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Pessary or progesterone to prevent preterm birth in women with short cervical length: protocol of the 4-6 year follow-up of a randomised controlled trial (Quadruple-P)

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Manuscripts

Pessary or progesterone to prevent preterm birth in women with short cervical length: protocol of the 4-6 year follow-up of a randomised controlled trial (Quadruple-P)

Emilie V.J. van Limburg Stirum MD^{1,2}, Larissa I. van der Windt MD^{1,2}, Charlotte E. van Dijk MD^{1,2}, Anneloes L. van Baar PhD³, Aleid G. Leemhuis MD PhD⁴, Madelon van Wely PhD^{1,2}, Marjon A. de Boer MD PhD^{2,5}, Janneke van 't Hooft MD PhD^{1,2}, Martijn A. Oudijk MD PhD^{2,5}, Eva Pajkrt MD PhD^{1,2},
Quadruple-P study group

1. Amsterdam UMC location University of Amsterdam, Department of Obstetrics and Gynaecology, Meibergdreef 9, Amsterdam, The Netherlands
2. Amsterdam Reproduction & Development, Amsterdam, The Netherlands
3. Utrecht University, Child and Adolescent studies, Utrecht, the Netherlands
4. Emma Children's Hospital, Amsterdam UMC location University of Amsterdam, Department of Neonatology and Paediatrics, Amsterdam Reproduction & Development research institute, Meibergdreef 9, Amsterdam, the Netherlands
5. Amsterdam UMC location Vrije Universiteit Amsterdam, Department of Obstetrics and Gynaecology, De Boelelaan 1117, Amsterdam, the Netherlands

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Corresponding author: Emilie van Limburg Stirum, Amsterdam UMC, University of Amsterdam, Department of Obstetrics and Gynaecology, room H4-240, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands. Tel: +31 (0)20 5661470, E-mail: e.v.vanlimburgstirum@amsterdamumc.nl

Abstract

Introduction:

Vaginal progesterone and a cervical pessary are both interventions to prevent preterm birth (PTB) in high risk pregnancies. Thus far, beneficial or harmful effects of these interventions on long-term child health and development are described, but the literature is scarce. With this follow-up study, we intent to investigate if progesterone or a pessary is superior for the prevention of PTB considering the child's health on the long term.

Methods and analysis:

This study is a follow-up study of the Quadruple-P trial; a multicentre, randomised clinical trial (NL42926.018.13, Eudractnumber 2013-002884-24) which randomizes women with an asymptomatic midtrimester short cervix to daily progesterone or a pessary for the prevention of PTB. All children born to mothers who participated in the Quadruple-P study (n=628 singletons and n=332 multiples) will be eligible for follow-up at 4-6 years of corrected age. Children will be assessed using parental questionnaires. Main outcomes are child (neuro)development and behavior. Other outcomes include child mortality, growth and general health. A composite of adverse child outcomes will be compared between the progesterone and pessary groups reporting odds ratio and the corresponding 95% Confidence Interval. Analyses will be performed separately for singletons and multiples and using the intention to treat approach.

Ethics and dissemination:

The Medical Research Ethics Committee from Amsterdam UMC confirmed that de Medical Research Involving Human Subjects Act (WMO) did not apply to our study (W20_481 #20.531). Results will be published in a peer-reviewed journal and shared with stakeholders and participants. This protocol is published before analysis of the results.

Registration details:

This follow-up study is registered in the Netherlands Trial Register (NL9646). The original trial is partly funded by Stichting Achmea Gezondheidszorg (SAG), a Dutch health foundation founded by insurance company Achmea (Z475) and partly by "Stop te vroeg bevallen", a foundation that stimulates and supports medical research regarding prevention of preterm birth. This follow-up study is also funded by "Stop te vroeg bevallen".

Key words: follow-up, long-term, pessary, preterm birth, progesterone

Article Summary

- This study will be one of very few studies collecting long-term follow-up data after progesterone and pessary application during pregnancy in context of a randomised controlled trial.
- We will evaluate if a pessary or progesterone is superior for the prevention of preterm birth considering the child's health on the long-term for both singleton and multiple pregnancies.
- We will use two validated questionnaires to assess the child's development and daily functioning on all developmental domains, in combination with questionnaires on behaviour and health.
- In our follow-up population several children will already have passed the age for the validated questionnaires, which could result in detection of children with severe developmental delays, but a few with milder problems might be missed.

Introduction

Background and rationale

Prevention of preterm birth (PTB) is of utmost importance to reduce neonatal mortality and morbidity.¹ Several prenatal interventions to prevent PTB (e.g. progesterone and a pessary) have been investigated with mixed evidence regarding effectiveness in different groups of (high risk) pregnancies.²⁻⁷

Progesterone promotes uterine quiescence by a range of actions including inhibition of prostaglandin activity, reduction of contraction associated proteins and decreasing oxytocin receptors.⁸ In addition, it inhibits cervical ripening by regulating the extracellular matrix metabolism.⁹ These range of actions result in its effectiveness to prevent preterm birth. In singletons at risk for PTB (i.e. previous PTB or midtrimester short cervix), vaginal progesterone significantly reduces the risk of birth before 34 weeks (Relative Risk (RR) 0.78, 95% Confidence Interval (CI) 0.68-0.90).² In multiples with a midtrimester short cervix, evidence suggests that progesterone decreases the risk of birth before 34 weeks as well (RR 0.68, 95% CI 0.46–0.99).¹⁰ In unselected singleton or multiple pregnancies (i.e. no previous PTB nor midtrimester short cervix), there is no convincing evidence of effect from vaginal progesterone.^{2, 11}

Another intervention used for prevention of PTB is a cervical pessary. By altering the axis of the cervical canal and displace the weight of the uterus from the cervix, a pessary may prevent the cervix from shortening and dilation and conserve the mucus plug (a barrier for ascending infections).¹²⁻¹⁴ Although several randomised controlled trials (RCTs) have shown a reduction of PTB in singletons with a midtrimester short cervix^{15, 16}, a recent meta-analysis did not show significant reduction (RR 0.80, 95% CI 0.43-1.49).¹⁷ The ProTWIN trial assessed the effect of a cervical pessary in multiple pregnancies, and in a subgroup with a midtrimester short cervix. They observed a reduction of PTB before 32 weeks of gestation (RR 0.49, 95% CI 0.24-0.97) and improvement of neonatal outcomes with 60% was shown.⁵ However, two recent RCTs comparing a cervical pessary (n=250 and n=157) versus no intervention (n=253 and n=158) showed no significant reduction of preterm birth or adverse neonatal outcomes in women with a twin pregnancy and a midtrimester short cervix.^{7, 18}

Besides the importance of finding more solid evidence of effectiveness of these obstetric interventions for the prevention of preterm birth, it is necessary to expand the scope beyond immediate neonatal period to the long-term child's health and development. Especially, since previous studies demonstrated that interventions performed during pregnancy can have unexpected harmful long-term effects which may not be apparent at birth.^{19, 20} At this moment, only a minority of studies on prenatal exposure to progesterone or pessary have published long term results of the children. To date, there are approximately 150 RCTs on progesterone use for the prevention of

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3 preterm birth. A recently published systematic review identified seven studies (5% of the total
4 amount of studies on progesterone) evaluating long-term effects of prenatal progesterone exposure.
5 This review found no evidence of long-term beneficial or harmful effects, but concluded that the
6 results were based on heterogeneous studies, using different assessments, varying from screening
7 tools to face to face assessments with a follow-up age ranging from 6 months to 8 years.²¹ To date,
8 there are approximately 50 RCTs on pessary use for the prevention of preterm birth, of which only
9 two studies (4%) published follow-up information so far.^{22, 23} Follow-up of the ProTWIN study showed
10 improvement of child survival without affecting neurodevelopment at three years of corrected age of
11 the children from women with a midtrimester short cervix treated with a pessary compared to no
12 pessary.²² At four years of corrected age, follow-up data showed no benefits or harmful effects of
13 pessary use regarding child outcome, however, results suggest favourable outcomes for children of
14 women with a midtrimester short cervix.²⁴ Tran et al²³ performed follow-up of children born to
15 women with a multiple pregnancy and midtrimester short cervix, randomised to vaginal
16 progesterone (n=150) or cervical pessary (n=150), at three years of age. They showed a poor child
17 outcome in 10.5% of the pessary group versus 15.8% in the progesterone group (RR 0.66, 95% CI
18 0.43-1.01). The data so far is not robust enough to exclude potential harm on long term from pessary
19 or progesterone, or any potential benefit on either one of these interventions. This implies the need
20 for further follow-up research on progesterone and pessary exposure during pregnancy. In 2014, a
21 multicentre randomised trial (Quadruple-P trial) started to evaluate the effectiveness of
22 progesterone versus a pessary in singleton and multiple pregnancies with an asymptomatic
23 midtrimester short cervix for prevention of PTB.²⁵ This trial allows optimal comparison of the long-
24 term outcomes of exposure to progesterone versus pessary in singleton and multiple pregnancies.
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41 Objectives

42 We aim to assess the long-term effects of in utero exposure to progesterone versus a pessary on
43 child (neuro)development and behaviour at 4-6 years of corrected age. With this follow-up study, we
44 intent to investigate if progesterone or a pessary is superior for the prevention of PTB considering
45 child's health on the long-term.
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Methods and analysis

Study setting

This study will be a follow-up study of a multicentre randomised clinical trial (Quadruple-P trial, NL42926.018.13, Eudractnumber 2013-002884-24) conducted across 21 Dutch hospitals. In the Quadruple-P trial, singletons with an asymptomatic short cervix (≤ 35 mm) at 18-22 weeks of gestation or multiples with an asymptomatic short cervix (< 38 mm) at 16-22 weeks of gestation are randomised to daily vaginal progesterone versus a pessary continued until 36 weeks of gestation. The Quadruple-P trial has a superiority design and in singletons a pessary is compared with vaginal progesterone as standard care, while in multiples vaginal progesterone is compared with a pessary as standard intervention. Outcomes include adverse perinatal outcomes, PTB rate and maternal morbidity, measured until 10 weeks after expected due date. The Quadruple-P study started in 2014 and finished in the first quartile of 2022 for the singletons. For the multiples recruitment of patients is still ongoing while writing this protocol. Eventually 628 singleton pregnancies and 332 multiple pregnancies will be potentially included in this trial. Long term follow-up of the Quadruple-P study was announced in the original trial protocol.²⁵

The follow-up study will be an observational study performed within the Dutch consortium for Healthcare evaluation and Research in Obstetrics and Gynecology and coordinated from the Amsterdam University Medical Centre. Data of this follow-up study will be linked to maternal and neonatal data of the Quadruple-P trial. The study protocol has been developed according to the "Standard Protocol Items: Recommendations for Interventional Trials" (SPIRIT) criteria.

Participants/eligibility criteria

The study population consists of participants of the original Quadruple-P trial and their children. Both singleton- and multiple pregnancies (of whom at least one child is alive) will be eligible for inclusion. Assessment will be performed when children are 4-6 years of corrected age. Only participants who did not refuse to be approached for follow-up research will be approached for this follow-up study. Since the questionnaires in this follow-up study are in Dutch, and the original patient information in both Dutch and English, participants of the original trial who are not able to read Dutch will be excluded from this follow-up study.

Study design

Good clinical practice (GCP) trained research nurses from the local hospital (all involved in the NVOG consortium for research in obstetrics) will verify the medical records of mother and child(ren) for the

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3 possible occurrence of death and to obtain contact details. Using the Dutch Personal Records
4 Database (BRP), a database containing records of all registered citizens of the Netherlands,
5 occurrence of death and up to date contact details will be crosschecked. Thereafter, research nurses
6 will send out information letters and informed consent forms by post. After receiving written
7 informed consent of both parents/caregivers, participants will be contacted by phone to get the
8 opportunity to ask questions, discuss informed consent and to be informed that they can withdraw
9 consent to participate at any time with no reason. Participants will be asked to once fill out four
10 questionnaires when their child is 4-6 years old. Questionnaires will be sent by e-mail and
11 parents/caregivers will be asked to fill out the questionnaires online.
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19 **Blinding**

20 No participants or researchers are blinded in the original Quadruple-P trial. In this follow-up study,
21 researchers involved in data entry are blinded for allocation.
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26 **Patient involvement**

27 Members of the Parents of preterm children Association (care4neo.nl) have been actively involved by
28 our research team and they have stressed the importance of follow-up research. In 2017, members
29 were asked to fill out an online survey including questions about parents' concerns on their child's
30 development and most important long-term outcomes of complications during pregnancy (e.g.
31 preterm birth). Seventy-five members filled out the online questionnaire of whom 85% percent
32 stated to have concerns on their child's long-term development. In the members' opinion, child's
33 school attainment and cognitive development, behaviour problems or psychological problems, motor
34 skills, respiratory problems, general health, growth, and medication use were the most important
35 outcomes to assess in follow-up research. In 2019, our research team also organised a focus group
36 for women who delivered preterm. This focus group showed comparable outcomes. The results of
37 the questionnaire and focus group have primarily determined our choice in main outcome variables
38 of this follow-up study.
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50 **Outcomes**

51 The main outcomes of this follow-up study are child (neuro)development and behaviour disabilities.
52 Other outcomes include child mortality, growth and general health. We will assess all outcomes using
53 parental questionnaires and will report the outcomes as a separate outcome, as well as a composite
54 outcome as described below. We will present data as continuous scores (with mean and Standard
55 Deviation (SD), or median with Interquartile Range IQR) and dichotomised scores (based on the
56 predefined cut-off scores), see table 1. We will document data for singletons and multiples
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separately.

Table 1. Overview of the child outcomes and measurements.

Outcome	Method of measurement	Definition	Measurements
Neurodevelopment	Ages and Stages Questionnaire 4 th edition (ASQ-4)	Scores of the 5 domains: - communication - gross motor skills - fine motor skills - problem-solving skills - personal-social skills	Mean (SD) Abnormal: ≥2 SD in any domain or multiple domains <1 SD below mean Mildly abnormal: ≥1 and <2 SD in one domain below mean
	Vineland screener	Total adaptive functioning score based on 4 domains: - communication - social skills - daily living skills - motor skills	Mean (SD) Abnormal: ≤10th percentile of the population Mildly abnormal: 11-25th percentile of the population
Behaviour	Strength and Difficulties Questionnaire (SDQ)	Total difficulties score based on 4 subscales: - Conduct problems - Emotional symptoms - Hyperactivity - Peer relationships	Mean (SD) Abnormal: >90th percentile of the population Mildly abnormal: 80-90 percentile of the population
Mortality	Medical records and the Dutch Personal Records Database	Perinatal mortality and death up to 7 years of age.	Number (%)~
General health	General health questionnaire*	Height	Mean (SD) Abnormal: 1.6 SDS above or below target height range
		BMI	Mean (SD) Abnormal: ^{26, 27} - underweight - overweight - obesity
		Hospital admissions/medication/surgeries	Number (%)

SD: standard deviation. **BMI:** Body Mass Index.

~ The denominator changes into all children born to participants of the original Quadruple-P study.

* This questionnaire was developed by our research team that is specialised in follow-up research of obstetric intervention studies. The questionnaire has been used in multiple follow-up studies.^{24, 28-30}

(Neuro)development

- ASQ-4: The Ages and Stages Questionnaire (ASQ) is a screening tool to monitor child development by measuring five domains: communication, gross and fine motor skills, problem-solving skills and personal-social skills. The fourth and thereby newest version of the ASQ will be used for this follow-up study and can be used till 6 years of age. The Dutch version of the ASQ-4 is currently being validated, using a Dutch reference group to identify mean score and SDs.³¹

Interpretation: scores of ≥ 1 SD below the mean of the ASQ normative data in two or more domains, or ≥ 2 below the normative mean in at least one domain will be considered abnormal. Results will be considered as mildly abnormal when the scores are ≥ 1 and < 2 SD in one domain below mean.

Children > 6 years of age with a mildly abnormal score will be considered abnormal.

- Vineland screener: The Vineland screener is a tool to assess adaptive functioning (defined as the collection of conceptual, social and practical skills that have been learned by people in order to function in everyday life) of children from 0 to 6 years. The tool exists of 72 questions concerning everyday behavior and covers four domains: communication, social, motor and daily living skills. The total adaptive functioning score is the sum of these four domains.^{32, 33}

Interpretation: A total adaptive functioning score of ≤ 99 and ≤ 111 is considered abnormal (≤ 10 th percentile of the population) for children 4-5 years and 5-6 years of age respectively. In children > 6 years of age a score ≤ 115 will be considered abnormal. A total adaptive functioning score of ≤ 107 and ≤ 115 will be considered mildly abnormal (11-25th percentile of the population) for children 4-5 years and 5-6 years of age respectively. A mildly abnormal score will not be calculated for children > 6 years of age.

Behaviour disabilities

SDQ parent report: The Strengths & Difficulties Questionnaire (SDQ) is a screening tool to identify behavioral problems in children concerning five subscales: emotional problems, conduct problems, hyperactivity, peer problems and prosocial behavior. The validated Dutch translation of the SDQ version 4-17 years will be used. A total difficulties score can be calculated summing the first four subscales, leaving out pro-social behavior.^{34, 35}

Interpretation: A Total Difficulty Score of ≥ 15 is considered abnormal (> 90 th percentile). A Total Difficulty Score of 11-14 is considered mildly abnormal (80-90th percentile).

Mortality

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3 Child death (i.e. perinatal mortality and death up to 7 years of age). Medical records and the Dutch
4 Personal Records Database will be used to verify the number of deceased children.
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8 General health

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10 General health questionnaire: we used the “general health questionnaire” which is used in several
11 previous obstetric follow-up studies performed by the nationwide obstetric consortium.^{24, 36, 37} In the
12 general health questionnaire women will be asked about child growth (i.e. child longitudinal height
13 and weight measurements performed at regular visits at Children’s Healthcare Centres at the age of
14 three months, two years and four years) and health related problems (i.e. need for surgery, hospital
15 admissions, medication use and reported medical conditions). Women will also be asked for
16 information about occurrence and outcome of subsequent pregnancies.
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21 *Interpretation:*

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23 - Growth: Based on Dutch reference values, we will present height as standard deviation scores and
24 dichotomous outcome (normal/abnormal score). An abnormal score is defined as 1.6 SD above or
25 below target height range.³⁸ We will calculate the body-mass index (BMI) and will report BMI as a
26 continuous value and as a proportion of children who are underweight, overweight or obese based
27 on Dutch reference data.^{26, 27}
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31 - Health related problems: we will show the number of child’s medical diagnoses, hospital
32 admissions, medication (used) and history of surgery and will classify them per organ system.
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36 Composite outcomes

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38 Composite of adverse child outcome is defined as:

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40 - abnormal: if the score in ASQ-4 or Vineland screener is abnormal for children up to 6 years of age or
41 mildly abnormal for children >6 years, the score in SDQ is abnormal, or the occurrence of child death,
42 as defined above.
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46 - mildly abnormal: if the scores in ASQ-4 or Vineland screener or SDQ questionnaire are mildly
47 abnormal as defined above.
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50 **Sample size**

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52 In line with the original trial, this follow-up has a superiority design. The original study has included
53 628 singleton pregnancies (314 participants in each group) and will include 332 multiples (166
54 participants in each group, i.e. at least 332 children in each group). Although the number of eligible
55 participants for our follow-up study will be fixed, we can calculate the minimum number of
56 participants needed to find significant difference. We considered 0.5 SD as clinically important
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3 difference for the main outcomes (i.e. 6.05 points Vineland screener³¹ and 2.41 points SDQ³³).³⁸
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5 Therefore, we would need a sample size of 64 participants per study group to achieve a power of
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7 80% and a 2-sided alpha of 0.05 and 86 per study group when we use a conservative alpha of 0.05/3
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9 in view of the three main outcomes as measured by different questionnaires.

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11 Based on prior follow-up studies using questionnaires, we expect to realize a follow-up rate of 30-
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13 50%. When only 30% of the participants of the original trial will participate in this follow-up study
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15 (n=189 singletons and n=100 multiples, i.e. 200 children), we will still have enough power to detect a
16
17 clinically important difference of the main outcomes.

18 19 **Statistical analysis**

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21 Analyses will be performed separately for singletons and multiples. Difference in baseline
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23 characteristics including sociodemographic background of the families of Quadruple-P follow-up
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25 participants in progesterone and pessary group will be measured using unpaired T-test, Mann-
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27 Whitney U test, Chi-square test or Fisher's exact test when appropriate. Like so, characteristics of
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29 follow-up participants will be compared with those lost to follow-up to detect any attrition bias. A
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31 two-sided P-value <0.05 will be considered as statistically significant. We will perform multiple
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33 imputation to approach the problem of missing data using maternal characteristics (e.g. ethnicity,
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35 age, smoking during pregnancy and education) and neonatal outcomes (e.g. gestational age at birth,
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37 birthweight, sex and neonatal sepsis) as predictive variables. We will perform a best and worst-case
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39 scenario analysis if the loss to follow-up is substantially high.

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41 For the main outcomes (neurodevelopment) and behaviour, we will report mean scores
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43 with SDs and abnormal/mildly abnormal scores of the subscales and total scores of the ASQ-4,
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45 Vineland screener and SDQ. For the outcome mortality, the denominator should be changed into all
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47 children born to participants of the original Quadruple-P study. In case data of survival is incomplete,
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49 multiple imputation can be considered in sensitivity analysis. For the outcome concerning general
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51 health, we will mention the outcomes as previously described. Composite of (mildly) abnormal child
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53 outcome will be reported for the progesterone and pessary group.

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55 A directed acyclic graph (DAG) analysis will be constructed to assess potential
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57 confounders. Identified confounders may be corrected using a linear or logistic regression. In
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59 singletons, comparison between progesterone and pessary group will be done using an independent-
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samples T-test, Mann Whitney U test, Chi-square test or Fisher's exact test, as appropriate. Odds
ratio (OR) and the corresponding 95% Confidence Interval (95% CI) for the (mildly) abnormal
outcomes will be reported. For multiple pregnancies we will account for multiple children from the

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3 same pregnancy by using generalized linear mixed effects model (GLMM). All analyses will be
4 performed according to the intention-to-treat principle using SPSS or R.
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7 **Additional analyses**

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9 We will perform sensitivity analysis for the composite of adverse child outcome between
10 progesterone and pessary group (i.e. mortality or abnormal developmental outcome). Analysis will
11 be performed for singletons and multiples separately.
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14 A subgroup analyses will be done comparing children of women with $\geq 80\%$ compliance versus $< 80\%$
15 compliance to progesterone or pessary. Because not all questionnaires are validated for the use up
16 to and including 6 years of age, a subgroup analyse of children < 6 years will also be performed.
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20 **Data management**

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22 All data will be handled confidentially and participants are registered pseudonymised by a 6 digit
23 number. If necessary, investigators have access to the keycode to identify subjects. Procedures of
24 this follow-up study will all be in accordance with the Dutch Personal Data Protection Act.
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Discussion

This follow-up study will evaluate long-term child health and development after two frequently used obstetric interventions in pregnancy to prevent preterm birth, vaginal progesterone and cervical pessary. Long-term follow-up is of utmost importance, since short-term success of an intervention does not guarantee beneficial effects for child on the long term and can even have harmful effects.^{19, 20, 39} Thus far, only 16% of obstetric randomised controlled trials performed long-term follow-up.⁴⁰ To ensure best obstetric care for mother and child, each obstetric intervention study should aim to perform follow-up.

We will perform follow-up during early childhood (4-6 years of age). Early childhood is a very sensitive period for developing cognitive ability, language, social and motor skills. Determining developmental delay or neurodevelopmental disorders at this age will therefore be a reliable predictor for functioning later in life.^{41, 42}

In our follow-up study, we will use two different questionnaires to explore child (neuro)development (i.e. ASQ-4 questionnaire and Vineland screener). These questionnaires may complement each other and, therefore, might give better insight in child's functioning. This information could be used in further follow-up research. Thereby, we contribute to the validation of the ASQ 4 questionnaire for the Dutch population. Validation will be completed before the end of the follow-up study. Both questionnaires are suitable for children up to 6 years of age. In our follow-up population several children will already have passed this age before the start of the study. As a result, this may lead to overestimation of the results. However, children with severe developmental delays will still be detected and other questionnaires used (i.e. SDQ and general health questionnaire) are applicable for children beyond 6 years of age. A sub analysis will be performed for only those children who had the appropriate age range for the validated questionnaires.

Ethics and dissemination

This follow-up study is registered at the Dutch Trial Registry (number NL9646, date August 3rd 2021). The Medical Research Ethics Committee from Amsterdam UMC confirmed that de Medical Research Involving Human Subjects Act (WMO) did not apply to our study (W20_481 #20.531). Results will be published in a peer-reviewed journal and shared with stakeholders and participants. This protocol is published before analysis of the results. After analysis and publication, data of this study will be available from the corresponding author upon reasonable request.

Authors' contributions

EvLS, LvdW, EvD, AvB, AL, MvW, MdB, JvH, MO en EP were all involved in conception and design of the study and protocol. The manuscript was drafted by EvLS, LvdW, AL, JvH and EP. The manuscript was reviewed and argued by all authors and all authors approved the final version of the manuscript. The implementation of this follow-up study is made possible in cooperation with the Quadruple-P study group.

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Competing interests statement

No author reported any conflicts of interest.

References

1. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet*. 2008;371(9606):75-84.
2. Group E. Evaluating Progestogens for Preventing Preterm birth International Collaborative (EPPPIC): meta-analysis of individual participant data from randomised controlled trials. *Lancet*. 2021;397(10280):1183-94.
3. Dodd JM, Jones L, Flenady V, Cincotta R, Crowther CA. Prenatal administration of progesterone for preventing preterm birth in women considered to be at risk of preterm birth. *Cochrane Database Syst Rev*. 2013(7):CD004947.
4. Romero R, Conde-Agudelo A, Da Fonseca E, O'Brien JM, Cetingoz E, Creasy GW, Hassan SS, Nicolaides KH. Vaginal progesterone for preventing preterm birth and adverse perinatal outcomes in singleton gestations with a short cervix: a meta-analysis of individual patient data. *Am J Obstet Gynecol*. 2018;218(2):161-80.
5. Liem S, Schuit E, Hegeman M, Bais J, de Boer K, Bloemenkamp K, Brons J, Duvekot H, Bijvank BN, Franssen M, Gaugler I, de Graaf I, Oudijk M, Papatsonis D, Pernet P, Porath M, Scheepers L, Sikkema M, Sporcken J, Visser H, van Wijngaarden W, Woiski M, van Pampus M, Mol BW, Bekedam D. Cervical pessaries for prevention of preterm birth in women with a multiple pregnancy (ProTWIN): a multicentre, open-label randomised controlled trial. *Lancet*. 2013;382(9901):1341-9.
6. Conde-Agudelo A, Romero R, Nicolaides KH. Cervical pessary to prevent preterm birth in asymptomatic high-risk women: a systematic review and meta-analysis. *Am J Obstet Gynecol*. 2020;223(1):42-65.e2.
7. Norman JE, Norrie J, MacLennan G, Cooper D, Whyte S, Chowdhry S, Cunningham-Burley S, Neilson AR, Mei XW, Smith JB, Shennan A, Robson SC, Thornton S, Kilby MD, Marlow N, Stock SJ, Bennett PR, Denton J. The Arabin pessary to prevent preterm birth in women with a twin pregnancy and a short cervix: the STOPPIT 2 RCT. *Health Technol Assess*. 2021;25(44):1-66.
8. Di Renzo GC, Tosto V, Tsibizova V, Fonseca E. Prevention of Preterm Birth with Progesterone. *J Clin Med*. 2021;10(19).
9. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. *Science*. 2014;345(6198):760-5.
10. Romero R, Conde-Agudelo A, Rehal A, Da Fonseca E, Brizot ML, Rode L, Serra V, Cetingoz E, Syngelaki A, Tabor A, Perales A, Hassan SS, Nicolaides KH. Vaginal progesterone for the prevention of preterm birth and adverse perinatal outcomes in twin gestations with a short cervix: an updated individual patient data meta-analysis. *Ultrasound Obstet Gynecol*. 2022;59(2):263-6.
11. Brizot ML, Hernandez W, Liao AW, Bittar RE, Francisco RPV, Krebs VLJ, Zugaib M. Vaginal progesterone for the prevention of preterm birth in twin gestations: a randomized placebo-controlled double-blind study. *Am J Obstet Gynecol*. 2015;213(1):82 e1- e9.
12. Vitsky M. Simple treatment of the incompetent cervical os. *Am J Obstet Gynecol*. 1961;81:1194-7.
13. Becher N, Adams Waldorf K, Hein M, Ulbjerg N. The cervical mucus plug: structured review of the literature. *Acta Obstet Gynecol Scand*. 2009;88(5):502-13.
14. Hein M, Helmig RB, Schonheyder HC, Ganz T, Ulbjerg N. An in vitro study of antibacterial properties of the cervical mucus plug in pregnancy. *Am J Obstet Gynecol*. 2001;185(3):586-92.
15. Goya M, Pratcorona L, Merced C, Rodo C, Valle L, Romero A, Juan M, Rodriguez A, Munoz B, Santacruz B, Bello-Munoz JC, Llubra E, Higuera T, Cabero L, Carreras E, Pesario Cervical para Evitar Prematuridad Trial G. Cervical pessary in pregnant women with a short cervix (PECEP): an open-label randomised controlled trial. *Lancet*. 2012;379(9828):1800-6.
16. Saccone G, Maruotti GM, Giudicepietro A, Martinelli P, Italian Preterm Birth Prevention Working G. Effect of Cervical Pessary on Spontaneous Preterm Birth in Women With Singleton Pregnancies and Short Cervical Length: A Randomized Clinical Trial. *JAMA*. 2017;318(23):2317-24.

17. Conde-Agudelo A, Romero R, Nicolaidis KH. Cervical pessary to prevent preterm birth in asymptomatic high-risk women: a systematic review and meta-analysis. *Am J Obstet Gynecol*. 2020;223(1):42-65 e2.
18. Groussolles M, Winer N, Sentilhes L, Biquart F, Massoud M, Vivanti AJ, Bouchghoul H, Rozenberg P, Olivier P, Desbriere R, Chauleur C, Perrotin F, Coatleven F, Fuchs F, Bretelle F, Tsatsaris V, Salomon LJ, Sananes N, Kayem G, Houflin-Debarge V, Schmitz T, Benoist G, Arnaud C, Ehlinger V, Vayssière C. Arabin pessary to prevent adverse perinatal outcomes in twin pregnancies with a short cervix: a multicenter randomized controlled trial (PESSARONE). *Am J Obstet Gynecol*. 2022.
19. Kenyon S, Pike K, Jones DR, Brocklehurst P, Marlow N, Salt A, Taylor DJ. Childhood outcomes after prescription of antibiotics to pregnant women with spontaneous preterm labour: 7-year follow-up of the ORACLE II trial. *Lancet*. 2008;372(9646):1319-27.
20. van der Heyden JL, Willekes C, van Baar AL, van Wassenaer-Leemhuis AG, Pajkrt E, Oudijk MA, Porath MM, Duvekot HJ, Bloemenkamp KW, Groenewout M, Woiski M, Nij Bijvank B, Bax CJ, van 't Hooft J, Sikkema MJ, Akerboom BM, Mulder TA, Nijhuis JG, Mol BW, van der Ham DP. Behavioural and neurodevelopmental outcome of 2-year-old children after preterm premature rupture of membranes: follow-up of a randomised clinical trial comparing induction of labour and expectant management. *Eur J Obstet Gynecol Reprod Biol*. 2015;194:17-23.
21. Simons NE, Leeuw M, Van't Hooft J, Limpens J, Roseboom TJ, Oudijk MA, Pajkrt E, Finken M, Painter RC. The long-term effect of prenatal progesterone treatment on child development, behaviour and health: a systematic review. *BJOG*. 2021;128(6):964-74.
22. van 't Hooft J, van der Lee JH, Opmeer BC, van Wassenaer-Leemhuis AG, van Baar AL, Bekedam DJ, Steenis LJP, Liem S, Schuit E, Cuijpers C, Bleeker E, Vinke ME, Simons N, de Graaf IM, Mol BWJ, van de Beek C. Pessary for prevention of preterm birth in twin pregnancy with short cervix: 3-year follow-up study. *Ultrasound Obstet Gynecol*. 2018;51(5):621-8.
23. Tran VTT, Nguyen NA, Nguyen NT, Vo TTM, Uong TS, Nguyen HT, Nguyen NT, Nguyen LMT, Nguyen MHN, Nguyen LK, Vuong LN, Mol B, Dang VQ. 15 Long-term development of children born to women with twin pregnancies treated with pessary or progesterone. *American Journal of Obstetrics & Gynecology*. 2021;224(2):S10.
24. Simons NE, van de Beek C, van der Lee JH, Opmeer BC, van Wassenaer-Leemhuis AG, van Baar AL, Steenis L, Liem S, Schuit E, Bekedam D, Mol BWJ, Van't Hooft J. Child outcomes after placement of a cervical pessary in women with a multiple pregnancy: A 4-year follow-up of the ProTWIN trial. *Acta Obstet Gynecol Scand*. 2019;98(10):1292-300.
25. van Zijl MD, Koullali B, Naaktgeboren CA, Schuit E, Bekedam DJ, Moll E, Oudijk MA, van Baal WM, de Boer MA, Visser H, van Drongelen J, van de Made FW, Vollebregt KC, Muller MA, Bekker MN, Brons JTJ, Sueters M, Langenveld J, Franssen MT, Schuitemaker NW, van Beek E, Scheepers HCJ, de Boer K, Tepe EM, Huisjes AJM, Hooker AB, Verheijen ECJ, Papatsonis DN, Mol BWJ, Kazemier BM, Pajkrt E. Pessary or Progesterone to Prevent Preterm delivery in women with short cervical length: the Quadruple P randomised controlled trial. *BMC Pregnancy Childbirth*. 2017;17(1):284.
26. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *Bmj*. 2000;320(7244):1240-3.
27. Cole TJ, Flegal KM, Nicholls D, Jackson AA. Body mass index cut offs to define thinness in children and adolescents: international survey. *Bmj*. 2007;335(7612):194.
28. van Winden T, Klumper J, Kleinrouweler CE, Tichelaar MA, Naaktgeboren CA, Nijman TA, van Baar AL, van Wassenaer-Leemhuis AG, Roseboom TJ, Van't Hooft J, Roos C, Mol BW, Pajkrt E, Oudijk MA. Effects of tocolysis with nifedipine or atosiban on child outcome: follow-up of the APOSTEL III trial. *BJOG*. 2020;127(9):1129-37.
29. de Ruigh AA, Simons NE, Van 't Hooft J, van Wassenaer-Leemhuis AG, Aarnoudse-Moens CSH, van Wely M, van Baaren GJ, Vlemmix F, van der Ham DP, van Teeffelen ASP, Mol BW, Roseboom TJ, Pajkrt E. Child outcomes after induction of labour or expectant management in women with preterm prelabour rupture of membranes between 34 and 37 weeks of gestation: study protocol of the PPROMEXIL Follow-up trial. A long-term follow-up study of the randomised controlled trials PPROMEXIL and PPROMEXIL-2. *BMJ Open*. 2021;11(6):e046046.

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2
3 30. Simons NE, van Limburg Stirum EVJ, van Wassenaer-Leemhuis AG, Finken MJJ, Aarnoudse-
4 Moens CSH, Oosterlaan J, van Baar A, Roseboom TJ, Lim AC, van Wely M, de Boer MA, Painter RC,
5 Pajkrt E, Oudijk MA, van THJ. Long-term follow-up of children exposed in-utero to progesterone
6 treatment for prevention of preterm birth: study protocol of the AMPHIA follow-up. *BMJ Open*.
7 2021;11(9):e053066.
8
9 31. Ontwikkeling Voorop 2020, accessed 1 February 2022, <https://www.ontwikkelingvoorop.nl/>.
10 32. van Duijn G, Dijkhoorn Y, Noens I, Scholte E, van Berckelaer-Onnes I. Vineland Screener 0-12
11 years research version (NL). Constructing a screening instrument to assess adaptive behaviour. *Int J*
12 *Methods Psychiatr Res*. 2009;18(2):110-7.
13 33. Sparrow SS, Carter AS, Cicchetti DV. Vineland Screener 0-6 jaar. Handleiding (Nederlandse
14 bewerking van E.M. Scholte, G. van Duin, Y. Dijkhoorn, I. Noens & I.A. van Berckelaer-Onnes).
15 Hogrefe Uitgevers B.V., Amsterdam, The Netherlands. 2019.
16 34. Maurice-Stam H, Haverman L, Splinter A, van Oers HA, Schepers SA, Grootenhuis MA. Dutch
17 norms for the Strengths and Difficulties Questionnaire (SDQ) - parent form for children aged 2-
18 18 years. *Health Qual Life Outcomes*. 2018;16(1):123.
19 35. Theunissen MHCWd, M.; Grieken van, A.; Mieloo, C. (2016). Handleiding , voor vhgvdSbdJV,
20 het signalering van psychosociale problemen bij 3-17 jarigen. TNO L.
21 36. de Ruigh AA, Simons NE, van 't Hooft J, van Teeffelen AS, Duijnhoven RG, van Wassenaer-
22 Leemhuis AG, Aarnoudse-Moens C, van de Beek C, Oepkes D, Haak MC, Woiski M, Porath MM, Derks
23 JB, van Kempen L, Roseboom TJ, Mol BW, Pajkrt E. Child outcomes after amniocentesis compared
24 with no intervention in women with second-trimester rupture of membranes: a long-term follow-up
25 study of the PROMEXIL-III trial. *Bjog*. 2021;128(2):292-301.
26 37. Cuijpers CJJ, Van't Hooft J, Schneeberger C, Van Der Lee JH, Simons NE, Van Os MA, Van Der
27 Ven J, De Groot CJM, Mol BWJ, Van Wassenaer-Leemhuis AG. Progesterone for prevention of
28 preterm birth in women with short cervical length: 2-year infant outcomes. *Ultrasound Obstet*
29 *Gynecol*. 2021;57(3):431-9.
30 38. Netherlands Organisation for applied scientific research (TNO). JGZ-Richtlijn Lengtegroei
31 [Dutch]. 2019. [Cited 2021 Aug 10]. Available from: [https://www.ncj.nl/richtlijnen/alle-](https://www.ncj.nl/richtlijnen/alle-richtlijnen/richtlijn/lengtegroei-2019)
32 [richtlijnen/richtlijn/lengtegroei-2019](https://www.ncj.nl/richtlijnen/alle-richtlijnen/richtlijn/lengtegroei-2019).
33 39. Thorp JA, O'Connor M, Jones AM, Hoffman EL, Belden B. Does perinatal phenobarbital
34 exposure affect developmental outcome at age 2? *Am J Perinatol*. 1999;16(2):51-60.
35 40. Teune MJ, van Wassenaer AG, Malin GL, Asztalos E, Alfirevic Z, Mol BW, Opmeer BC. Long-
36 term child follow-up after large obstetric randomised controlled trials for the evaluation of perinatal
37 interventions: a systematic review of the literature. *Bjog*. 2013;120(1):15-22.
38 41. Currie J SM, Manivong P, Roos LL. Child health and young adult outcomes. *Journal of Human*
39 *Resources*. 2010;45(3):517-548.
40 42. Boyle CA, Decouflé P, Yeargin-Allsopp M. Prevalence and health impact of developmental
41 disabilities in US children. *Pediatrics*. 1994;93(3):399-403.
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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number:
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	Additional file 2
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	2, 18
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	18
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	n/a
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	4-5
	6b	Explanation for choice of comparators	4-5
Objectives	7	Specific objectives or hypotheses	5

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2	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, noninferiority, exploratory)	6, 10
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8	Methods: Participants, interventions, and outcomes			
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10	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	6
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15	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
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21	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	n/a
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26		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	n/a
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31		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	n/a
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36		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a
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39	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	7-10
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48	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)	6-7
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54	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	10-11
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2 Recruitment 15 Strategies for achieving adequate participant enrolment to reach target sample size 6-7
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5 **Methods: Assignment of interventions (for controlled trials)**
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7 Allocation:

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9 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 n/a
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18 Allocation concealment mechanism 16b Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned n/a
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24 Implementation 16c Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions n/a
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28 Blinding (masking) 17a Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how 7
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33 17b If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial n/a
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38 **Methods: Data collection, management, and analysis**

39 Data collection methods 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 6-10
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49 18b Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols n/a
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54 Data management 19 Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol 12
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2	Statistical	20a	Statistical methods for analysing primary and secondary	11-12
3	methods		outcomes. Reference to where other details of the	
4			statistical analysis plan can be found, if not in the protocol	
5				
6		20b	Methods for any additional analyses (eg, subgroup and	12
7			adjusted analyses)	
8				
9		20c	Definition of analysis population relating to protocol non-	11
10			adherence (eg, as randomised analysis), and any	
11			statistical methods to handle missing data (eg, multiple	
12			imputation)	
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15	Methods: Monitoring			
16				
17	Data monitoring	21a	Composition of data monitoring committee (DMC);	n/a
18			summary of its role and reporting structure; statement of	
19			whether it is independent from the sponsor and competing	
20			interests; and reference to where further details about its	
21			charter can be found, if not in the protocol. Alternatively,	
22			an explanation of why a DMC is not needed	
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25		21b	Description of any interim analyses and stopping	n/a
26			guidelines, including who will have access to these interim	
27			results and make the final decision to terminate the trial	
28				
29	Harms	22	Plans for collecting, assessing, reporting, and managing	n/a
30			solicited and spontaneously reported adverse events and	
31			other unintended effects of trial interventions or trial	
32			conduct	
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35	Auditing	23	Frequency and procedures for auditing trial conduct, if any,	n/a
36			and whether the process will be independent from	
37			investigators and the sponsor	
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40	Ethics and dissemination			
41				
42	Research ethics	24	Plans for seeking research ethics committee/institutional	1, 14
43	approval		review board (REC/IRB) approval	
44				
45	Protocol	25	Plans for communicating important protocol modifications	n/a
46	amendments		(eg, changes to eligibility criteria, outcomes, analyses) to	
47			relevant parties (eg, investigators, REC/IRBs, trial	
48			participants, trial registries, journals, regulators)	
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51	Consent or assent	26a	Who will obtain informed consent or assent from potential	6-7
52			trial participants or authorised surrogates, and how (see	
53			Item 32)	
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55		26b	Additional consent provisions for collection and use of	n/a
56			participant data and biological specimens in ancillary	
57			studies, if applicable	
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2	Confidentiality	27	How personal information about potential and enrolled	12
3			participants will be collected, shared, and maintained in	
4			order to protect confidentiality before, during, and after the	
5			trial	
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7	Declaration of	28	Financial and other competing interests for principal	18
8	interests		investigators for the overall trial and each study site	
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10	Access to data	29	Statement of who will have access to the final trial dataset,	14
11			and disclosure of contractual agreements that limit such	
12			access for investigators	
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15	Ancillary and	30	Provisions, if any, for ancillary and post-trial care, and for	n/a
16	post-trial care		compensation to those who suffer harm from trial	
17			participation	
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19	Dissemination	31a	Plans for investigators and sponsor to communicate trial	14
20	policy		results to participants, healthcare professionals, the public,	
21			and other relevant groups (eg, via publication, reporting in	
22			results databases, or other data sharing arrangements),	
23			including any publication restrictions	
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26		31b	Authorship eligibility guidelines and any intended use of	18
27			professional writers	
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29		31c	Plans, if any, for granting public access to the full protocol,	14
30			participant-level dataset, and statistical code	
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33	Appendices			
34				
35	Informed consent	32	Model consent form and other related documentation	Additional file 3
36	materials		given to participants and authorised surrogates	
37				
38	Biological	33	Plans for collection, laboratory evaluation, and storage of	n/a
39	specimens		biological specimens for genetic or molecular analysis in	
40			the current trial and for future use in ancillary studies, if	
41			applicable	
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*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.

BMJ Open

Pessary or progesterone to prevent preterm birth in women with short cervical length: protocol of the 4-6 year follow-up of a randomised controlled trial (Quadruple-P)

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Secondary Subject Heading:	Epidemiology, Paediatrics
Keywords:	Maternal medicine < OBSTETRICS, Fetal medicine < OBSTETRICS, Developmental neurology & neurodisability < PAEDIATRICS

SCHOLARONE™
Manuscripts

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3 1 **Pessary or progesterone to prevent preterm birth in women with short**
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5 2 **cervical length: protocol of the 4-6 year follow-up of a randomised**
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7 3 **controlled trial (Quadruple-P)**
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10 5 Emilie V.J. van Limburg Stirum MD^{1,2}, Larissa I. van der Windt MD^{1,2}, Charlotte E. van Dijk MD^{1,2},
11 6 Anneloes L. van Baar PhD³, Aleid G. Leemhuis MD PhD⁴, Madelon van Wely PhD^{1,2}, Marjon A. de Boer
12 7 MD PhD^{2,5}, Janneke van 't Hooft MD PhD^{1,2}, Martijn A. Oudijk MD PhD^{2,5}, Eva Pajkrt MD PhD^{1,2},
13 8 Quadruple-P study group
14 9

- 15 10 1. Amsterdam UMC location University of Amsterdam, Department of Obstetrics and
16 11 Gynaecology, Meibergdreef 9, Amsterdam, The Netherlands
17 12 2. Amsterdam Reproduction & Development, Amsterdam, The Netherlands
18 13 3. Utrecht University, Child and Adolescent studies, Utrecht, The Netherlands
19 14 4. Emma Children's Hospital, Amsterdam UMC location University of Amsterdam, Department
20 15 of Neonatology and Paediatrics, Amsterdam Reproduction & Development research institute,
21 16 Meibergdreef 9, Amsterdam, the Netherlands
22 17 5. Amsterdam UMC location Vrije Universiteit Amsterdam, Department of Obstetrics and
23 18 Gynaecology, De Boelelaan 1117, Amsterdam, the Netherlands
24 19

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37 26 **Corresponding author:** Emilie van Limburg Stirum, Amsterdam UMC, University of Amsterdam,
38 27 Department of Obstetrics and Gynaecology, room H4-240, Meibergdreef 9, 1105 AZ Amsterdam, the
39 28 Netherlands. Tel: +31 (0)20 5661470, E-mail: e.v.vanlimburgstirum@amsterdamumc.nl
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33 **Abstract**

34 **Introduction:**

35 Vaginal progesterone and a cervical pessary are both interventions that are investigated for the
36 prevention of preterm birth (PTB). Thus far, beneficial or harmful effects of these interventions on
37 long-term child health and development are described, but there is no follow-up study comparing
38 these two interventions in a head to head comparison. With this follow-up study, we intent to
39 investigate if progesterone or a pessary is superior for the prevention of PTB considering the child's
40 health at 4-6 years of corrected age.

41

42 **Methods and analysis:**

43 This study is a follow-up study of the Quadruple-P trial; a multicentre, randomised clinical trial
44 (NL42926.018.13, Eudractnumber 2013-002884-24) which randomizes women with an asymptomatic
45 midtrimester short cervix to daily progesterone or a pessary for the prevention of PTB. All children
46 born to mothers who participated in the Quadruple-P study (n=628 singletons and n=332 multiples)
47 will be eligible for follow-up at 4-6 years of corrected age. Children will be assessed using parental
48 questionnaires. Main outcomes are child (neuro)development and behavior. Other outcomes include
49 child mortality, growth and general health. A composite of adverse child outcomes will be compared
50 between the progesterone and pessary groups reporting odds ratio and the corresponding 95%
51 Confidence Interval. Analyses will be performed separately for singletons and multiples and using the
52 intention to treat approach.

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54 **Ethics and dissemination:**

55 The Medical Research Ethics Committee from Amsterdam UMC confirmed that de Medical Research
56 Involving Human Subjects Act (WMO) did not apply to our study (W20_481 #20.531). Results will be
57 published in a peer-reviewed journal and shared with stakeholders and participants. This protocol is
58 published before analysis of the results.

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60 **Registration details:**

61 This follow-up study is registered in the Netherlands Trial Register (NL9646). The original trial is partly
62 funded by Stichting Achmea Gezondheidszorg (SAG), a Dutch health foundation founded by
63 insurance company Achmea (Z475) and partly by "Stop te vroeg bevallen", a foundation that
64 stimulates and supports medical research regarding prevention of preterm birth. This follow-up study
65 is also funded by "Stop te vroeg bevallen".

66 **Key words:** follow-up, long-term, pessary, preterm birth, progesterone

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3 **68 Article Summary**
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6 **69** • This study will be one of very few studies collecting long-term follow-up data after
7 **70** progesterone and pessary application during pregnancy in context of a randomised
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9 **71** controlled trial.
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11 **72** • We will evaluate if a pessary or progesterone is superior for the prevention of preterm birth
12 **73** considering the child's health on the long-term for both singleton and multiple pregnancies.
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14 **74** • We will use two validated questionnaires to assess the child's development and daily
15 **75** functioning on all developmental domains, in combination with questionnaires on behaviour
16 **76** and health.
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18 **77** • In our follow-up population several children will already have passed the age for the
19 **78** validated questionnaires, which could result in detection of children with severe
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21 **79** developmental delays, but a few with milder problems might be missed.
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81 Introduction

82 Background and rationale

83 Prevention of preterm birth (PTB) is of utmost importance to reduce neonatal mortality and
84 morbidity.[1] Several prenatal interventions to prevent PTB (e.g. progesterone and a pessary) have
85 been investigated with mixed evidence regarding effectiveness in different groups of (high risk)
86 pregnancies.[2-7]

87 Progesterone promotes uterine quiescence by a range of actions including inhibition of
88 prostaglandin activity, reduction of contraction associated proteins and decreasing oxytocin
89 receptors.[8] In addition, it inhibits cervical ripening by regulating the extracellular matrix
90 metabolism.[9] These range of actions result in its effectiveness to prevent preterm birth. In
91 singletons at risk for PTB (i.e. previous PTB or midtrimester short cervix), vaginal progesterone
92 significantly reduces the risk of birth before 34 weeks (Relative Risk (RR) 0.78, 95% Confidence
93 Interval (CI) 0.68-0.90).[2] In multiples with a midtrimester short cervix, evidence suggests that
94 progesterone decreases the risk of birth before 34 weeks as well (RR 0.68, 95% CI 0.46–0.99).[10] In
95 unselected singleton or multiple pregnancies (i.e. no previous PTB nor midtrimester short cervix),
96 there is no convincing evidence of effect from vaginal progesterone.[2, 11]

97 Another intervention used for prevention of PTB is a cervical pessary. By altering the axis of
98 the cervical canal and displace the weight of the uterus from the cervix, a pessary may prevent the
99 cervix from shortening and dilation and conserve the mucus plug (a barrier for ascending
100 infections).[12-14] Although several randomised controlled trials (RCTs) have shown a reduction of
101 PTB in singletons with a midtrimester short cervix[15, 16], a recent meta-analysis did not show
102 significant reduction (RR 0.80, 95% CI 0.43-1.49).[17] The ProTWIN trial assessed the effect of a
103 cervical pessary in multiple pregnancies, and in a subgroup with a midtrimester short cervix. They
104 observed a reduction of PTB before 32 weeks of gestation (RR 0.49, 95% CI 0.24-0.97) and
105 improvement of neonatal outcomes with 60% was shown.[5] However, two recent RCTs comparing a
106 cervical pessary (n=250 and n=157) versus no intervention (n=253 and n=158) showed no significant
107 reduction of preterm birth or adverse neonatal outcomes in women with a twin pregnancy and a
108 midtrimester short cervix.[7, 18]

109 Besides the importance of finding more solid evidence of effectiveness of these obstetric
110 interventions for the prevention of preterm birth, it is necessary to expand the scope beyond
111 immediate neonatal period to the long-term child's health and development. Especially, since
112 previous studies demonstrated that interventions performed during pregnancy can have unexpected
113 harmful long-term effects which may not be apparent at birth.[19, 20] At this moment, only a
114 minority of studies on prenatal exposure to progesterone or pessary have published long term

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3 115 results of the children. To date, there are approximately 150 RCTs on progesterone use for the
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5 116 prevention of preterm birth. A recently published systematic review identified seven studies (5% of
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7 117 the total amount of studies on progesterone) evaluating long-term effects of prenatal progesterone
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9 118 exposure. This review found no evidence of long-term beneficial or harmful effects, but concluded
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11 119 that the results were based on heterogeneous studies, using different assessments, varying from
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13 120 screening tools to face to face assessments with a follow-up age ranging from 6 months to 8
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15 121 years.[21] To date, there are approximately 50 RCTs on pessary use for the prevention of preterm
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17 122 birth, of which only two studies (4%) published follow-up information so far.[22, 23] Follow-up of the
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19 123 ProTWIN study showed improvement of child survival without affecting neurodevelopment at three
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21 124 years of corrected age of the children from women with a midtrimester short cervix treated with a
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23 125 pessary compared to no pessary.[22] At four years of corrected age, follow-up data showed no
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25 126 benefits or harmful effects of pessary use regarding child outcome, however, results suggest
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27 127 favourable outcomes for children of women with a midtrimester short cervix .[24] Tran et al[23]
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29 128 performed follow-up of children born to women with a multiple pregnancy and midtrimester short
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31 129 cervix, randomised to vaginal progesterone (n=150) or cervical pessary (n=150), at three years of age.
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33 130 They showed a poor child outcome in 10.5% of the pessary group versus 15.8% in the progesterone
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35 131 group (RR 0.66, 95% CI 0.43-1.01). The data so far is not robust enough to exclude potential harm on
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37 132 long term from pessary or progesterone, or any potential benefit on either one of these
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39 133 interventions. This implies the need for further follow-up research on progesterone and pessary
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41 134 exposure during pregnancy. In 2014, a multicentre randomised trial (Quadruple-P trial) started to
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43 135 evaluate the effectiveness of progesterone versus a pessary in singleton and multiple pregnancies
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45 136 with an asymptomatic midtrimester short cervix for prevention of PTB.[25] This trial allows optimal
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47 137 comparison of the long-term outcomes of exposure to progesterone versus pessary in singleton and
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49 138 multiple pregnancies.

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140 **Objectives**

141 We aim to assess the long-term effects of in utero exposure to progesterone versus a pessary on
142 child (neuro)development and behaviour at 4-6 years of corrected age. With this follow-up study, we
143 intent to investigate if progesterone or a pessary is superior for the prevention of PTB considering
144 child's health on the long-term.

146 **Methods and analysis**

147 **Study setting**

148 This study will be a follow-up study of a multicentre randomised clinical trial (Quadruple-P trial,
149 NL42926.018.13, Eudractnumber 2013-002884-24) conducted across 21 Dutch hospitals. In the
150 Quadruple-P trial, singletons with an asymptomatic short cervix ($\leq 35\text{mm}$) at 18-22 weeks of gestation
151 or multiples with an asymptomatic short cervix ($< 38\text{mm}$) at 16-22 weeks of gestation are randomised
152 to daily vaginal progesterone versus a pessary continued until 36 weeks of gestation. The Quadruple-
153 P trial has a superiority design and in singletons a pessary is compared with vaginal progesterone as
154 standard care, while in multiples vaginal progesterone is compared with a pessary as standard
155 intervention. Outcomes include adverse perinatal outcomes, PTB rate and maternal morbidity,
156 measured until 10 weeks after expected due date. The Quadruple-P study started in 2014 and
157 finished in the first quartile of 2022 for the singletons. For the multiples recruitment of patients is
158 still ongoing while writing this protocol. Eventually 628 singleton pregnancies and 332 multiple
159 pregnancies will be potentially included in this trial. Long term follow-up of the Quadruple-P study
160 was announced in the original trial protocol.[25]

161
162 The follow-up study will be an observational study performed within the Dutch consortium for
163 Healthcare evaluation and Research in Obstetrics and Gynecology and coordinated from the
164 Amsterdam University Medical Centre. Data of this follow-up study will be linked to maternal and
165 neonatal data of the Quadruple-P trial. The study protocol has been developed according to the
166 “Standard Protocol Items: Recommendations for Interventional Trials” (SPIRIT) criteria.

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168 **Participants/eligibility criteria**

169 The study population consists of participants of the original Quadruple-P trial and their children. In
170 the original Quadruple-P trial participants gave informed consent for follow-up research. Both
171 singleton- and multiple pregnancies (of whom at least one child is alive) will be eligible for inclusion.
172 Assessment will be performed when children are 4-6 years of corrected age. However, some children
173 born to mothers of the Quadruple-P study are already 7 years of corrected age before the start of the
174 follow-up study. We will not exclude these children from the follow-up but will separate this data in
175 sensitivity analysis (see statistical analysis). Since the questionnaires in this follow-up study are in
176 Dutch, and the original patient information in both Dutch and English, participants of the original trial
177 who are not able to read Dutch will be excluded from this follow-up study.

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179 **Study design**

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3 180 Good clinical practice (GCP) trained research nurses from the local hospital (all involved in the NVOG
4 181 consortium for research in obstetrics) will verify the medical records of mother and child(ren) for the
5 182 possible occurrence of death and to obtain contact details. Using the Dutch Personal Records
6 183 Database (BRP), a database containing records of all registered citizens of the Netherlands,
7 184 occurrence of death and up to date contact details will be crosschecked. Thereafter, research nurses
8 185 will send out information letters and informed consent forms by post or email when child(ren) are 4-
9 186 6 years of corrected age. After receiving informed consent of parents/caregivers, participants will be
10 187 contacted by phone to get the opportunity to ask questions, discuss informed consent and to be
11 188 informed that they can withdraw consent to participate at any time with no reason. If the research
12 189 team does not receive any response, research nurses of the local hospital will contact women by
13 190 phone or email to verify if women received the information letter and want to participate in the
14 191 follow-up. Participants will be asked to fill out four questionnaires once when their child is 4-6 years
15 192 old. This will take no longer than 40 minutes for all questionnaires. Questionnaires will be sent by e-
16 193 mail and parents/caregivers will be asked to fill out the questionnaires online. If a questionnaire is
17 194 incomplete, participants will be kindly asked by phone or email to complete the questionnaire.

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196 **Blinding**

31 197 No participants or researchers are blinded in the original Quadruple-P trial. In this follow-up study,
32 198 researchers involved in data entry are blinded for allocation.

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200 **Patient involvement**

36 201 Members of the Parents of preterm children Association (care4neo.nl) have been actively involved by
37 202 our research team and they have stressed the importance of follow-up research. In 2017, members
38 203 were asked to fill out an online survey including questions about parents' concerns on their child's
39 204 development and most important long-term outcomes of complications during pregnancy (e.g.
40 205 preterm birth). Seventy-five members filled out the online questionnaire of whom 85% percent
41 206 stated to have concerns on their child's long-term development. In the members' opinion, child's
42 207 school attainment and cognitive development, behaviour problems or psychological problems, motor
43 208 skills, respiratory problems, general health, growth, and medication use were the most important
44 209 outcomes to assess in follow-up research. In 2019, our research team also organised a focus group
45 210 for women who delivered preterm. This focus group showed comparable outcomes. The results of
46 211 the questionnaire and focus group have primarily determined our choice in main outcome variables
47 212 of this follow-up study.

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3 **214 Outcomes**

4 **215** The main outcomes of this follow-up study are child (neuro)development and behaviour disabilities.

5 **216** Other outcomes include child mortality, growth and general health. We will assess all outcomes using

6 **217** parental questionnaires and will report the outcomes as a separate outcome, as well as a composite

7 **218** outcome as described below. We will present data as continuous scores (with mean and Standard

8 **219** Deviation (SD), or median with Interquartile Range IQR) and dichotomised scores (based on the

9 **220** predefined cut-off scores), see table 1. We will document data for singletons and multiples

10 **221** separately.

11 **222**

12 **223** Table 1. Overview of the child outcomes and measurements.

Outcome	Method of measurement	Definition	Measurements
Neurodevelopment	Ages and Stages Questionnaire 4 th edition (ASQ-4)	Scores of the 5 domains: - communication - gross motor skills - fine motor skills - problem-solving skills - personal-social skills	Mean (SD) Abnormal: ≥2 SD in any domain or multiple domains <1 SD below mean Mildly abnormal: ≥1 and <2 SD in one domain below mean
	Vineland screener	Total adaptive functioning score based on 4 domains: - communication - social skills - daily living skills - motor skills	Mean (SD) Abnormal: ≤10th percentile of the population Mildly abnormal: 11-25th percentile of the population
Behaviour	Strength and Difficulties Questionnaire (SDQ)	Total difficulties score based on 4 subscales: - Conduct problems - Emotional symptoms - Hyperactivity - Peer relationships	Mean (SD) Abnormal: >90th percentile of the population Mildly abnormal: 80-90 percentile of the population
Mortality	Medical records and the Dutch Personal Records Database	Perinatal mortality and death up to 7 years of age.	Number (%)~
General health	General health questionnaire*	Height	Mean (SD) Abnormal: 1.6 SDS above or below target height range
		BMI	Mean (SD) Abnormal: [26, 27]

			- underweight - overweight - obesity
		Hospital admissions/medication/surgeries	Number (%)

224 **SD:** standard deviation. **BMI:** Body Mass Index.

225 ~ The denominator changes into all children born to participants of the original Quadruple-P study.

226 * This questionnaire was developed by our research team that is specialised in follow-up research of obstetric
227 intervention studies. The questionnaire has been used in multiple follow-up studies.[24, 28-30]

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229 (Neuro)development

230 - ASQ-4: The Ages and Stages Questionnaire (ASQ) is a screening tool to monitor child development
231 by measuring five domains: communication, gross and fine motor skills, problem-solving skills and
232 personal-social skills. The fourth and thereby newest version of the ASQ will be used for this follow-
233 up study and can be used till 6 years of age. The Dutch version of the ASQ-4 is currently being
234 validated, using a Dutch reference group to identify mean score and SDs.[31]

235 *Interpretation:* scores of ≥ 1 SD below the mean of the ASQ normative data in two or more domains,
236 or ≥ 2 below the normative mean in at least one domain will be considered abnormal. Results will be
237 considered as mildly abnormal when the scores are ≥ 1 and < 2 SD in one domain below mean.

238 Children > 6 years of age with a mildly abnormal score will be considered abnormal.

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240 - Vineland screener: The Vineland screener is a tool to assess adaptive functioning (defined as the
241 collection of conceptual, social and practical skills that have been learned by people in order to
242 function in everyday life) of children from 0 to 6 years. The tool exists of 72 questions concerning
243 everyday behavior and covers four domains: communication, social, motor and daily living skills. The
244 total adaptive functioning score is the sum of these four domains.[32, 33]

245 *Interpretation:* A total adaptive functioning score of ≤ 99 and ≤ 111 is considered abnormal (≤ 10 th
246 percentile of the population) for children 4-5 years and 5-6 years of age respectively. In children > 6
247 years of age a score ≤ 115 will be considered abnormal. A total adaptive functioning score of ≤ 107
248 and ≤ 115 will be considered mildly abnormal (11-25th percentile of the population) for children 4-5
249 years and 5-6 years of age respectively. A mildly abnormal score will not be calculated for children > 6
250 years of age.

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252 Behaviour disabilities

253 SDQ parent report: The Strengths & Difficulties Questionnaire (SDQ) is a screening tool to identify
254 behavioral problems in children concerning five subscales: emotional problems, conduct problems,
255 hyperactivity, peer problems and prosocial behavior. The validated Dutch translation of the SDQ

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3 256 version 4-17 years will be used. A total difficulties score can be calculated summing the first four
4 257 subscales, leaving out pro-social behavior.[34, 35]

5 258 *Interpretation:* A Total Difficulty Score of ≥ 15 is considered abnormal ($>90^{\text{th}}$ percentile). A Total
6 259 Difficulty Score of 11-14 is considered mildly abnormal (80-90th percentile).
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11 261 Mortality

12 262 Child death (i.e. perinatal mortality and death up to 7 years of age). Medical records and the Dutch
13 263 Personal Records Database will be used to verify the number of deceased children.
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18 265 General health

19 266 General health questionnaire: we used the “general health questionnaire” which is used in several
20 267 previous obstetric follow-up studies performed by the nationwide obstetric consortium.[24, 36, 37]

21 268 In the general health questionnaire women will be asked about child growth (i.e. child’s last
22 269 measured longitudinal height and weight) and health related problems (i.e. need for surgery, hospital
23 270 admissions, medication use and reported medical conditions). Women will also be asked for
24 271 information about occurrence and outcome of subsequent pregnancies.
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30 272 *Interpretation:*

31 273 - Growth: Based on Dutch reference values, we will present height as standard deviation scores and
32 274 dichotomous outcome (normal/abnormal score). An abnormal score is defined as 1.6 SD above or
33 275 below target height range.[38] We will calculate the body-mass index (BMI) and will report BMI as a
34 276 continuous value and as a proportion of children who are underweight, overweight or obese based
35 277 on Dutch reference data.[26, 27]

36 278 - Health related problems: we will show the number of child’s medical diagnoses, hospital
37 279 admissions, medication (used) and history of surgery and will classify them per organ system.
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46 281 Composite outcomes

47 282 Composite of adverse child outcome is defined as:

48 283 - abnormal:

49 284 - if the score in ASQ-4 or Vineland screener is abnormal for children up to 6 years of age, as
50 285 defined above.

51 286 - if the score in ASQ-4 or Vineland screener is mildly abnormal for children >6 years, as defined
52 287 above.

53 288 - if the score in SDQ is abnormal, as defined above.

54 289 - the occurrence of child death.
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3 291 - mildly abnormal: if the scores in ASQ-4 or Vineland screener or SDQ questionnaire are mildly
4 292 abnormal as defined above.

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8 294 **Sample size**

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10 295 In line with the original trial, this follow-up has a superiority design. The original study has included
11 296 628 singleton pregnancies (314 participants in each group) and will include 332 multiples (166
12 297 participants in each group, i.e. at least 332 children in each group). Although the number of eligible
13 298 participants for our follow-up study will be fixed, we can calculate the minimum number of
14 299 participants needed to find significant difference. We considered 0.5 SD as clinically important
15 300 difference for the main outcomes (0.5 SD difference on ASQ-4, Vineland screener and SDQ).
16 301 Therefore, we would need a sample size of 64 participants per study group to achieve a power of
17 302 80% and a 2-sided alpha of 0.05 and 86 per study group when we use a conservative alpha of 0.05/3
18 303 in view of the three main outcomes as measured by different questionnaires.

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20 304 Based on prior follow-up studies using questionnaires, we expect to realize a follow-up rate of 30-
21 305 50%. When only 30% of the participants of the original trial will participate in this follow-up study
22 306 (n=189 singletons and n=100 multiples, i.e. 200 children), we will still have enough power to detect a
23 307 clinically important difference of the main outcomes.

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26 309 **Statistical analysis**

27 310 Analyses will be performed separately for singletons and multiples. Difference in baseline
28 311 characteristics including sociodemographic background of the families of Quadruple-P follow-up
29 312 participants in progesterone and pessary group will be measured using unpaired T-test, Mann-
30 313 Whitney U test, Chi-square test or Fisher's exact test when appropriate. Similarly, characteristics of
31 314 follow-up participants will be compared with those lost to follow-up to detect any attrition bias. A
32 315 two-sided P-value <0.05 will be considered as statistically significant. We will perform multiple
33 316 imputation to approach the problem of missing data using maternal characteristics (e.g. ethnicity,
34 317 age, smoking during pregnancy and education) and neonatal outcomes (e.g. gestational age at birth,
35 318 birthweight, sex and neonatal sepsis) as predictive variables. We will perform a best and worst-case
36 319 scenario analysis if the loss to follow-up is more than 20%.[39]

37 320 For the main outcomes (neurodevelopment) and behaviour, we will report mean scores
38 321 with SDs and abnormal/mildly abnormal scores of the subscales and total scores of the ASQ-4,
39 322 Vineland screener and SDQ. For the outcome mortality, the denominator should be changed into all
40 323 children born to participants of the original Quadruple-P study. In case data of survival is incomplete,
41 324 multiple imputation can be considered in sensitivity analysis. For the outcome concerning general

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3 325 health, we will mention the outcomes as previously described. Composite of (mildly) abnormal child
4 326 outcome will be reported for the progesterone and pessary group.

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6 327 A directed acyclic graph (DAG) analysis will be constructed to assess potential confounders.
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8 328 Identified confounders may be corrected using a linear or logistic regression. In singletons,
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10 329 comparison between progesterone and pessary group will be done using an independent-samples T-
11 330 test, Mann Whitney U test, Chi-square test or Fisher's exact test, as appropriate. Odds ratio (OR) and
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13 331 the corresponding 95% Confidence Interval (95% CI) for the (mildly) abnormal outcomes will be
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15 332 reported. For multiple pregnancies we will account for multiple children from the same pregnancy by
16 333 using generalized linear mixed effects model (GLMM). All analyses will be performed according to the
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18 334 intention-to-treat principle using SPSS or R.

20 21 335 **Additional analyses**

22 336 We will perform sensitivity analysis for the composite of adverse child outcome between
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24 337 progesterone and pessary group (i.e. mortality or abnormal developmental outcome). Analysis will
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26 338 be performed for singletons and multiples separately.

27 339 A subgroup analyses will be done comparing children of women with $\geq 80\%$ compliance versus $< 80\%$
28 340 compliance to progesterone or pessary. Because not all questionnaires are validated for the use up
29 341 to and including 6 years of age, a subgroup analyse of children < 6 years will also be performed.

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34 343 **Data management**

35 344 All data will be handled confidentially and participants are registered pseudonymised by a 6 digit
36 345 number. If necessary, investigators have access to the keycode to identify subjects. Procedures of
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38 346 this follow-up study will all be in accordance with the Dutch Personal Data Protection Act.
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347 Discussion

348 This follow-up study will evaluate long-term child health and development after two
349 frequently used obstetric interventions in pregnancy to prevent preterm birth, vaginal progesterone
350 and cervical pessary. Long-term follow-up is of utmost importance, since short-term success of an
351 intervention does not guarantee beneficial effects for child on the long term and can even have
352 harmful effects.[19, 20, 40] Thus far, only 16% of obstetric randomised controlled trials performed
353 long-term follow-up.[41] To ensure best obstetric care for mother and child, each obstetric
354 intervention study should aim to perform follow-up.

355 We will perform follow-up during early childhood (4-6 years of age). Early childhood is a very
356 sensitive period for developing cognitive ability, language, social and motor skills. Determining
357 developmental delay or neurodevelopmental disorders at this age will therefore be a reliable
358 predictor for functioning later in life. [42, 43]

359 In our follow-up study, we will use two different questionnaires to explore child
360 (neuro)development (i.e. ASQ-4 questionnaire and Vineland screener). These questionnaires may
361 complement each other and, therefore, might give better insight in child's functioning. This
362 information could be used in further follow-up research. Thereby, we contribute to the validation of
363 the ASQ 4 questionnaire for the Dutch population. Validation will be completed before the end of the
364 follow-up study. Both questionnaires are suitable for children up to 6 years of age. In our follow-up
365 population several children will already have passed this age before the start of the study. As a result,
366 this may lead to overestimation of the results. However, children with severe developmental delays
367 will still be detected and other questionnaires used (i.e. SDQ and general health questionnaire) are
368 applicable for children beyond 6 years of age. A sub analysis will be performed for only those children
369 who had the appropriate age range for the validated questionnaires.

371 **Ethics and dissemination**

372 This follow-up study is registered at the Dutch Trial Registry (number NL9646, date August 3rd 2021).
373 The Medical Research Ethics Committee from Amsterdam UMC confirmed that de Medical Research
374 Involving Human Subjects Act (WMO) did not apply to our study (W20_481 #20.531). If outcomes
375 indicate abnormal child development, this will be discussed with a neonatologist and/or
376 (neuro)psychologist from our research team. If applicable, parents will be contacted by email or
377 phone about the results of their child(ren) and will be referred to their general practitioner for
378 further assistance.

379 Results will be published in a peer-reviewed journal and shared with stakeholders and
380 participants. This protocol is published before analysis of the results. After analysis and publication,
381 data of this study will be available from the corresponding author upon reasonable request.

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383 **Authors' contributions**

384 EvLS, LvdW, EvD, AvB, AL, MvW, MdB, JvH, MO, EP and Quadruple-P study group were all involved in
385 conception and design of the study and protocol. The manuscript was drafted by EvLS, LvdW, AL, JvH
386 and EP and the Quadruple-P study group was involved planning the follow-up. The manuscript was
387 reviewed and argued by EvLS, LvdW, EvD, AvB, AL, MvW, MdB, JvH, MO and EP and all approved the
388 final version of the manuscript. The implementation of this follow-up study is made possible in
389 cooperation with the Quadruple-P study group.

390 **Funding statement**

391 This follow-up is supported by "Stop te vroeg bevallen", a foundation that stimulates and supports
392 medical research regarding prevention of preterm birth. "Stop te vroeg bevallen" has no role in study
393 design, data collection, management, analysis and interpretation of data of this follow-up, nor in
394 writing or submission of this manuscript.

395 **Competing interests statement**

396 No author reported any conflicts of interest.

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399 **References**

- 400 1. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth.
401 *Lancet*. 2008;371(9606):75-84.
- 402 2. Group E. Evaluating Progestogens for Preventing Preterm birth International Collaborative
403 (EPPPIC): meta-analysis of individual participant data from randomised controlled trials. *Lancet*.
404 2021;397(10280):1183-94.
- 405 3. Dodd JM, Jones L, Flenady V, Cincotta R, Crowther CA. Prenatal administration of
406 progesterone for preventing preterm birth in women considered to be at risk of preterm birth.
407 *Cochrane Database Syst Rev*. 2013(7):CD004947.
- 408 4. Romero R, Conde-Agudelo A, Da Fonseca E, O'Brien JM, Cetingoz E, Creasy GW, et al. Vaginal
409 progesterone for preventing preterm birth and adverse perinatal outcomes in singleton gestations
410 with a short cervix: a meta-analysis of individual patient data. *Am J Obstet Gynecol*. 2018;218(2):161-
411 80.
- 412 5. Liem S, Schuit E, Hegeman M, Bais J, de Boer K, Bloemenkamp K, et al. Cervical pessaries for
413 prevention of preterm birth in women with a multiple pregnancy (ProTWIN): a multicentre, open-
414 label randomised controlled trial. *Lancet*. 2013;382(9901):1341-9.
- 415 6. Conde-Agudelo A, Romero R, Nicolaidis KH. Cervical pessary to prevent preterm birth in
416 asymptomatic high-risk women: a systematic review and meta-analysis. *Am J Obstet Gynecol*.
417 2020;223(1):42-65.e2.
- 418 7. Norman JE, Norrie J, MacLennan G, Cooper D, Whyte S, Chowdhry S, et al. The Arabin pessary
419 to prevent preterm birth in women with a twin pregnancy and a short cervix: the STOPPIT 2 RCT.
420 *Health Technol Assess*. 2021;25(44):1-66.
- 421 8. Di Renzo GC, Tosto V, Tsbizova V, Fonseca E. Prevention of Preterm Birth with Progesterone.
422 *J Clin Med*. 2021;10(19).
- 423 9. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. *Science*.
424 2014;345(6198):760-5.
- 425 10. Romero R, Conde-Agudelo A, Rehal A, Da Fonseca E, Brizot ML, Rode L, et al. Vaginal
426 progesterone for the prevention of preterm birth and adverse perinatal outcomes in twin gestations
427 with a short cervix: an updated individual patient data meta-analysis. *Ultrasound Obstet Gynecol*.
428 2022;59(2):263-6.
- 429 11. Brizot ML, Hernandez W, Liao AW, Bittar RE, Francisco RPV, Krebs VJ, et al. Vaginal
430 progesterone for the prevention of preterm birth in twin gestations: a randomized placebo-
431 controlled double-blind study. *Am J Obstet Gynecol*. 2015;213(1):82 e1- e9.
- 432 12. Vitsky M. Simple treatment of the incompetent cervical os. *Am J Obstet Gynecol*.
433 1961;81:1194-7.
- 434 13. Becher N, Adams Waldorf K, Hein M, Uldbjerg N. The cervical mucus plug: structured review
435 of the literature. *Acta Obstet Gynecol Scand*. 2009;88(5):502-13.
- 436 14. Hein M, Helmig RB, Schonheyder HC, Ganz T, Uldbjerg N. An in vitro study of antibacterial
437 properties of the cervical mucus plug in pregnancy. *Am J Obstet Gynecol*. 2001;185(3):586-92.
- 438 15. Goya M, Pratorcorona L, Merced C, Rodo C, Valle L, Romero A, et al. Cervical pessary in
439 pregnant women with a short cervix (PECEP): an open-label randomised controlled trial. *Lancet*.
440 2012;379(9828):1800-6.
- 441 16. Saccone G, Maruotti GM, Giudicepietro A, Martinelli P, Italian Preterm Birth Prevention
442 Working G. Effect of Cervical Pessary on Spontaneous Preterm Birth in Women With Singleton
443 Pregnancies and Short Cervical Length: A Randomized Clinical Trial. *JAMA*. 2017;318(23):2317-24.
- 444 17. Conde-Agudelo A, Romero R, Nicolaidis KH. Cervical pessary to prevent preterm birth in
445 asymptomatic high-risk women: a systematic review and meta-analysis. *Am J Obstet Gynecol*.
446 2020;223(1):42-65 e2.
- 447 18. Groussolles M, Winer N, Sentilhes L, Biquart F, Massoud M, Vivanti AJ, et al. Arabin pessary
448 to prevent adverse perinatal outcomes in twin pregnancies with a short cervix: a multicenter
449 randomized controlled trial (PESSARONE). *Am J Obstet Gynecol*. 2022.

- 1
2
3 450 19. Kenyon S, Pike K, Jones DR, Brocklehurst P, Marlow N, Salt A, et al. Childhood outcomes after
4 451 prescription of antibiotics to pregnant women with spontaneous preterm labour: 7-year follow-up of
5 452 the ORACLE II trial. *Lancet*. 2008;372(9646):1319-27.
- 6 453 20. van der Heyden JL, Willekes C, van Baar AL, van Wassenaer-Leemhuis AG, Pajkrt E, Oudijk
7 454 MA, et al. Behavioural and neurodevelopmental outcome of 2-year-old children after preterm
8 455 premature rupture of membranes: follow-up of a randomised clinical trial comparing induction of
9 456 labour and expectant management. *Eur J Obstet Gynecol Reprod Biol*. 2015;194:17-23.
- 11 457 21. Simons NE, Leeuw M, Van't Hooft J, Limpens J, Roseboom TJ, Oudijk MA, et al. The long-term
12 458 effect of prenatal progesterone treatment on child development, behaviour and health: a systematic
13 459 review. *BJOG*. 2021;128(6):964-74.
- 14 460 22. van 't Hooft J, van der Lee JH, Opmeer BC, van Wassenaer-Leemhuis AG, van Baar AL,
15 461 Bekedam DJ, et al. Pessary for prevention of preterm birth in twin pregnancy with short cervix: 3-
16 462 year follow-up study. *Ultrasound Obstet Gynecol*. 2018;51(5):621-8.
- 18 463 23. Tran VTT, Nguyen NA, Nguyen NT, Vo TTM, Uong TS, Nguyen HT, et al. 15 Long-term
19 464 development of children born to women with twin pregnancies treated with pessary or
20 465 progesterone. *American Journal of Obstetrics & Gynecology*. 2021;224(2):S10.
- 21 466 24. Simons NE, van de Beek C, van der Lee JH, Opmeer BC, van Wassenaer-Leemhuis AG, van
22 467 Baar AL, et al. Child outcomes after placement of a cervical pessary in women with a multiple
23 468 pregnancy: A 4-year follow-up of the ProTWIN trial. *Acta Obstet Gynecol Scand*. 2019;98(10):1292-
24 469 300.
- 25 470 25. van Zijl MD, Koullali B, Naaktgeboren CA, Schuit E, Bekedam DJ, Moll E, et al. Pessary or
26 471 Progesterone to Prevent Preterm delivery in women with short cervical length: the Quadruple P
27 472 randomised controlled trial. *BMC Pregnancy Childbirth*. 2017;17(1):284.
- 29 473 26. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child
30 474 overweight and obesity worldwide: international survey. *Bmj*. 2000;320(7244):1240-3.
- 31 475 27. Cole TJ, Flegal KM, Nicholls D, Jackson AA. Body mass index cut offs to define thinness in
32 476 children and adolescents: international survey. *Bmj*. 2007;335(7612):194.
- 33 477 28. van Winden T, Klumper J, Kleinrouweler CE, Tichelaar MA, Naaktgeboren CA, Nijman TA, et
34 478 al. Effects of tocolysis with nifedipine or atosiban on child outcome: follow-up of the APOSTEL III trial.
35 479 *BJOG*. 2020;127(9):1129-37.
- 37 480 29. de Ruigh AA, Simons NE, Van 't Hooft J, van Wassenaer-Leemhuis AG, Aarnoudse-Moens CSH,
38 481 van Wely M, et al. Child outcomes after induction of labour or expectant management in women
39 482 with preterm prelabour rupture of membranes between 34 and 37 weeks of gestation: study
40 483 protocol of the PPROMEXIL Follow-up trial. A long-term follow-up study of the randomised controlled
41 484 trials PPROMEXIL and PPROMEXIL-2. *BMJ Open*. 2021;11(6):e046046.
- 42 485 30. Simons NE, van Limburg Stirum EVJ, van Wassenaer-Leemhuis AG, Finken MJJ, Aarnoudse-
43 486 Moens CSH, Oosterlaan J, et al. Long-term follow-up of children exposed in-utero to progesterone
44 487 treatment for prevention of preterm birth: study protocol of the AMPHIA follow-up. *BMJ Open*.
45 488 2021;11(9):e053066.
- 47 489 31. Ontwikkeling Voorop 2020, accessed 1 February 2022, <https://www.ontwikkelingvoorop.nl/>.
- 48 490 32. van Duijn G, Dijkxhoorn Y, Noens I, Scholte E, van Berckelaer-Onnes I. Vineland Screener 0-12
49 491 years research version (NL). Constructing a screening instrument to assess adaptive behaviour. *Int J*
50 492 *Methods Psychiatr Res*. 2009;18(2):110-7.
- 51 493 33. Sparrow SS, Carter AS, Cicchetti DV. Vineland Screener 0-6 jaar. Handleiding (Nederlandse
52 494 bewerking van E.M. Scholte, G. van Duin, Y. Dijkxhoorn, I. Noens & I.A. van Berckelaer-Onnes).
53 495 Hogrefe Uitgevers B.V., Amsterdam, The Netherlands. 2019.
- 54 496 34. Maurice-Stam H, Haverman L, Splinter A, van Oers HA, Schepers SA, Grootenhuis MA. Dutch
55 497 norms for the Strengths and Difficulties Questionnaire (SDQ) - parent form for children aged 2-
56 498 18 years. *Health Qual Life Outcomes*. 2018;16(1):123.
- 58 499 35. Theunissen MHCWd, M.; Grieken van, A.; Mieloo, C. (2016). Handleiding , voor vhgvdSbdJV,
59 500 het signalering van psychosociale problemen bij 3-17 jarigen. TNO L.

- 1
2
3 501 36. de Ruigh AA, Simons NE, van 't Hooft J, van Teeffelen AS, Duijnhoven RG, van Wassenaer-
4 502 Leemhuis AG, et al. Child outcomes after amnioinfusion compared with no intervention in women
5 503 with second-trimester rupture of membranes: a long-term follow-up study of the PROMEXIL-III trial.
6 504 *Bjog*. 2021;128(2):292-301.
- 7 505 37. Cuijpers CJJ, Van't Hooft J, Schneeberger C, Van Der Lee JH, Simons NE, Van Os MA, et al.
8 506 Progesterone for prevention of preterm birth in women with short cervical length: 2-year infant
9 507 outcomes. *Ultrasound Obstet Gynecol*. 2021;57(3):431-9.
- 10 508 38. Netherlands Organisation for applied scientific research (TNO). JGZ-Richtlijn Lengtegroei
11 509 [Dutch]. 2019. [Cited 2021 Aug 10]. Available from: <https://www.ncj.nl/richtlijnen/alle-richtlijnen/richtlijn/lengtegroei-2019>.
- 12 510 39. Schulz KF, Grimes DA. Sample size slippages in randomised trials: exclusions and the lost and
13 511 wayward. *Lancet*. 2002;359(9308):781-5.
- 14 512 40. Thorp JA, O'Connor M, Jones AM, Hoffman EL, Belden B. Does perinatal phenobarbital
15 513 exposure affect developmental outcome at age 2? *Am J Perinatol*. 1999;16(2):51-60.
- 16 514 41. Teune MJ, van Wassenaer AG, Malin GL, Asztalos E, Alfirovic Z, Mol BW, et al. Long-term child
17 515 follow-up after large obstetric randomised controlled trials for the evaluation of perinatal
18 516 interventions: a systematic review of the literature. *Bjog*. 2013;120(1):15-22.
- 19 517 42. Currie J SM, Manivong P, Roos LL. Child health and young adult outcomes. *Journal of Human*
20 518 *Resources*. 2010;45(3):517-548.
- 21 519 43. Boyle CA, Decouflé P, Yeargin-Allsopp M. Prevalence and health impact of developmental
22 520 disabilities in US children. *Pediatrics*. 1994;93(3):399-403.
- 23 521

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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number:
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	Additional file 2
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	2, 18
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	18
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	n/a
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	4-5
	6b	Explanation for choice of comparators	4-5
Objectives	7	Specific objectives or hypotheses	5

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2	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, noninferiority, exploratory)	6, 10
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8	Methods: Participants, interventions, and outcomes			
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10	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	6
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15	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6
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21	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	n/a
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26		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	n/a
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31		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	n/a
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36		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a
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39	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	7-10
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48	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)	6-7
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54	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	10-11
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2 Recruitment 15 Strategies for achieving adequate participant enrolment to reach target sample size 6-7
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5 **Methods: Assignment of interventions (for controlled trials)**
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7 Allocation:

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9 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 n/a
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18 Allocation concealment mechanism 16b Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned n/a
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24 Implementation 16c Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions n/a
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28 Blinding (masking) 17a Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how 7
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33 17b If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial n/a
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38 **Methods: Data collection, management, and analysis**

39 Data collection methods 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 6-10
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49 18b Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols n/a
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54 Data management 19 Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol 12
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2	Statistical	20a	Statistical methods for analysing primary and secondary	11-12
3	methods		outcomes. Reference to where other details of the	
4			statistical analysis plan can be found, if not in the protocol	
5				
6		20b	Methods for any additional analyses (eg, subgroup and	12
7			adjusted analyses)	
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9		20c	Definition of analysis population relating to protocol non-	11
10			adherence (eg, as randomised analysis), and any	
11			statistical methods to handle missing data (eg, multiple	
12			imputation)	
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15	Methods: Monitoring			
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17	Data monitoring	21a	Composition of data monitoring committee (DMC);	n/a
18			summary of its role and reporting structure; statement of	
19			whether it is independent from the sponsor and competing	
20			interests; and reference to where further details about its	
21			charter can be found, if not in the protocol. Alternatively,	
22			an explanation of why a DMC is not needed	
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25		21b	Description of any interim analyses and stopping	n/a
26			guidelines, including who will have access to these interim	
27			results and make the final decision to terminate the trial	
28				
29	Harms	22	Plans for collecting, assessing, reporting, and managing	n/a
30			solicited and spontaneously reported adverse events and	
31			other unintended effects of trial interventions or trial	
32			conduct	
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35	Auditing	23	Frequency and procedures for auditing trial conduct, if any,	n/a
36			and whether the process will be independent from	
37			investigators and the sponsor	
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40	Ethics and dissemination			
41				
42	Research ethics	24	Plans for seeking research ethics committee/institutional	1, 14
43	approval		review board (REC/IRB) approval	
44				
45	Protocol	25	Plans for communicating important protocol modifications	n/a
46	amendments		(eg, changes to eligibility criteria, outcomes, analyses) to	
47			relevant parties (eg, investigators, REC/IRBs, trial	
48			participants, trial registries, journals, regulators)	
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51	Consent or assent	26a	Who will obtain informed consent or assent from potential	6-7
52			trial participants or authorised surrogates, and how (see	
53			Item 32)	
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55		26b	Additional consent provisions for collection and use of	n/a
56			participant data and biological specimens in ancillary	
57			studies, if applicable	
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2	Confidentiality	27	How personal information about potential and enrolled	12
3			participants will be collected, shared, and maintained in	
4			order to protect confidentiality before, during, and after the	
5			trial	
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7	Declaration of	28	Financial and other competing interests for principal	18
8	interests		investigators for the overall trial and each study site	
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10	Access to data	29	Statement of who will have access to the final trial dataset,	14
11			and disclosure of contractual agreements that limit such	
12			access for investigators	
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15	Ancillary and	30	Provisions, if any, for ancillary and post-trial care, and for	n/a
16	post-trial care		compensation to those who suffer harm from trial	
17			participation	
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19	Dissemination	31a	Plans for investigators and sponsor to communicate trial	14
20	policy		results to participants, healthcare professionals, the public,	
21			and other relevant groups (eg, via publication, reporting in	
22			results databases, or other data sharing arrangements),	
23			including any publication restrictions	
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26		31b	Authorship eligibility guidelines and any intended use of	18
27			professional writers	
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29		31c	Plans, if any, for granting public access to the full protocol,	14
30			participant-level dataset, and statistical code	
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33	Appendices			
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35	Informed consent	32	Model consent form and other related documentation	Additional file 3
36	materials		given to participants and authorised surrogates	
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38	Biological	33	Plans for collection, laboratory evaluation, and storage of	n/a
39	specimens		biological specimens for genetic or molecular analysis in	
40			the current trial and for future use in ancillary studies, if	
41			applicable	
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*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.