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Long-term follow-up of children exposed in-utero to progesterone treatment for prevention of preterm birth: study protocol of the AMPHIA follow-up

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ABSTRACT

Introduction Preterm birth is one of the main problems in obstetrics, and the most important cause of neonatal mortality, morbidity and neurodevelopmental impairment. Multiple gestation is an important risk factor for preterm birth, with up to 50% delivering before 37 weeks. Progesterone has a role in maintaining pregnancy and is frequently prescribed to prevent (recurrent) preterm birth and improve pregnancy outcomes in high-risk patients. However, little is known about its long-term effects in multiple gestations. The objective of this follow-up study is to assess long-term benefits and harms of prenatal exposure to progesterone treatment in multiple gestations on child development.

Methods and analysis This is a follow-up study of a multicentre, double-blind, placebo-controlled randomised trial (AMPHIA trial, ISRCTN40512715). Between 2006 and 2009 women with a multiple gestation were randomised at 16–20 weeks of gestation to weekly injections 250 mg 17α-hydroxyprogesterone caproate or placebo, until 36 weeks of gestation or delivery. The current long-term follow-up will assess all children (n=1355) born to mothers who participated in the AMPHIA trial, at 11–14 years of age, with internationally validated questionnaires, completed by themselves, their parents and their teachers.

Main outcomes are child cognition and behaviour Additional outcomes are death (perinatal and up to age 14), gender identity, educational performance and health-related problems. We will use intention-to-treat analyses comparing experimental and placebo group. To adjust for the correlation between twins, general linear mixed-effects models will be used.

Ethics and dissemination Amsterdam UMC MEC provided a waiver for the Medical Research Involving Human Subjects Act (W20_234#20.268). Results will be disseminated through peer-reviewed journals and summaries shared with stakeholders, patients and participants. This protocol is published before analysis of the results.

Strengths and limitations of this study

- This follow-up study is the first to evaluate outcomes in early adolescence (11–14 years) after maternal progesterone administration in pregnancy.
- Children will be evaluated using internationally validated questionnaires, filled out by themselves, their parents and their teachers, using local normative data.
- We will collect data on school performance at the end of primary school, using a well-validated nationwide registration system.
- A focus group meeting of women who delivered preterm identified the most essential long-term outcomes after obstetrical interventions, which are used in this study and, therefore, making our results of utmost importance for daily clinical practice.
- The main limitation is that even though questionnaires are highly feasible and relatively inexpensive assessment tools, face-to-face assessment to evaluate development in children could be more useful to detect mild problems.

Trial registration number NL8933.

INTRODUCTION

Background and rationale

Preterm birth, defined as birth before 37 weeks of gestation, is one of the main problems in obstetrics and is the most important cause of neonatal morbidity and mortality.[1] It complicates 5%–13% of all pregnancies worldwide. Most importantly, children born preterm more often have neurodevelopmental deficits including impairments in cognitive, motor, behavioural and emotional functioning in

early adolescence.[2–5] Progesterone has a role in maintaining pregnancy. Antenatal progesterone treatment is effective in preventing preterm birth, and consequently improving neonatal outcomes among women considered at high risk of preterm delivery, for example, due to a history of preterm birth or short cervix in mid trimester.[6 7] Multiple gestation is an important risk factor of preterm birth, with 53% of multiple gestations in the Netherlands born before 37 weeks of gestation.[8] Consequently, we evaluated the effect of the synthetic form of progesterone, 17α hydroxyprogesterone caproate (17-OHPC), on preterm birth prevention in multiple gestations in the AMPHIA trial. We found that 17-OHPC did not prevent neonatal morbidity or preterm birth in multiple gestations. The findings of our study were confirmed by other trials, and showed that progesterone did not reduce the risk of preterm birth in multiple gestations.[9]

Animal models suggest that natural progestogens have neuroprotective properties.[10 11] One of the neuroactive metabolites derived from natural progesterone is allopregnanolone. Pharmacological suppression of allopregnanolone might increase cell death in the fetal brain.[11 12] Furthermore, rats treated with progesterone following a traumatic brain injury, stroke or for Alzheimer's disease showed improved memory, learning and sensorimotor outcomes compared with untreated rats.[13] Animal studies also suggested that perinatal progesterone exposure may alter behaviour. Male rat pups treated neonatally with progesterone showed demasculinisation of behaviour patterns, whereas in female rat pups progesterone was suggested to protect against the virilising effects of sex hormones.[14 15] 17-OHPC is relatively resilient to metabolism by classical pathways. Treatment with 17-OHPC, as compared with natural progesterone, is therefore accompanied by lower quantities of progesterone metabolites, including allopregnanolone, leading to a distinct activation pattern of progesterone receptors.[16] The unintended effects of synthetic 17-OHPC on the developing brain might therefore be different from those observed after natural progesterone.

Previous studies found no evidence for long-term developmental effects of prenatal progesterone exposure in children aged up to 8 years.[17–23] The included studies had used different tests and ages of follow-up, precluding aggregation of evidence. Of these studies, three reported on the follow-up of children following progesterone or placebo use in twin pregnancies.[19 22 23] Though the majority of children appear to have a normal development, these studies suggest progesterone use might lead to lower neurodevelopmental concerns. As expected, the attrition rates at the age of six were high in the studies, limiting the validity of the results and underscoring the necessity of further follow-up studies in children. Moreover, no studies evaluated the long-term effect of progesterone use in multiple gestations in late childhood or early adolescence, when more complex and differentiated cognitive functions and behavioural and emotional behaviour become apparent.

To contribute to this knowledge gap, we will study the cognitive, behaviour, mortality, gender identity, educational performance and health-related problems in children born from mothers that participated in the AMPHIA trial, in children between 11 and 14 years old.

Objectives

The aim of this follow-up study is to assess the long-term effect of prenatal exposure of progesterone treatment versus placebo in multiple gestations on child development (ie, cognition, behaviour, mortality, gender identity, educational performance and health-related problems) in early adolescence.

METHODS AND ANALYSIS

Study setting

Description of original AMPHIA trial

This is a follow-up study of a multicentre, double-blind, placebo-controlled randomised controlled trial (AMPHIA trial, ISRCTN40512715, MEC 05/102).[24] Between 2006 and 2009 women with a multiple gestation were randomised to receive weekly injections of either 250 mg 17-OHPC or placebo, starting between 16 and 20 weeks of gestation and continued until 36 weeks of gestation or delivery, whichever came first. In the AMPHIA trial participants were followed until discharge from the hospital. Participants, caregivers and investigators of the original trial were blinded for treatment allocation. The number of women that requested to unblind their treatment allocation after the trial is unknown. The primary outcome of the AMPHIA trial was a composite measure of adverse neonatal outcome (ie, severe respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular haemorrhage, necrotising enterocolitis, sepsis and death). This was present in 110 children (16%) born to mothers in the progesterone group, and in 80 children (12%) born to mothers in the placebo group (relative risk 1.34, 95% CI 0.95 to 1.89). Mean gestational age at delivery was 35.4 weeks for the progesterone group and 35.7 weeks for the placebo group (p value 0.32).[24]

Current follow-up study

In this long-term follow-up study we will assess children born to mothers following their participation in the AMPHIA trial, and therefore exposed to 17-OHPC or placebo as fetuses. Data collected in this follow-up study will be linked to maternal, obstetrical and neonatal data collected during the AMPHIA trial. The study protocol is designed, constructed and reported according to the recommendations given in the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) (see online supplemental file 1, SPIRIT checklist for reporting randomised trials and online supplemental file 2, SPIRIT schematic diagram of enrolment).

Participants/eligibility criteria

The study population consists of children (n=1355) born to mothers (n=671) who participated in the AMPHIA trial between 11–14 years of age and attending secondary school.

Exclusion criteria

Not able to read and speak Dutch (ie, not able to give informed consent and fill out questionnaires for this follow-up study).

Study design

The follow-up study will be performed within the Dutch consortium for Healthcare Evaluation and Research in Obstetrics and Gynaecology (NVOG Consortium; https://zorgevaluatienederland.nl/NVOG). Research nurses from participating hospitals will be asked to cross-check medical records for possible deaths (of women and offspring) before contacting mothers and their children for participating in this follow-up study. All mothers will be contacted by mail (see online supplemental file 3). Children have to sign their own informed consent in addition to the parental informed consent form if they are ≥12 years of age. After receiving informed consent of both parents and child(ren), a weblink to the online questionnaires will be sent by email. Additionally, this informed consent will allow us to contact teachers or tutors of the children, asking them to participate in an online questionnaire. First inclusion was on 29 September 2020. The expected end date will be in December 2023.

Blinding

In the follow-up study, investigators involved in data collection will be blinded for treatment allocation. We expect that most participants are still blinded for treatment allocation. Blinding status will be asked during follow-up. Women who request unblinding during this follow-up study, will be offered allocation information after completion of the follow-up measurements.

Patient involvement

Our research team has involved patients in establishing several follow-up studies, all evaluating perinatal interventions to improve maternal and neonatal health. Most of these studies evaluated long-term neurodevelopmental outcomes after interventions to prevent preterm birth. Late neurodevelopmental morbidity is one of the core outcomes mentioned in the core outcome set for studies investigating prevention of preterm birth.[25] The Dutch patient organisation for parents with a (very) preterm born child (care4neo.nl) participated in an online survey emphasising the importance and willingness to participate in follow-up studies. Furthermore, our team organised a focus group meeting in 2019 with mothers of children born preterm, to explore the different aspects of long-term development of their children, and their opinion on crucial outcomes that should be assessed in future long-term follow-up studies. The results of this focus group meeting have driven the choices of outcomes in this follow-up study (see online supplemental file 4, GRIPP2 short form.

Outcomes

The main outcomes of this follow-up study are cognitive as well as behavioural and emotional functioning. All outcomes will be evaluated using questionnaires completed by parents, children and teachers. We will report the outcomes of these questionnaires for each informant (eg, cognition reported by parents, children and teachers) and as a composite (eg, abnormal score on cognitive questionnaire by at least one informant). Finally, we will present a composite outcome consisting of abnormal score(s) on cognition and/or behavioural and emotional functioning. See table 1 for an overview of all questionnaires, outcomes and the analyses that will be used. We will report means of continuous scores (with SD) as well as dichotomised scores on basis of the cut-off for abnormal outcome. Abnormal outcomes are defined as ≤−2SD or 'within clinical range' following the scoring manual of the questionnaires used.

Cognition

- Executive function: The child's executive functions will be assessed with the Behaviour Rating Inventory of Executive Function (BRIEF) screener.[26] This questionnaire evaluates the executive functions of children between 11 and 17 years of age in 14 items (self-report) or 18 items (parent report) on a 3-point Likert scale. Mean scores will be compared and scores will be transformed in a total score percentile rank, with a higher score indicating weaker executive functioning. Norm scores are age and gender dependent and a score of 1.5 SD above the mean of the Dutch reference group is considered abnormal.[27]
- Current school functioning:
  - Level of education will be asked in a questionnaire filled out by parents.
  - Need for additional help/support inside or outside the classroom will be answered by the current teacher/mentor and parent of the child. Additional help is further specified in help with mathematics, reading, writing, physical education, social skills and/or speech therapy.
- Learning progress primary school: The child's teacher in primary school will be asked to send results of the *cito test scores of the final 6 years at primary school*. The Dutch pupil monitoring system (*cito*) developed by the National Institute for Educational Measurement assesses academic performance thought primary school. Most primary schools in the Netherlands use these exams to evaluate the students learning progress. Scores of the cito exams will give us information on the learning progress of the child in primary school on the following subjects: mathematics, spelling, technical reading and reading comprehension . Furthermore, we will collect the eighth grade final cito test score (*cito eindtoets*) or another final test if applicable.

Table 1 Overview of questionnaires, outcomes and analyses of results

	Questionnaire	Participant	Outcome(s)	Outcome measurement
Cognition	Behaviour Rating Inventory of Executive Function (BRIEF) Screener[26]	Parents	Total score executive function	Mean (SD) Cut-off according to age and gender
	BRIEF Screener Self Report[26]	Child	Total score executive function	Mean (SD) Cut-off according to age and gender
	Additional questions (part of general health questionnaire)	Parents	Current level of education Repeating a class/grade in primary/secondary school	Number (%) of children per educational level including special education. Number (%) of children who repeated a grade.
	Additional questions	Teacher secondary school Parents	Additional help inside the classroom or outside the classroom	Number (%)
	Citoscores* primary school	Teacher primary school	Learning progress primary school from third until eighth grade (ability scores)	Mean (SD) grade score from third until eighth grade. Longitudinal relation between learning progress and intervention/placebo. Final eighth grade score equal or below 'level E' (≤10 percentile)
	Educational performance at the end of primary school	Central Bureau of Statistics	Final eighth grade score	Final eighth grade score equal or below 'level E' (≤10 percentile)
Behaviour	Strength and Difficulties Questionnaire[28]	Parents	Total difficulties score	Mean (SD) Cut-off according to age and gender Cut-off according to subscale
	SDQ[28]	Children	Total difficulties score	Mean (SD) Cut-off according to age and gender
	SDQ[28]	Teacher secondary school	Total difficulties score	Mean (SD) Cut-off according to age and gender
	Strengths and Weaknesses of ADHD symptoms and Normal-behaviour rating scale[41]	Parents	Attention deficit hyperactivity/impulsiveness combined	Mean (SD) Cut-off <5% (mean+1.65 sd, cut-off; ADHD-C 2.11, ADHD-I 2.48, ADHD-HI 2.00)
Mortality	Medical records and the Dutch Personal Records Database	Child		Number (%)
Gender identity	Gender Identity Questionnaire[31]	Parents		Mean (SD) Cut-off according to age
General health and sociodemographic characteristics	General Health Questionnaire†	Parents		Number (%)

*National pupil monitoring system scores of the Central Institute for Test Development.[42]
†A general health questionnaire, developed by our team and used in several other follow-up studies, includes questions on children's current and previous health (including hospital visits, medication and mental health).
ADHD-C, attention deficit hyperactivity disorder-combined; ADHD-HI, attention deficit hyperactivity disorder-hyperactivity/impulsiveness.; ADHD-I, attention deficit hyperactivity disorder-inattentive.

- Grade repetition and special education: repeating a class/grade in primary and/or secondary school or following special education: This will be answered by the parents.
- Composite cognitive outcomes, at least one of the following applies:
  - Abnormal BRIEF screener (parent or self-report).
  - Attending special education.

Behaviour

- Behaviour: the Strength and Difficulties Questionnaire (SDQ) 4–17 years screens for behavioural problems in children and consists of 25 items that inform on five subscales (emotional problems, conduct problems, hyperactivity, peer problems and prosocial behaviour).[28] The first four subscales give a total difficulties score. A higher total difficulties score indicates more problems. The SDQ will be filled out by parents, children and teachers. A total difficulty score >90th

percentile of the Dutch reference group is considered abnormal.[29]

- ADHD symptoms: the Strengths and Weaknesses of ADHD symptoms and Normal-behaviour (SWAN) rating consists of 18 items addressing attention deficit and hyperactivity/impulsiveness on a 7-point Likert scale.[30] A lower the score on the rating scale, the more attention problems the child has. Cut-off score of <5% is considered abnormal.
- Composite behavioural problems, at least one of the following applies:
  - Abnormal SDQ (parent, child or teacher report).

Composite abnormal cognition and/or behaviour development

As there are many questionnaires reflecting cognition and behaviour in children, and not one outcome is the most important outcome, we included a composite outcome to make a distinction for the most affected children. This will consist of a composite of abnormal cognition and/or behaviour if the child scores abnormal on at least one of the following outcomes:

- Abnormal executive function (BRIEF parent or self-report).
- Attending special education.
- Behavioural problems (SDQ parent, child or teacher report).

Mortality

- Mortality is defined as perinatal death (from 16 weeks of gestation) and death up to 14 years of age. Medical records and the Dutch Personal Records Database will be used to verify the number of deceased children.

Gender identity

- The Gender Identity Questionnaire for Children covers a range of gender-typed behaviour questions in 16 items, rating on a 5-point Likert scale.[31] These behaviours correspond to various features of the core phenomenology of the Diagnostic and Statistical Manual of Mental Disorders (DSM) diagnosis of Gender Dysphoria. Lower scores reflect more cross-gendered behaviour. The DSM threshold for diagnostic status validated in a Dutch population is used (mean 2.21).[31]

General health

- In a general health questionnaire, developed by our team and used in several previous follow-up studies,[32–34] questions will be asked on health and growth (concerning child's current and previous health, including hospital visits, medication and mental health). Health-related problems (assessed in terms of the need for medical specialist consultation and/or paramedical care, medication used in the past 12 months, hospital admissions and/or need for surgery) will be clustered in categories to provide insight in the range of health-related problems.

Sociodemographic information

- The general health questionnaire also addresses demographic information on both parents and child(ren).

Educational performance at the end of primary school

We will collect data on the school performance at the end of primary school (*cito eindtoets*) in all children born in the AMPHIA trial. This analysis will be carried out within the secure microdata environment of Statistic Netherlands (Central bureau of Statistics).[35] Anonymous records of the AMPHIA trial will be linked to the educational results within the secure environment and enables comparison between progesterone and placebo group, without linking it to the individual participant.

Sample size

In the AMPHIA trial, 681 children (336 mothers) were born in the progesterone group, and 674 children (335 mothers) in the placebo group. Since the follow-up is based on this randomised controlled trial, the maximum number of possible participants is fixed. We calculated the minimum number of participants that need to be assessed to find a clinically significant difference, defined as a difference of 0.5 SD, for the main outcomes in offspring.

With a sample size of at 63 per study group we would be able detect a mean difference of 0.5 SD (5.4 points for cognition (BRIEF screener)[26 27] and 3.1 point for behaviour (SDQ)[36] with a power of 80% and a two-sided alpha of 0.05.

Current practices show a follow-up rate between 30%–70% in follow-up studies using solely questionnaires. Therefore, even at 30% attrition of AMPHIA participants (n=407 children), we will have enough power to detect a clinically important difference of 0.5 SD in mean scores of cognitive and behavioural outcomes.

Statistical analysis

Differences in demographic characteristics of maternal and perinatal outcomes will be compared for participants of the AMPHIA follow-up between progesterone and placebo groups using unpaired t-test, Mann Whitney U test, χ^2 test or Fisher's exact test when appropriate. To estimate presence of attrition bias, we will compare baseline characteristics of follow-up participants to the group of subjects lost to follow-up. All analyses will be based on the intention-to-treat principle. For the outcomes cognition, behaviour and the composite outcome we will report means of continuous scores (with SDs) as well as dichotomised scores on basis of the cut-off for abnormal outcome (see table 1). Our analyses will focus on the results from children assessed at follow-up (complete case analysis).[37]

To adjust for the correlation between children from the same pregnancy we will analyse all data comparing progesterone versus placebo group with a general linear mixed-effects model. For the linear outcomes this will provide us

Table 2 WHO trial registration data set

Primary registry and trial identifying number	Trial NL8933
Date of registration in primary registry	29 September 2020
Secondary identifying numbers	n/a
Source(s) of monetary or material support	Amsterdam Reproduction and Development Institute, V.000296.
Primary sponsor	Academic Medical Center, Amsterdam, The Netherlands.
Secondary sponsor(s)	n/a
Contact for public queries	Drs Emilie van Limburg Stirum amphia-fu@amsterdamumc.nl
Contact for scientific queries	Dr Janneke van 't Hooft j.vanthooft1@amsterdamumc.nl
Public title	AMPHIA follow-up.
Scientific title	Long-term follow-up of children exposed in-utero to progesterone for prevention of preterm birth: follow-up of the AMPHIA trial.
Countries of recruitment	The Netherlands.
Health condition(s) or problem(s) studied	Multiple gestations is a strong risk factor for preterm birth, with an incidence of delivery before 37 weeks of gestation of 53% in the Netherlands. Progesterone is extensively studied as an intervention to prevent preterm birth, not only in singleton pregnancies, but also in other high risk pregnancies (such as multiple gestations). However, little is known about the long-term effect of progesterone on the fetus and the development and health of the child later in life.
Intervention(s)	n/a
Key inclusion and exclusion criteria	The AMPHIA follow-up study population consists of all women that participated in the AMPHIA trial (n=671) and their children (n=1355) between 11–14 years of age.
Study type	Follow-up of a randomised controlled trial.
Date of first enrolment	29 September 2020
Sample size	In the AMPHIA trial, 681 children (336 mothers) were born in the progesterone group, and 674 children (335 mothers) in the placebo group. Since the follow-up is based on this randomised controlled trial, the maximum number of possible participants is fixed. We calculated the minimum number of participants that need to be assessed to find a clinically significant difference, defined as a difference of 0.5 SD, for the main outcomes in offspring. With a sample size of at 94 per study group we would be able detect a mean difference of 0.5 SD (5.4 points for cognition and 3.1 point for behaviour) with a power of 80% and a two-sided alpha of 0.01 to enable multiple testing. Current practices show a follow-up rate between 30%–70% in follow-up studies using solely questionnaires. Therefore, even at 30% attrition of AMPHIA participants (n=405), we will have enough power to detect a clinical important difference of 0.5 SD in mean scores of cognitive and behavioural outcomes.
Recruitment status	Open for patient inclusion.
Primary outcome(s)	Cognition and behaviour in children between 11–14 years of age.
Key secondary outcomes	Death (perinatal and death up to 14 years of age), gender identity and health-related problems (including information on surgery, hospital admission and medication use). Educational performance at the end of primary school for all children born to women who participated in the AMPHIA trial (regardless of participation in follow-up).
Ethics review	The Medical Ethics Committee of the Amsterdam UMC confirmed that the Medical Research Involving Human Subjects Act does not apply to the AMPHIA follow-up (W20_234 # 20.268, date of confirmation 20 May 2020).
Completion date	n/a
Summary results	n/a
Individual patient data sharing statement	n/a

mean differences with their corresponding 95% CI and for the dichotomous outcomes, OR and 95% CI.

For the health-related outcomes only one predetermined analysis will be performed in each health-related category (≥3 admissions/medication/surgeries) to reduce the number of tests.

For the outcome of mortality the denominator needs to be changed in the analysis. In this analysis, all randomised children will be included in the denominator. In case the researchers have been able to collect data on mortality in all children (including loss to follow-up) no imputation technique is needed. In case it is not possible to collect

mortality outcome for all participants, multiple imputation techniques will be considered (depending on the presence of sufficient data to apply this techniques).

Subgroup and/or sensitivity analyses

We will analyses a composite outcome of death or survival with composite abnormal cognitive and/or behaviour development. Combining mortality data with survival of children with a severe developmental disability will help in providing the full scope of relevant outcomes from the start of randomisation until up to 14 years of age. The denominator in this analyses will be all children born in the AMPHIA trial, instead of the number of children included in follow-up. However, this outcome has some challenges due to the high probability of loss to follow-up (as is the case in many obstetrical follow-up studies after several years) and therefore the inevitable need to deal with missing data. This will be done by either applying multiple imputation techniques or, in case of a high loss to follow-up, a worst-case and best-case scenario analyses.

Furthermore, in our main analyses we evaluate a composite of children who score abnormal in at least one of the questionnaires per outcome. Additionally, we will evaluate the number of children that score abnormal in all questionnaires per outcome (eg, for the composite cognition, it will be defined as an abnormal score in the BRIEF screener parent and self-report).

In addition, we will analyse the number of children with a −1 SD score or within 'subclinical' range, because a mild delay in children's development could also interfere with daily activities.

Data management

To ensure confidentiality, a subject identification code will be used as an identifier. The key to this code is only known by the research team. Handling of the personal data will comply within the General Data Protection Regulation and The Dutch Medical Treatment Contracts Act. An electronic case report from (Castor EDC)[38] linked to the unique identification code will be used for data collection and documentation. All questionnaires are filled out through the same data management system (Castor EDC), and directly linked to the individual participant by unique identification code. All data will be stored for 15 years, according to national guidelines.

DISCUSSION

Evidence on the long-term effects of in utero exposure to progesterone treatment is scarce. Although no harmful or beneficial effects are described in literature, research is limited up to the age of 7 years.[17] Progesterone is used for multiple indications and is increasingly prescribed in pregnancy. Patients have indicated the need to know more about the long-term effects of obstetrical interventions in respect to child's behaviour and cognition.

Long-term neurodevelopmental outcome is considered one of the core outcomes in studies evaluating preterm birth prevention.[25] However, follow-up studies are time consuming and often beyond the scope of the original randomised controlled trial.[39 40] A systematic review showed that only 16% of randomised controlled trials evaluating obstetrical interventions performed a follow-up study.[40] The Dutch consortium for Healthcare Evaluation and Research in Obstetrics and Gynaecology took on this task to execute long-term follow-up of all trials performed, even though funding for this type of research is often lacking.

The use of parent and child report questionnaires has the advantage that they are relatively inexpensive and highly feasible. We use mainly internationally validated questionnaires, to increase comparability of results to previous international studies. However, not all questionnaires used have a validated (English) translation. For this reason we will exclude non-Dutch participants. Nonetheless, the number of non-Dutch speaking participants in the original trial is low, but it could cause a minor selection bias in our follow-up study.

This follow-up study will add valuable information to the existing evidence of long-term effects of prenatal progesterone up to 14 years after exposure in multiple pregnancies. This data will provide information for the increasing number of women taking progesterone in pregnancy and help clinicians to counsel parents with the best available evidence on the risks and benefits of this medication.

Ethics approval and consent to participate

The AMPHIA follow-up aims to assess long-term childhood outcomes of the AMPHIA trial (ISRCTN40512715). The Medical Ethics Committee of the Amsterdam UMC provided a waiver for the Medical Research Involving Human Subjects Act for the proposed study (W20_234 # 20.268, date of confirmation 20 May 2020) (see https://zorgevaluatienederland.nl/evaluations/amphia-follow-up for more information).

Written informed consent will be obtained from both parents prior to participation. Children ≥12 years of age have to sign their own informed consent, in addition to the parental informed consent form (see online supplemental file 3). A copy of the informed consent form(s) will be given to parents and/or children. Participants (parents, children or teachers) can leave the study at any time for any reason if they wish to do so without any consequences.

Dissemination

No arrangements have been made concerning public disclosure. The follow-up study is registered at the Dutch Trial Registry. Date of registration 29 September 2020. For the WHO trial registration data set, see table 2.

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REFERENCES

1 Goldenberg RL, Culhane JF, Iams JD, *et al*. Preterm birth 1: epidemiology and causes of preterm birth. *Obstetric Anesthesia* 2009;29:6–7.
2 Allotey J, Zamora J, Cheong-See F, *et al*. Cognitive, motor, behavioural and academic performances of children born preterm: a meta-analysis and systematic review involving 64 061 children. *BJOG* 2018;125:16–25.
3 Aarnoudse-Moens CSH, Weisglas-Kuperus N, van Goudoever JB, *et al*. Meta-nalysis of neurobehavioral outcomes in very preterm and/or very low birth weight children. *Pediatrics* 2009;124:717–28.
4 Burnett AC, Anderson PJ, Cheong J, *et al*. Prevalence of psychiatric diagnoses in preterm and full-term children, adolescents and young adults: a meta-analysis. *Psychol Med* 2011;41:2463–74.
5 Fitzallen GC, Sagar YK, Taylor HG, *et al*. Anxiety and depressive disorders in children born preterm: a meta-analysis. *J Dev Behav Pediatr* 2021;42:154–62.
6 Dodd JM, Jones L, Flenady V, *et al*. 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.
7 Stewart LA, Simmonds M, Duley L, *et al*. Evaluating progestogens for preventing preterm birth international collaborative (EPPPIC): meta-analysis of individual participant data from randomised controlled trials. *Lancet* 2021;397:1183–94.
8 Perined, 2020. Available: https://www.peristat.nl/
9 Dodd JM, Grivell RM, OBrien CM, *et al*. Prenatal administration of progestogens for preventing spontaneous preterm birth in women with a multiple pregnancy. *Cochrane Database Syst Rev* 2019;2019. doi:10.1002/14651858.CD012024.pub3. [Epub ahead of print: 20 Nov 2019].
10 Hill M, Pašková A, Kančeva R, *et al*. Steroid profiling in pregnancy: a focus on the human fetus. *J Steroid Biochem Mol Biol* 2014;139:201–22.
11 Yawno T, Hirst JJ, Castillo-Melendez M, *et al*. Role of neurosteroids in regulating cell death and proliferation in the late gestation fetal brain. *Neuroscience* 2009;163:838–47.
12 Hirst JJ, Kelleher MA, Walker DW, *et al*. Neuroactive steroids in pregnancy: key regulatory and protective roles in the foetal brain. *J Steroid Biochem Mol Biol* 2014;139:144–53.
13 Singh M, Su C. Progesterone and neuroprotection. *Horm Behav* 2013;63:284–90.
14 Hull EM, Franz JR, Snyder AM, *et al*. Perinatal progesterone and learning, social and reproductive behavior in rats. *Physiol Behav* 1980;24:251–6.
15 Shapiro BH, Goldman AS, Bongiovanni AM, *et al*. Neonatal progesterone and feminine sexual development. *Nature* 1976;264:795–6.
16 Byrns MC. Regulation of progesterone signaling during pregnancy: implications for the use of progestins for the prevention of preterm birth. *J Steroid Biochem Mol Biol* 2014;139:173–81.
17 Simons NE, Leeuw M, Van't Hooft J, *et al*. The long-term effect of prenatal progesterone treatment on child development, behaviour and health: a systematic review. *BJOG* 2021;128:964–74.

18 Klein K, Rode L, Nicolaides KH, *et al*. Vaginal micronized progesterone and risk of preterm delivery in high-risk twin pregnancies: secondary analysis of a placebo-controlled randomized trial and meta-analysis. *Ultrasound Obstet Gynecol* 2011;38:281–7.
19 McNamara HC, Wood R, Chalmers J, *et al*. Stoppit baby follow-up study: the effect of prophylactic progesterone in twin pregnancy on childhood outcome. *PLoS One* 2015;10:e0122341.
20 Norman JE, Marlow N, Messow C-M, *et al*. Vaginal progesterone prophylaxis for preterm birth (the opptimum study): a multicentre, randomised, double-blind trial. *Lancet* 2016;387:2106–16.
21 Northen AT, Norman GS, Anderson K, *et al*. Follow-up of children exposed in utero to 17 alpha-hydroxyprogesterone caproate compared with placebo. *Obstet Gynecol* 2007;110:865–72.
22 Rode L, Klein K, Nicolaides KH, *et al*. Prevention of preterm delivery in twin gestations (predict): a multicenter, randomized, placebo-controlled trial on the effect of vaginal micronized progesterone. *Ultrasound Obstet Gynecol* 2011;38:272–80.
23 Vedel C, Larsen H, Holmskov A, *et al*. Long-term effects of prenatal progesterone exposure: neurophysiological development and hospital admissions in twins up to 8 years of age. *Ultrasound Obstet Gynecol* 2016;48:382–9.
24 Lim AC, Schuit E, Bloemenkamp K, *et al*. 17α-hydroxyprogesterone caproate for the prevention of adverse neonatal outcome in multiple pregnancies: a randomized controlled trial. *Obstet Gynecol* 2011;118:513–20.
25 van 't Hooft J, Duffy JMN, Daly M, *et al*. A core outcome set for evaluation of interventions to prevent preterm birth. *Obstet Gynecol* 2016;127:49–58.
26 Baron IS, Gioia GA, Isquith PK, Guy SC. Behavior rating inventory of executive function. *Child Neuropsychol* 2000;6:235–8.
27 Huizinga M, Smidts DP. Age-related changes in executive function: a normative study with the Dutch version of the behavior rating inventory of executive function (brief). *Child Neuropsychol* 2011;17:51–66.
28 Theunissen M, de Wolff M, Vugteveen J. *Handleiding voor hhet gebruik van de strengths and difficulties questionnaire bij adolescenten (12 – 17 jaar) binnen de Jeugdgezondheidszorg Vragenlijst voor Het signaleren van psychosociale problemen*. Leiden: TNO innovation for life, 2019.
29 Theunissen MHC, MSdd W, Avv G. *Handleiding voor Het gebruik van de strengths and difficulties questionnaire binnen de Jeugdgezondheidszorg*. TNO, 2016. https://www.captise.nl/Portals/1/Downloads/SDQ_Handleiding%20SDQ.pdf?ver=2016-10-28-053202-200
30 Swanson JM, Schuck S, Porter MM, *et al*. Categorical and dimensional definitions and evaluations of symptoms of ADHD: history of the SNAP and the wan rating scales. *Int J Educ Psychol Assess* 2012;10:51–70.
31 Cohen-Kettenis PT, Wallien M, Johnson LL, Fau WM, *et al*. A parent-report gender identity questionnaire for children: a cross-national, cross-clinic comparative analysis. *Clin Child Psychol Psychiatry* 2006;11:397–405.
32 Cuijpers CJJ, Van't Hooft J, Schneeberger C, *et al*. Progesterone for prevention of preterm birth in women with short cervical length: 2-year infant outcomes. *Ultrasound Obstet Gynecol* 2021;57:431–9.
33 de Ruigh AA, Simons NE, van 't Hooft J, *et al*. Child outcomes after amnioinfusion compared with no intervention in women with second-trimester rupture of membranes: a long-term follow-up study of the PROMEXIL-III trial. *BJOG* 2021;128:292–301.
34 Simons NE, van de Beek C, van der Lee JH, *et al*. 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;98:1292–300.
35 Algemeen, 2021. Available: https://www.cbs.nl/nl-nl/onze-diensten/maatwerk-en-microdata
36 Vugteveen J, de Bildt A, Theunissen M, *et al*. Validity aspects of the strengths and difficulties questionnaire (SDQ) adolescent self-report and parent-report versions among Dutch adolescents. *Assessment* 2021;28:601–16.
37 Hughes RA, Heron J, Sterne JAC, *et al*. Accounting for missing data in statistical analyses: multiple imputation is not always the answer. *Int J Epidemiol* 2019;48:1294–304.
38 Castor, 2021. Available: https://www.castoredc.com/
39 Marlow N, Doyle LW, Anderson P, *et al*. Assessment of long-term neurodevelopmental outcome following trials of medicinal products in newborn infants. *Pediatr Res* 2019;86:567–72.
40 Teune MJ, van Wassenaer AG, Malin GL, *et al*. Long-term child follow-up after large obstetric randomised controlled trials for the evaluation of perinatal interventions: a systematic review of the literature. *BJOG* 2013;120:15–22.
41 Polderman TJC, Derks EM, Hudziak JJ, *et al*. Across the continuum of attention skills: a twin study of the swan ADHD rating scale. *J Child Psychol Psychiatry* 2007;48:1080–7.
42 Twilhaar ES, de Kieviet JF, van Elburg RM, *et al*. Academic trajectories of very preterm born children at school age. *Arch Dis Child Fetal Neonatal Ed* 2019;104:F419–23.