CSI is a continuous outcome, linear and non-linear functions will be
51
52 299 compared using restricted cubic splines. A lower Akaike's Information Criterion (AIC) or overall p-
53
54 300 value will determine which functional form is chosen.(25) If there is censoring (i.e. loss-to-follow-
55
56 301 up), a Cox proportional hazards model for time to delivery including a time-varying covariate for
57
58 302 CSI will be used. As a sensitivity analysis, a comparison of either of these models with a joint

1
2
3 303 survival model will be done (combining a Cox model for time to delivery with a linear mixed model
4
5 304 for CSI measurements).

6
7 305 For the S-STIPP cohort, a logistic regression will be used to determine the relationship between
8
9 306 input variables and a dichotomous outcome.

10
11
12 307

13
14 308 **Subgroup analysis**

15
16
17 309 Subgroup analysis are planned for participants and treatments that may potentially effect cervical
18
19 310 stiffness, in order to assess their impact on the CSI:

- 20
21 311 - Nulliparous versus multiparous women
22
23 312 - Singleton versus multiple pregnancies
24
25 313 - Women with previous cervical surgery versus women without
26
27 314 - Women with a (abdominal or cervical) cerclage in current pregnancy versus no cerclage
28
29 315 - Women treated with progesterone versus no treatment

30
31 316 *A-STIPP cohort subgroup analysis*

32
33 317 Subgroup of interest in asymptomatic participants are:

- 34
35
36 318 - Women with a short cervix (≤ 25 mm) during screening vs. women with a long cervix
37
38 319 (> 25 mm)
39
40 320 - Women who received additional treatment (pessary or cerclage) vs. no treatment.

41
42
43 321 *S-STIPP cohort subgroup analysis*

44
45 322 Subgroup of interest in symptomatic participants are performed in groups based on clinical risk
46
47 323 stratification:

- 48
49 324 - Cervical length ≥ 30 mm
50
51 325 - Cervical length ≥ 15 and < 30 mm with negative fFN
52
53 326 - Cervical length ≥ 15 and < 30 mm with positive fFN
54
55 327 - Cervical length < 15 mm

56
57 328

58
59 329 **Monitoring and safety**

1
2
3 330 An independent Data and Safety Monitoring Board (DSMB) is assigned to safeguard the safety of
4
5 331 the study participants and provide recommendations.
6

7 332 Since the measurement with the Pregnotia System is minimally invasive, the risk of adverse
8
9 333 events (AEs) related to the measurement is small. However, any AEs and serious adverse
10
11 334 events (SAEs) will be reported. If evaluation by the DSMB demonstrates increased safety risks
12
13 335 within the study, the DSMB can always advice to stop the study.
14

15 336

16 337 **Data management**

17
18 338 Data will be collected using an accredited electronic data capture system (Castor). To protect the
19
20 339 privacy of the participant, personal data is encrypted. Data cannot be traced back to participants
21
22 340 in reports and publications about the study. All personal data is protected according to the General
23
24 341 Data Protection Regulation (GDPR and Dutch privacy regulation (AVG)).
25

26 342 All agreements regarding data sharing are defined in a signed Clinical Trial Agreement (CTA), GDPR
27
28 343 are applicable to this agreement.
29

30 344

31 345 **Clinical impact**

32
33 346 This STIPP study will provide evidence on the value of the cervical stiffness as a single clinical
34
35 347 marker and in combination with other clinical markers such as cervical length to predict the
36
37 348 risk of spontaneous PTB in groups of pregnant women with an increased risk of PTB.
38
39 349

40 350 **Patient and public involvement**

41
42 351 The patient organisation care4Neo was informed about the study and was favourable about
43
44 352 purpose of the study.
45
46 353

47 354 **Ethics and dissemination**

48
49 355 The Medical Ethics Committee of the Amsterdam UMC has given approval for this research
50
51 356 (METC2022.0226). All participants will give written and oral informed consent prior to entry
52
53 357 to the study and will be made aware participation is completely voluntary. The results of the
54
55 358 study will be submitted for publication in a peer-reviewed journal.
56
57 359

58 359

59 360

361 **Contributors**

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

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373 **Competing interests**

374 None declared.

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For peer review only

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

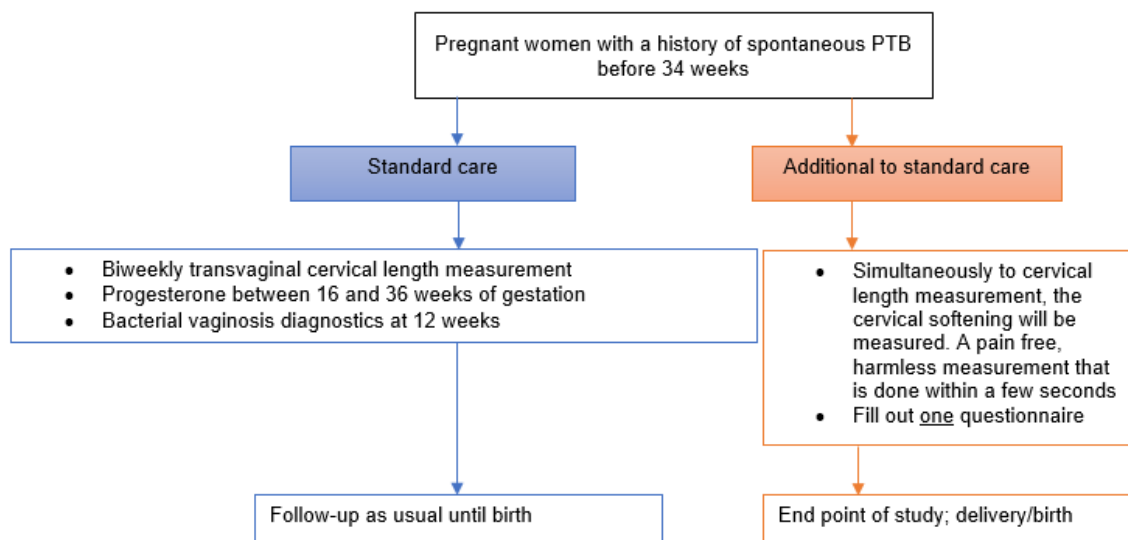
Table 3. Predictor variables

	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

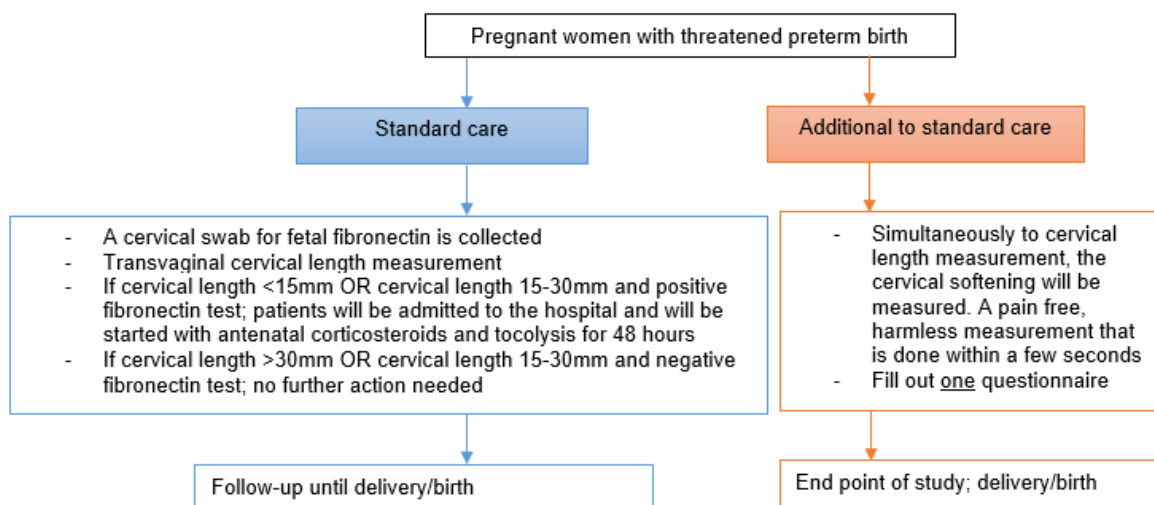
S-STIPP only.

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 dissatisfaction 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.

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

In your methods section, say that you used the SPIRIT reporting 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

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

1	Trial registration	#2a	Trial identifier and registry name. If not yet registered,	2
2			name of intended registry	
3				
4				
5				
6	Trial registration:	#2b	All items from the World Health Organization Trial	Abstract/
7			Registration Data Set	protocol
8	data set			
9				
10				
11	Protocol version	#3	Date and version identifier	2
12				
13				
14				
15	Funding	#4	Sources and types of financial, material, and other	14
16			support	
17				
18				
19				
20	Roles and	#5a	Names, affiliations, and roles of protocol contributors	1
21				
22	responsibilities:			
23				
24	contributorship			
25				
26				
27				
28	Roles and	#5b	Name and contact information for the trial sponsor	1
29				
30	responsibilities:			
31				
32	sponsor contact			
33				
34	information			
35				
36				
37				
38	Roles and	#5c	Role of study sponsor and funders, if any, in study	14
39			design; collection, management, analysis, and	
40	responsibilities:		interpretation of data; writing of the report; and the	
41			decision to submit the report for publication, including	
42	sponsor and funder		whether they will have ultimate authority over any of	
43			these activities	
44				
45				
46				
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48				
49				
50				
51				
52	Roles and	#5d	Composition, roles, and responsibilities of the	NA
53			coordinating centre, steering committee, endpoint	
54	responsibilities:		adjudication committee, data management team, and	
55				
56	committees			
57				
58				
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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

1	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If	6
2				
3				
4			applicable, eligibility criteria for study centres and	
5				
6			individuals who will perform the interventions (eg,	
7				
8			surgeons, psychotherapists)	
9				
10				
11	Interventions:	#11a	Interventions for each group with sufficient detail to	7
12				
13	description		allow replication, including how and when they will be	
14				
15				
16			administered	
17				
18				
19	Interventions:	#11b	Criteria for discontinuing or modifying allocated	NA
20				
21	modifications		interventions for a given trial participant (eg, drug dose	
22				
23			change in response to harms, participant request, or	
24				
25			improving / worsening disease)	
26				
27				
28				
29	Interventions:	#11c	Strategies to improve adherence to intervention	NA
30				
31	adherence		protocols, and any procedures for monitoring adherence	
32				
33			(eg, drug tablet return; laboratory tests)	
34				
35				
36	Interventions:	#11d	Relevant concomitant care and interventions that are	7
37				
38	concomitant care		permitted or prohibited during the trial	
39				
40				
41				
42	Outcomes	#12	Primary, secondary, and other outcomes, including the	8/9
43				
44			specific measurement variable (eg, systolic blood	
45				
46			pressure), analysis metric (eg, change from baseline,	
47				
48			final value, time to event), method of aggregation (eg,	
49				
50			median, proportion), and time point for each outcome.	
51				
52				
53			Explanation of the clinical relevance of chosen efficacy	
54				
55			and harm outcomes is strongly recommended	
56				
57				
58				
59				
60				

1 2 3 4 5 6 7 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
10 11 12 13 14 15 16 17 18 19 20	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
21 22 23 24 25	Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	NA
26 27 28 29 30 31 32 33 34 35	Methods: Assignment of interventions (for controlled trials)			
36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52	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
53 54 55 56 57 58 59 60	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: [#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): [#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

1	Data collection plan:	#18b	Plans to promote participant retention and complete	NA
2				
3	retention		follow-up, including list of any outcome data to be	
4			collected for participants who discontinue or deviate	
5			from intervention protocols	
6				
7				
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10				
11	Data management	#19	Plans for data entry, coding, security, and storage,	12
12			including any related processes to promote data quality	
13			(eg, double data entry; range checks for data values).	
14			Reference to where details of data management	
15			procedures can be found, if not in the protocol	
16				
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23	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary	10
24			outcomes. Reference to where other details of the	
25			statistical analysis plan can be found, if not in the	
26			protocol	
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33	Statistics: additional	#20b	Methods for any additional analyses (eg, subgroup and	10
34	analyses		adjusted analyses)	
35				
36				
37				
38				
39	Statistics: analysis	#20c	Definition of analysis population relating to protocol non-	NA
40	population and		adherence (eg, as randomised analysis), and any	
41	missing data		statistical methods to handle missing data (eg, multiple	
42			imputation)	
43				
44				
45				
46				
47				
48	Methods: Monitoring			
49				
50				
51	Data monitoring:	#21a	Composition of data monitoring committee (DMC);	12
52	formal committee		summary of its role and reporting structure; statement of	
53			whether it is independent from the sponsor and	
54				
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1 competing interests; and reference to where further
 2 details about its charter can be found, if not in the
 3 protocol. Alternatively, an explanation of why a DMC is
 4 not needed
 5
 6
 7
 8
 9

10	Data monitoring:	#21b	Description of any interim analyses and stopping	NA
11	interim analysis		guidelines, including who will have access to these	
12			interim results and make the final decision to terminate	
13			the trial	
14				
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20	Harms	#22	Plans for collecting, assessing, reporting, and managing	12
21			solicited and spontaneously reported adverse events	
22			and other unintended effects of trial interventions or trial	
23			conduct	
24				
25				
26				
27				
28				
29				
30	Auditing	#23	Frequency and procedures for auditing trial conduct, if	NA
31			any, and whether the process will be independent from	
32			investigators and the sponsor	
33				
34				
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38	Ethics and			
39	dissemination			
40				
41				
42				
43	Research ethics	#24	Plans for seeking research ethics committee /	12
44	approval		institutional review board (REC / IRB) approval	
45				
46				
47				
48	Protocol	#25	Plans for communicating important protocol	NA
49	amendments		modifications (eg, changes to eligibility criteria,	
50			outcomes, analyses) to relevant parties (eg,	
51			investigators, REC / IRBs, trial participants, trial	
52			registries, journals, regulators)	
53				
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1	Consent or assent	#26a	Who will obtain informed consent or assent from	11
2				
3				
4			potential trial participants or authorised surrogates, and	
5				
6			how (see Item 32)	
7				
8				
9	Consent or assent:	#26b	Additional consent provisions for collection and use of	NA
10				
11	ancillary studies		participant data and biological specimens in ancillary	
12				
13			studies, if applicable	
14				
15				
16	Confidentiality	#27	How personal information about potential and enrolled	12
17				
18			participants will be collected, shared, and maintained in	
19				
20			order to protect confidentiality before, during, and after	
21				
22			the trial	
23				
24				
25				
26	Declaration of	#28	Financial and other competing interests for principal	14
27				
28	interests		investigators for the overall trial and each study site	
29				
30				
31				
32	Data access	#29	Statement of who will have access to the final trial	12
33				
34			dataset, and disclosure of contractual agreements that	
35				
36			limit such access for investigators	
37				
38				
39	Ancillary and post	#30	Provisions, if any, for ancillary and post-trial care, and	NA
40				
41	trial care		for compensation to those who suffer harm from trial	
42				
43			participation	
44				
45				
46				
47	Dissemination policy:	#31a	Plans for investigators and sponsor to communicate trial	12
48				
49	trial results		results to participants, healthcare professionals, the	
50				
51			public, and other relevant groups (eg, via publication,	
52				
53			reporting in results databases, or other data sharing	
54				
55			arrangements), including any publication restrictions	
56				
57				
58				
59				
60				

1 Dissemination policy: [#31b](#) Authorship eligibility guidelines and any intended use of NA
 2
 3 authorship professional writers
 4
 5

6 Dissemination policy: [#31c](#) Plans, if any, for granting public access to the full NA
 7
 8 reproducible protocol, participant-level dataset, and statistical code
 9
 10 research
 11
 12

13 Appendices

14
 15
 16
 17 Informed consent [#32](#) Model consent form and other related documentation Appendix
 18
 19 materials given to participants and authorised surrogates 3
 20
 21

22
 23 Biological specimens [#33](#) Plans for collection, laboratory evaluation, and storage NA
 24
 25 of biological specimens for genetic or molecular analysis
 26
 27 in the current trial and for future use in ancillary studies,
 28
 29 if applicable
 30
 31

32
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