BMJ Open Short-term oestrogen as a strategy to prevent postpartum depression in high-risk women: protocol for the double-blind, randomised, placebo-controlled MAMA clinical trial

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ABSTRACT

Introduction Postpartum depression affects 10%–15% of women and has a recurrence rate of 40% in subsequent pregnancies. Women who develop postpartum depression are suspected to be more sensitive to the rapid and large fluctuations in sex steroid hormones, particularly estradiol, during pregnancy and postpartum. This trial aims to evaluate the preventive effect of 3 weeks transdermal estradiol treatment immediately postpartum on depressive episodes in women at high risk for developing postpartum depression.

Methods and analysis The Maternal Mental Health Trial is a double-blind, randomised and placebo-controlled clinical trial. The trial involves three departments of obstetrics organised under Copenhagen University Hospital in Denmark. Women who are singleton pregnant with a history of perinatal depression are eligible to participate. Participants will be randomised to receive either transdermal estradiol patches (200 µg/day) or placebo patches for 3 weeks immediately postpartum. The primary outcome is clinical depression, according to the Diagnostic and Statistical Manual of Mental Disorders-V criteria of Major Depressive Disorder with onset at any time between 0 and 6 months postpartum. Secondary outcomes include, but are not limited to, symptoms of depression postpartum, exclusive breastfeeding, cortisol dynamics, maternal distress sensitivity and cognitive function. The primary statistical analysis will be performed based on the intention-to-treat principle. With the inclusion of 220 participants and a 20% expected dropout rate, we anticipate 80% power to detect a 50% reduction in postpartum depressive episodes while controlling the type 1 error at 5%.

Ethics and dissemination The study protocol is approved by the Regional Committees on Health Research Ethics in the Capital Region of Denmark, the Danish Medicines Agency and the Centre for Data Protection Compliance in the Capital Region of Denmark. We will present results at scientific meetings and in peer-reviewed journals and in other formats to engage policymakers and the public.

Strengths and limitations of this study

- First trial to evaluate short-term transdermal estradiol treatment to prevent postpartum depression in women at high risk.
- Double-blind, randomised placebo-controlled multi-centre design.
- It may pose a challenge for breastfeeding women to participate in a trial involving a drug.
- Evaluation of offspring neurodevelopmental and physical health consequences of intervention is limited due to a relatively short follow-up period of 6 months.

Trial registration number NCT04685148.

INTRODUCTION

Major Depressive Disorder (MDD) is currently the leading cause of disability worldwide¹ and affects more women than men.¹ Women are at increased risk for depression when the endogenous sex steroid hormone milieu changes such as in puberty, during late pregnancy to postpartum and during the menopausal transition.^{2,3} This includes postpartum depression (PPD) that affects 10%–15% of mothers and has a recurrence rate of 40%.^{4–6} PPD is a disabling disorder that affects the entire family, including infant development and future health.^{7–9}

The underlying risk and resilience mechanisms in MDD, including PPD, are far from clear.^{10–12} Consequently, current treatment and preventive strategies are suboptimal. Women who develop PPD might be particularly sensitive to the transition from high

levels of sex steroid hormones, in particular, estradiol, in pregnancy to low levels in the hormone withdrawal phase postpartum.^{2 13} Thus, PPD is most likely to have a distinct pathophysiology, which may provide a unique opportunity for protecting mental health by targeted short-term prevention in the immediate postpartum period. Intriguingly, recent human data have provided evidence for sex hormone manipulation to provoke subclinical depressive symptoms in about 12% of healthy volunteers. The phenomenon was linked to changes in estradiol, which were induced by the pharmacological manipulation with a gonadotrophin-releasing hormone agonist^{14 15} that first stimulates and subsequently suppresses ovarian hormone production, primarily estradiol, to menopausal levels. Estradiol affects critical domains and key brain regions known to be dysfunctional in women with MDD.^{13 16} Estradiol sensitivity appears to predispose to PPD, which can be demonstrated at the level of gene transcription in clinical cohorts^{3 17 18} and is also directly supported by recent research results in a sex hormone manipulation model.^{17 19} Such peripheral markers of estradiol sensitivity may, therefore, prove useful in identifying individuals at excess risk for PPD and may help direct preventive efforts. Also, the hypothalamic–pituitary–adrenal (HPA) axis appears to be involved in the dysregulation of perinatal mental health both in human studies,²⁰ possibly in interaction with psychological stressors and interaction with endocrine profiles,²¹ and in rats, where blunting of HPA-axis dynamics was related to depressive-like behaviour in a model of PPD.²² Furthermore, compromised serotonin signalling may be involved in the mechanisms by which sex hormone transitions add to the risk of developing depressive symptoms, at least in a certain subgroup of susceptible individuals.^{14 18 19} This has been shown in a human sex hormone manipulation study¹⁵ and may also affect HPA axis dynamic capacities.^{23 24} Evidence for serotonergic contributions to risk for depressive symptoms across peripartum also comes from rodent work, which, in particular, highlights estradiol-dependent changes in key features of the serotonin signalling system.^{25–27} Some disturbances of serotonin signalling may, from high estradiol stimulated states (ie, late pregnancy), carry over to the early postpartum phase. Also, earlier findings of ours^{14 28} and others²⁹ support that the brain architecture of hormonal transition includes key targetable features beyond serotonin that ostensibly contribute to an increased risk for depressive episodes and are most likely linked to the estradiol *withdrawal* phase.

Transdermal estradiol emerges as a promising preventive treatment for PPD.³⁰ Previously, a randomised controlled trial (RCT) showed effect of transdermal estradiol treatment on manifest PPD.³¹ Meanwhile, a recent pilot RCT with transdermal estradiol as a treatment for PPD failed to achieve its primary outcome but, notably, did reduce depressive symptoms postpartum compared with placebo.³² Another study aimed to evaluate the efficacy of transdermal estradiol compared with placebo as treatment of PPD, with sertraline as an antidepressant

comparator. However, this study was stopped as it revealed non-significant estradiol concentration differences between the treatment groups.³³ Furthermore, transdermal estradiol appears to be effective in preventing clinically significant depressive symptoms among perimenopausal women, which is another group of women who undergo a hormonal transition.³⁴

Rather than treating manifest depressive episodes postpartum, we propose a different approach: to target and potentially prevent early postpartum risk mechanisms and to direct this preventive strategy towards women at high risk. This immediate and early postpartum timing corresponds to the peak risk period and covers the peak of hormonal decline postpartum.^{4 35}

This trial aims (1) to evaluate the preventive effect of transdermal estradiol treatment for 3 weeks immediately postpartum on depressive episodes in a subgroup of women who are at high risk due to a history of perinatal depression and (2) to determine if a set of genomic biomarkers can identify women within this high-risk group who benefit from the intervention (or vice versa become depressed in the placebo group) and, thus inform future personalised prevention or treatment.

METHODS

The Maternal Mental Health (MAMA) Trial is designed as a double-blind, 1:1 randomised, placebo-controlled multicentre trial. The MAMA trial was designed in accordance with the Consolidated Standards of Reporting Trials recommendation for RCTs³⁶ (figure 1) and with the Standard Protocol Items: Recommendations for Interventional Trials guidelines for reporting trial protocols (additional file 1).³⁷

Study setting

The trial is conducted in a multicentre setting involving maternity wards at four university hospitals in the Capital Region of Denmark. The four maternity wards have more than 20 000 deliveries in total per year, of which a minimum of 250 are deliveries of women with previous perinatal depression (estimated by personal communication with midwives from the specialised team for women with psychiatric history and social challenges).³⁸

The three maternity units have teams of experienced midwives, nurses and obstetricians working with pregnant women with current or previous psychiatric disorders and collaborate with psychiatrists in severe cases. The teams at two maternity units include psychologists and all teams include social workers, who are specialised in taking care of women with psychiatric history and social challenges. At all three maternity units, women with a history of perinatal depression are offered to stay with their infant at the postnatal ward after the delivery, typically for 2–5 days.

Eligibility criteria

Women who are singleton pregnant in gestational week $\geq 34+0$ with a history of perinatal depression (onset

Figure 1 CONSORT flow diagram of MAMA trial. CONSORT, Consolidated Standards of Reporting Trials; MAMA, Maternal Mental Health.

during pregnancy or before 6 months postpartum) and aged 18–45 years are eligible to participate. The women must be unmedicated and otherwise untreated for MDD at inclusion.

Exclusion criteria include moderate to severe depression with onset in the current pregnancy; severe psychiatric disorders; previous suicide attempts without having a depressive episode; neurological disorders; severe somatic illness; risk factors for any thromboembolic disorders; deep vein thrombosis or pulmonary embolism in the current pregnancy; pregnancy-induced hypertension or preeclampsia; contraindication for estrogenic treatment; use of psychotropic drugs; non-fluency in Danish; prepregnancy body mass index >35 kg/m²; ongoing alcohol or illicit drug abuse; severe postpartum haemorrhage (>1500 mL); severe illness or perinatal death of the infant.

A full list of inclusion and exclusion criteria is provided in table 1.

Interventions

Participants will be randomised to either transdermal estradiol patches (200 µg/day, Vivelle Dot) or placebo

patches for 3 weeks. The patches will be administered by a research assistant either on the day of delivery or day after the delivery. After detailed instructions, the trial participants self-administer the patches two times weekly for 3 weeks. Participants confirm the subsequent administrations by filling in a form on the date for changing the patch to monitor adherence. If participants fail to administer their patches for 4 days or more of the 3-week period, they will be considered non-adherent and treated as such in the analyses.

We will perform an interim analysis on the first 40 participants to assess estradiol levels during transdermal estradiol treatment compared with placebo (ie, blood samples at 3 weeks postpartum). Should the interim analysis indicate that the treatment is insufficient for changing estradiol levels, we will consider if there are elements of the intervention that need to be modified, such as the dose or type of administration.

Side effects

Using transdermal estradiol for a limited time in the immediate postpartum period is not expected to pose any unacceptable risk or adverse side effects to the

Table 1 Inclusion and exclusion criteria of the MAMA trial

Inclusion	Exclusion
<ul style="list-style-type: none"> ▶ Singleton pregnant in gestational week $\geq 34+0$ ▶ History of perinatal depression ▶ Age between 18 and 45 years 	<ul style="list-style-type: none"> ▶ Moderate to severe depression with onset during the current pregnancy. ▶ Severe psychiatric disorders (eg, disorders with psychotic symptoms, schizophrenia, bipolar disorders, inpatient eating disorders and inpatient obsessive-compulsive disorders). ▶ Previous suicide attempts without having a depressive episode. ▶ Use of psychotropic pharmacology, except for short-term sleep support treatment. ▶ Severe somatic illness. ▶ History of or ongoing cancer. ▶ History of venous thromboembolism, myocardial infarction, cerebrovascular thromboembolism or thrombophilia, or other risk factors clinically assessed after thrombophilia screening. ▶ Deep vein thrombosis or pulmonary embolism in current pregnancy. ▶ Pregnancy-induced hypertension or preeclampsia. ▶ Other contraindications for oestrogen treatment (eg, acute liver failure, severe varicose veins). ▶ Non-fluent in Danish or pronounced vision or hearing loss. ▶ Prepregnancy BMI > 35 kg/m². ▶ Ongoing alcohol or drug abuse. ▶ Severe postpartum haemorrhage (> 1500 mL). ▶ Severe illness or perinatal death of the infant.

BMI, body mass index; MAMA, Maternal Mental Health.

participants. However, there are some known side effects to consider such as risk of deep vein thrombosis, which is already increased due to pregnancy and childbirth.³⁹ Women who are at a known increased risk of venous thromboembolism will not be included in the trial.

The most frequently reported side effects of transdermal estradiol treatment are headache, redness or irritation of the skin (where the patch was worn), bloating, nausea and abdominal pain.

There are only a few trials investigating potential side effects regarding breastfeeding. A study that investigated 100 µg transdermal estradiol per 24 hours found no traces in breast milk.⁴⁰ Another study evaluated between 50 and 200 µg transdermal estradiol and found no significant association between the mother's and the infant's serum estradiol level.⁴¹ In the same study, no associations were found between the infant's growth, measured by weight, length and head circumference and estradiol treatment.⁴¹ In the MAMA trial, participants will be monitored and assessed through a breastfeeding questionnaire developed for the purpose and by using both weight change as percentage of birth weight and frequency of exclusive breastfeeding (without supplemental nutrition) after 3 weeks as outcome parameters.

Participants will be prompted to contact the investigators if they experience any adverse side effects. Moreover, participants will be asked about side effects 1 week postpartum and 3 weeks postpartum (at the end of the trial drug administration). The investigator at each site will assess severity and possible association to trial medication. Serious adverse events will be reported to the sponsor

investigator, monitor, and relevant authorities immediately, and unblinding is permissible.

Outcomes

The primary outcome of interest is clinical depression, according to the Diagnostic and Statistical Manual of Mental Disorders criteria of MDD with onset at any time between week 0 and 6 months postpartum diagnosed by a medical doctor specialised in psychiatry.

The participants will be screened for depressive symptoms at the follow-up assessments 3 and 8–10 weeks postpartum by the Edinburgh Postnatal Depression Scale (EPDS) and the Major Depression Inventory (MDI). Moreover, the participants will be screened for depressive symptoms at 8–10 weeks postpartum by a semistructured interview using the 17 items Hamilton Depression Rating Scale (HAMD-17). In case the participants present depressive symptoms (EPDS score of ≥ 9 or MDI score ≥ 20 or HAMD-17 ≥ 11) or if there is a clinical suspicion without scores on EPDS, MDI or HAMD-17 over cut-off, the participants will be booked for a clinical psychiatric interview with a doctor specialised in psychiatry. The participants are also encouraged to contact the research team if they experience depressive symptoms outside the follow-up assessments. Moreover, if the participants are admitted to the hospital, the researchers get a notification through the regional e-health platform. At the follow-up assessment 6 months postpartum, the participants are also asked about any depressive episodes during the follow-up period.

Secondary outcomes include, but are not limited to, symptoms of depression postpartum, exclusive breastfeeding, cortisol dynamics, maternal distress sensitivity, cognitive function and developmental functioning of the infants. The secondary outcomes are described in [table 2](#).

Recruitment

The women are assessed for eligibility by a midwife or obstetrician when attending antenatal care at the outpatient clinic ([figure 2](#)). Eligible participants who verbally consent to receive more information about the trial are subsequently contacted by telephone for more detailed information about participating in the trial. On request, a face-to-face meeting is also possible. Written information is provided after the telephone (or face-to-face) information. A second call/face-to-face meeting is arranged to give the potential participant and her partner at time to consider participation and to have the opportunity to ask questions. Written informed consent will be obtained before inclusion in the MAMA trial.

Allocation and blinding

Participant allocation to active or placebo group will be conducted by the capital region pharmacy. The participants will be block randomised with a fixed block size (unknown to the investigator). Trial participants, clinical care providers, research assistants, investigators, outcome assessors and data analysts will all be blinded to allocation.

Participant timeline

Participation in the MAMA trial includes four visits lasting between 20 min and 3 hours. In addition, the participants are asked to answer an online questionnaire at 6 months postpartum ([table 3](#)).

All participants have follow-up assessments at 1 week postpartum (by telephone to assess any side effects), 3 weeks postpartum (at the hospital), 8–10 weeks postpartum (at the hospital) and 6 months postpartum (by telephone).

Baseline assessment at gestational week $\geq 34+0$

The participants receive a basic physical screening, including somatic status and medical history, blood pressure measurement and routine blood samples. Furthermore, at baseline, we perform cognitive testing, and the participants are asked to fill in the baseline questionnaires covering questions on sociodemographic, lifestyle and obstetric history ([table 3](#)). The participants will randomly undergo toxicology urine tests for detection of drug abuse within the last month.

Psychometrics

Depressive symptom severity will be assessed with a face-to-face, semistructured interviews (Hamilton Depression Rating Scale six items, HAM-D-6) by a trained healthcare professional.⁴²

Self-reported questionnaires are filled in at gestational week $\geq 34+0$ (for screening purposes and baseline

measurements) and 3 weeks, 8–10 weeks and 6 months postpartum ([table 3](#)).

Psychometric measures of psychological traits include trait questionnaires indexing personality (NEO Personality Inventory and State Trait Anxiety Inventory (STAI-trait)); early life stress information (Child Abuse and Trauma Scale); quality of parental bonding (Parental Bonding Instrument) and substance abuse and psychiatric family history (Online Stimulant and Family History Assessment).

Furthermore, psychometric measures of psychological states include questionnaires indexing mental well-being (WHO-5)⁴³; depressive symptoms (EPDS and MDI)⁴⁴; anxiety (STAI-state)⁴⁵; stress (Cohen Perceived Stress Scale, Cohen Parental Stress Scale, PSS); antenatal attachment (Maternal Antenatal Attachment Scale)⁴⁶; sleep quality (Pittsburgh Sleep Quality Index)⁴⁷; pleasure (Snaith-Hamilton Pleasure Scale), obsessive compulsive behaviour (Obsessive-Compulsive Inventory); PSS⁴⁸; parental reflection functioning (Parental Reflective Functioning Questionnaire)⁴⁹ and parental sense of competence (Parenting Sense of Competence) Scale.⁵⁰ Finally, the infants' social-emotional development is assessed by the mother and the assessor in cooperation by using the semistructured Ages & Stages Questionnaires, second edition.⁵¹

Neuropsychological tests

Neuropsychological testing covers a range of emotion-independent (cold) and emotion-dependent (hot) cognitive domains. Cognitive testing is placed two times in the study programme, at baseline and follow-up assessment week 8–10 postpartum.

Cold cognitive task domains include simple reaction time, declarative verbal memory (Verbal Affective Memory Test-24),⁵² working memory (WAIS-IV Letter-Number Sequence)⁵³ and cognitive flexibility (Intra-Extra Dimensional) and hot cognitive task domains include facial emotion recognition (ERT-e) and emotion detection threshold (IM).⁵⁴

Further infant emotion recognition (infant emotion detection) is assessed using the iMotions software, V.8.0, and integrated hardware.^{55 56} Two infant video clips, a 'distress' and a 'laughter' video, will be shown to the participants. The 'distress video' displays a 4-month old infant crying heavily, unattended by a caregiver. The 'laughter video' displays a mother and her quadruplets all laughing continuously. During the infant videos, participants' facial expressions and galvanic skin responses will be recorded using specialised software. Participants will be instructed to watch the videos passively, and the duration of the task is less than 2 min.

Also, set of 50 infant vocalisations of each 2 s duration in five intensities: most happy, moderately happy, neutral, moderately distressed and most distressed will be played and the women will rate how happy or distressed they think the infant is on a continuous numerical Likert scale ranging from -4 (most distressed) to $+4$ (most

Table 2 Secondary outcomes of the MAMA trial**Secondary outcomes**

<i>Maternal well-being</i>	<p>Level of depressive symptoms postpartum measured as mean continuous score on the Edinburgh Postnatal Depression Scale (EPDS) at 8–10 weeks postpartum.</p> <p>Level of depressive symptoms postpartum measured as mean continuous score at the Hamilton Depression Rating Scale, 6 items (HAM-D-6) at 8–10 weeks postpartum.</p> <p>Maternal mental well-being measured as mean continuous score at WHO-5 Well-Being Index (WHO-5).</p> <p>Level of anxiety postpartum measured as continuous score at the State Trait Anxiety Inventory (STAI) at 8–10 weeks postpartum.</p> <p>Maternal sleep quality rated by mean continuous score on the Pittsburgh Sleep Quality Index (PSQI) 8–10 weeks postpartum.</p>
<i>Maternal capacity</i>	<p>Level of maternal antenatal attachment to the unborn child rated by mean continuous score on the Maternal Antenatal Attachment Scale at third trimester of pregnancy.</p> <p>Level of parental stress measured as mean continuous score at the Parental Stress Scale (PSS) at 8–10 weeks postpartum.</p> <p>Level of parental reflection measured as mean continuous score at the Parental Reflective Functioning Questionnaire (PRFQ) and Parenting Sense of Competence scale (PSOC), 8–10 weeks postpartum.</p> <p>Proportion of women who exclusively breastfeed their infants at 8–10 weeks postpartum (questionnaire).</p>
<i>Maternal cognitive performance</i>	<p>Performance on non-emotional (cold) cognitive domains including: reaction time assessed with the Simple Reaction Time (SRT) task; declarative memory performance assessed with Verbal Affective Memory Test (VAMT-24); working memory performance assessed with WAIS-IV Letter-Number Sequence (LNS); and cognitive flexibility assessed with the Intra-Extra Dimensional Set Shifting task (IED).</p> <p>Performance on emotional (hot) cognitive domains including emotion recognition assessed with the Emotion Recognition Task-eyes (ERT) and emotion detection threshold assessed with the Emotional Intensity Morphing Task (IM).</p> <p>Performance on the iMotions Infant Emotion Test (maternal distress sensitivity and infant emotion detection (IET)) including eye-tracking and facial emotion analysis, and galvanic skin response to emotional infant vocalisations and videos.</p>
<i>Maternal biological markers</i>	<p>Genome-wide genetic polymorphisms, gene expression and DNA methylation levels from peripheral blood at baseline (third trimester of pregnancy) and 3 weeks postpartum.</p> <p>Cortisol dynamics measured as Cortisol Awakening Response (CAR) in saliva indexed as area under the curve with respect to baseline from 0 to 60 min from awakening at 3–5 weeks postpartum.</p> <p>Evening cortisol concentrations in saliva 3–5 weeks postpartum.</p> <p>Hair cortisol level dynamics, that is, concentration of cortisol in hair from mother, estimating cortisol exposure up to 6 months prior to delivery.</p> <p>Estradiol level in third trimester of pregnancy, that is, gestational week >34. Estradiol level in peripheral blood.</p> <p>Postpartum estradiol level in peripheral blood measured at 3 weeks postpartum.</p> <p>Changes in estradiol level in peripheral blood from baseline (third trimester of pregnancy) to 3 weeks postpartum.</p> <p>Postpartum progesterone level in peripheral blood measured at 3 weeks postpartum.</p> <p>Changes in progesterone level in peripheral blood from baseline (third trimester of pregnancy) to 3 weeks postpartum.</p> <p>Allopregnanolone level in third trimester of pregnancy, that is, gestational week >34. Allopregnanolone level in peripheral blood.</p> <p>Postpartum allopregnanolone level in peripheral blood measured at 3 weeks postpartum.</p> <p>Changes in allopregnanolone level in peripheral blood from baseline (third trimester of pregnancy) to 3 weeks postpartum.</p>
<i>Infant outcomes</i>	<p>Epigenetic markers for HPA axis control (stress hormone axis), FKBP5 methylation index, from the infant.</p> <p>Developmental functioning of the infants rated by mean continuous score at Bayley Scales of Infant and Toddler Development—third edition (Bayley-III).</p> <p>Social-emotional development in the infants rated by mean continuous score at the Ages and Stages Questionnaire—Social-Emotional, second edition (ASQ:SE-2).</p>

HPA, hypothalamic–pituitary–adrenal ; MAMA, Maternal Mental Health.

Figure 2 Recruitment pathways for the MAMA trial. MAMA, Maternal Mental Health.

happy).^{57–59} During the display of infant vocalisations and faces, participants' facial expressions will be recorded and rated using iMotions software and integrated hardware.

In addition, the infants' fine and gross motor function and prelinguistic behaviours will be assessed by Bayley Scales of Infant and Toddler Development—third edition (Bayley-III) at 8–10 weeks of age. The interaction lasts approximately 20 min.⁶⁰

Information from medical records

Information on pregnancy and delivery outcomes will be obtained from patient records. The information comprises: labour onset (spontaneous or induced); use of epidural anaesthesia during delivery (yes or no); duration of labour (measured in hours); mode of delivery (spontaneous delivery, vacuum extraction or caesarean section (emergency or planned)); blood loss at delivery (measured in ml); gestational age at delivery (measured in weeks and days); birth weight (measured in grams); Apgar score at 5 min; admission to neonatal intensive care unit; hospitalisation and complications postpartum.

Saliva

Saliva is collected to determine the total cortisol output across 1 day as well as dynamics of the HPA-axis, as indexed by Cortisol Awakening Response.^{61 62} Serial saliva samples will be sampled at home and collected 3–5 weeks postpartum. Participants will be instructed to take samples immediately after awakening in the morning and again after 15, 30, 45 and 60 min and at 22:00 before going to sleep. Saliva will be collected with Salivette Cortisol (Sarstedt, Nümbrecht, Germany). To ensure accurate saliva sampling, participants will be instructed about the procedure by trained study personnel and receive written take-home instructions for use. Compliance is assessed by a self-reported form.

Hair cortisol

Hair samples (20 mg) will be collected 0–1 day postpartum from the mother to determine cortisol levels

during pregnancy.⁶³ The concentration is determined in the 3 cm hair closest to the scalp and in the 3–6 cm hair from the scalp. Based on an average hair growth of 1 cm/month, this represents cortisol in the last 3 months and 3–6 months, respectively, before the sample is collected.

Blood samples

At baseline, all participants are screened for somatic disease markers to exclude somatic conditions with possible influence on depressive symptoms. Corresponding blood samples for determining relevant blood biomarkers including sex steroid hormones, DNA, PAXgene tubes for mRNA (for candidate biomarker^{18 64}) analyses are taken at inclusion in late pregnancy and at follow-up 3 weeks postpartum.

Serum estradiol, estrone and estriol concentrations will be quantified by a specific and validated by isotope dilution online TurboFlow-Liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodology.⁶⁵

DNA samples from the infant saliva

Buccal swabs from the infant are collected 0–1 day postpartum for epigenetic data and to determine genetic polymorphisms of importance for stress regulation.^{66 67}

Data management

Questionnaire and neuropsychological paper-and-pencil data are managed and stored using REDCap, a secure web application for managing online questionnaires.⁶⁸ Computer-based neuropsychological data are managed and stored in the Center for Integrated Molecular Brain Imaging (Cimbi) database while biological data are stored in the Cimbi biobank at Neurobiological Research Unit at Copenhagen University Hospital, Rigshospitalet.⁶⁹

Sample size

The recurrence (baseline) risk for PPD is 40% within 6 months postpartum.⁴ We calculated that a sample of 2*88 complete cases would provide the trial with 80% power (at a two-sided alpha level of 0.05) to detect a reduction in PPD of 50%. Thus, with a study number of 2*110, the design is considered solid and can tolerate 22% dropouts. Since women with a history of perinatal depression are at high risk of depressive episodes, we expect that at least 50 participants will develop manifest depressive episodes untreated and more will display subclinical depressive symptoms. This will allow for comparison of depressive episode frequencies between active and placebo groups and correlation analyses with relevant outcome parameters.

Statistical analysis

The primary statistical analysis will be performed based on the intention-to-treat principle. We will compare data on the primary outcome in terms of proportions of depressive episodes in the group with transdermal estradiol treatment versus placebo with a melded binomial test.⁷⁰ We will also report a p value from a Pearson's χ^2 test.

Table 3 Time schedule of enrolment, intervention and outcome measures of the MAMA trial

	Study period						
	Gestational week <34	Gestational week ≥34+0	0–1 day postpartum	1 week postpartum	3 weeks postpartum	8–10 weeks postpartum	6 month postpartum
Timepoint		Baseline			T1	T2	T3
Face-to-face meeting		X	X		X	X	
Enrolment							
Eligibility screening	X						
Informed consent		X					
Allocation			X				
Intervention							
Transdermal estradiol/placebo patch							
Assessment of side effects				X	X	X	
Outcomes and measures							
Medical history		X					
Obstetric history		X					
Socio-demography/lifestyle		X					
Blood samples		X			X		
Blood pressure		X	X		X		
Saliva sample mother (cortisol)					X		
Saliva sample infant (DNA)			X				
Hair sample mother			X				
HAMD-6		X				X	
STAI-AD		X					
CATS		X					
OS-FHAM short		X					
PBI (mother)		X					
PBI (father)		X					
NEO-P-IR		X					
EPDS		X			X	X	X
WHO-5		X			X	X	X
SHAPS		X			X	X	X
MDI		X			X	X	X
Cohen PSS		X			X	X	X
RRS		X			X	X	X
STAI		X			X	X	X
PSQI		X			X	X	X
OCI		X			X	X	X
MAAS		X					
PSS					X	X	X

Continued

Table 3 Continued

	Study period						
	Gestational week <34	Gestational week ≥34+0	0–1 day postpartum	1 week postpartum	3 weeks postpartum	8–10 weeks postpartum	6 month postpartum
PCOS					X	X	X
PRFQ							X
Breast feeding					X	X	X
ASQ:SE-2						X	
Bayley-III						X	
Neuropsychological tests		X				X	

ASQ:SE-2, Ages and Stages Questionnaire; Bayley-III, Bayley Scales of Infant and Toddler Development—third edition; CATS, Child Abuse and Trauma Scale; EPDS, Edinburgh Postnatal Depression Scale; MAAS, maternal Antenatal Attachment Scale; MDI, Major Depression Inventory; NEO-PI-R, Revised NEO Personality Inventory; OCI, Obsessive-Compulsive Inventory; PBI, Parental Bonding Instrument; PCOS, Parents' Sense of Competence Scale; PRFQ, Parental Reflective Functioning Questionnaire; PSQI, Pittsburgh Sleep Quality Index; PSS, Parental Stress Scale; Cohen PSS, Perceived Stress Scale; RRS, Rumination Response Scale; SHAPS, Snaith-Hamilton Pleasure Scale; OS-FHAM short, substance abuse and family history; STAI, State-Trait Anxiety Inventory—State; STAI-AD, State Trait Anxiety Inventory-Trait; WHO-5, WHO-5 Well-being index.

Demographic and other baseline variables will be displayed using descriptive statistics (eg, means, SD, median, IQR, proportions). We will test the group balance achieved by randomisation by appropriate statistical tests (eg, Student's *t* tests, Pearson's χ^2 tests).

Secondary outcomes with a continuous distribution will be compared between groups using the mean (Student's *t* test). Pearson's χ^2 test will be used for binary categorical data.

In case of participant dropout and when possible, we will use a mixed effect model to model the distribution of the outcome over time. It will enable us to include patients for which the outcome is missing (eg, depression score at 8–10 weeks) but have an intermediate measurement (eg, depression score at 3 weeks). We will test the mean or proportion difference between the two groups using a Wald test based on mixed-effects model estimates. Compared with complete-case analysis, this approach will be valid under weaker assumptions about the dropout mechanism and provide more precise estimates (ie, higher statistical power). Missing data due to participant drop-out will be handled using imputation when the cause of censoring makes the primary outcome predictable (eg, questionnaire information on psychological distress may inform expected primary outcome). Participants who experience depression at one assessment will be classified as depressed even when they may have missing values at other assessments. To deal with potential non-random dropout, we will use inverse probability of censoring weighting, where the censoring weights are estimated by a Cox regression with, for example, treatment group or depression severity as covariates.

As an attempt to estimate the causal treatment effect in the case that non-adherence is not occurring at random, we will also use an instrumental variable approach using randomisation as an instrument.^{71 72}

Development of novel genomic biomarkers for PPD

This will build on gene expression and/or DNA methylation profiles of genes involved in hormonal signalling pathways.

Oestrogen sensitivity as a predictor of estradiol patch versus placebo group differences in postpartum depressive episodes in high-risk women

Based on earlier observed gene expression profiles of women in third trimester who later developed a PPD^{3 17} and women who in a sex-hormone manipulation study responded with depressive symptoms,¹⁹ we will test if such patterns translate in the current study. Specifically, we will derive a polygenic gene expression profile score (PGES) based on the effect sizes of the previous studies and test if this 'cumulative PGES' is higher in estradiol patch-treated women who did *not* develop a postpartum depressive episode (defined as interview-based diagnosis) compared with women who did. Likewise, we will evaluate if women in the placebo group who developed a postpartum depressive episode has a higher 'cumulative PGES' relative to placebo-treated women who did not develop such an episode. Similarly, for the DNA methylation from the same set of transcripts, we will derive a combined polygenic DNA methylation profile score (PDMS) and perform the same set of analyses. All the above analyses will be repeated using EPDS scores at 8–10 weeks postpartum as the outcome measure of depressive symptoms in order to capture any patterns with subclinical depression.

An exploratory analysis will be performed on genome wide gene expression and DNA methylation profiles. The results for these analyses will be corrected for multiple testing using the Bonferroni method for the number of tests performed for gene expression and the study wide epigenome wide significant *p* value threshold

of $p < 9 \times 10^{-8}$.⁷³ First, individual gene-level analyses will be performed via linear mixed-effects models with random intercepts fitted to example gene expression/DNA methylation differences between the time points across the groups. Next, to determine whether the genes identified to be different across the groups are enriched for oestrogen-signalling genes, targeted examination of oestrogen signalling pathway genes will be performed via a Monte-Carlo approach. Finally, gene expression/DNA methylation dynamics across time will be examined via more advanced and statistically powerful methods such as K-means clustering and time-course Gene Set analysis⁷⁴ to identify sets of genes showing similar patterns via longitudinal gene set trajectories. Such gene set methods can be applied to the set of differentially expression/methylated genes from above or implemented on the predefined set of oestrogen pathway genes to identify specific group dynamics of genes over the two time points.

Oestrogen sensitivity as a predictor of recurrent PPD

Second, based on the same earlier observed gene expression/methylation profiles of women in third trimester who later developed a PPD, we will test whether such patterns translate in the current study. Thus, we will test if the 'cumulative PGES and PDMS' defined above are associated with the onset of a depressive episode (interview-based diagnosis). This analysis will disregard any potential effects of oestrogen versus placebo treatment.

Again, to capture potential patterns of associations with subclinical PPD, we will also perform the analysis using EPDS scores at 8–10 weeks postpartum.

Patient and public involvement

During the preparation and design phase of the MAMA trial, we interviewed pregnant women with a history of perinatal depression who would have been considered eligible participants. The interviews lasted approximately 30 min. They were asked about their willingness to participate in such trial, about possible concerns regarding the trial medication and the amount of time required to participate in the trial. In addition, they were asked to assess the recruitment strategy.

They provided valuable insight, which led to considerations of ethical issues as well as feasibility, resulting in changes in the number of questionnaires to fill out postpartum and in the recruitment strategy.

During the trial, participants are invited to comment on any concerns on the setup or discomfort regarding the intervention. The comments will be taken into considerations for possible adjustments. We will comply with the General Data Protection Regulation by sending e-mails with sensitive personal information to the participants through an online digital mailbox (e-Boks). Using personal registration numbers, e-Boks is subjected to very restrictive legislation and is controlled and approved by the Danish Data Protection Agency.

ETHICS AND DISSEMINATION

Ethical considerations

The short-term transdermal estradiol treatment is not expected to pose unacceptable or intolerable side effects, disrupt breastfeeding or pass to the infant in any dosages that may pose a risk to the infant. Should adverse side effects for mother or infant occur or be suspected, the treatment will be discontinued immediately. When removing the patch, serum concentrations of estradiol return to baseline levels within 24 hours. Participants who develop mental distress or depressive symptoms that approach clinical thresholds will be referred to relevant care by a trained clinician. All potential participants receive oral and written information about the trial and all enrolled participants will provide written informed consent prior to inclusion. The partner receives written information about the trial regarding the infant and is offered oral information. Both parents provide written informed consent for the participation of the infant. Participants and parents can at any time withdraw their consent.

All potentially sensitive personal data will be anonymised. The trial will adhere to the Declaration of Helsinki.⁷⁵

Approvals and registrations

The trial protocol is approved by the Regional Committees on Health Research Ethics in the Capital Region of Denmark (H-20036213), the Danish Medicines Agency (EudraCT: 2020-001592-33) and the Knowledge Centre on Data protection Compliance in the Capital Region of Denmark (P-2020-712). The manufacturers of estradiol patches and placebo patches have been notified about the trial, as standard procedure from Danish Medicines Agency. They have not contributed to the protocol, nor have they made financial contributions to the trial.

Dissemination

We will present results at scientific meetings and in peer-reviewed journals. Results will be presented to policy-makers and engage the public, for example, via news media.

The results of this trial may be integrated in future recommendations on the clinical management of women at high-risk for PPD.

Monitoring

The trial is monitored ongoing by the Danish units for Good Clinical Practice in accordance with International Conference on Harmonisation for Good Clinical Practice.

Trial status

The MAMA trial was initiated on 4 January 2021. The first participant was included on 3 February 2021, the inclusion period is expected to run for 2–3 years, and the trial is expected to be completed by December 2026.

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Contributors VGF conceptualised the MAMA Trial with support from co-authors. SH, HKH, KMR, EC, AKT, AJ, CB, AJB, KWM, MV, DSS, VHD, BO, EB, DM and VGF participated in creating the study design. SH and VGF made the first draft of the manuscript. SH, BO, DM and VGF participated in creating the statistical analysis plan. All authors reviewed and revised the manuscript critically for important intellectual content. All authors reviewed and approved the final manuscript as submitted.

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Competing interests VGF declares that she has received honorarium as a consultant for SAGE therapeutics and Lundbeck Pharma A/S.

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REFERENCES

- World Health Organization. *Depression and other common mental disorders: global health estimates*. Geneva.: WHO, 2017.
- Lokuge S, Frey BN, Foster JA, et al. Depression in women: windows of vulnerability and new insights into the link between estrogen and serotonin. *J Clin Psychiatry* 2011;72:e1563-9.
- Mehta D, Newport DJ, Frishman G, et al. Early predictive biomarkers for postpartum depression point to a role for estrogen receptor signaling. *Psychol Med* 2014;44:2309-22.
- Wisner KL, Perel JM, Peindl KS, et al. Timing of depression recurrence in the first year after birth. *J Affect Disord* 2004;78:249-52.
- Gavin NI, Gaynes BN, Lohr KN, et al. Perinatal depression: a systematic review of prevalence and incidence. *Obstet Gynecol* 2005;106:1071-83.
- Woody CA, Ferrari AJ, Siskind DJ, et al. A systematic review and meta-regression of the prevalence and incidence of perinatal depression. *J Affect Disord* 2017;219:86-92.
- Stein A, Pearson RM, Goodman SH, et al. Effects of perinatal mental disorders on the fetus and child. *Lancet* 2014;384:1800-19.
- Hammerton G, Mahedy L, Mars B, et al. Association between maternal depression symptoms across the first eleven years of their child's life and subsequent offspring suicidal ideation. *PLoS One* 2015;10:e0131885.
- Sloman J, Honvo G, Emonts P, et al. Consequences of maternal postpartum depression: a systematic review of maternal and infant outcomes. *Womens Health* 2019;15:1745506519844044.
- Craddock N, Forty L. Genetics of affective (mood) disorders. *Eur J Hum Genet* 2006;14:660-8.
- Forty L, Jones L, Macgregor S, et al. Familiality of postpartum depression in unipolar disorder: results of a family study. *Am J Psychiatry* 2006;163:1549-53.
- Mitchell C, Notterman D, Brooks-Gunn J, et al. Role of mother's genes and environment in postpartum depression. *Proc Natl Acad Sci U S A* 2011;108:8189-93.
- Barth C, Villringer A, Sacher J. Sex hormones affect neurotransmitters and shape the adult female brain during hormonal transition periods. *Front Neurosci* 2015;9:37.
- Frokjaer VG. Pharmacological sex hormone manipulation as a risk model for depression. *J Neurosci Res* 2020;98:1283-92.
- Frokjaer VG, Pinborg A, Holst KK, et al. Role of serotonin transporter changes in depressive responses to sex-steroid hormone manipulation: a positron emission tomography study. *Biol Psychiatry* 2015;78:534-43.
- Comasco E, Frokjaer VG, Sundström-Poromaa I. Functional and molecular neuroimaging of menopause and hormone replacement therapy. *Front Neurosci* 2014;8:388.
- Mehta D, Grewen K, Pearson B, et al. Genome-Wide gene expression changes in postpartum depression point towards an altered immune landscape. *Transl Psychiatry* 2021;11:155.
- Guintivano J, Arad M, Gould TD, et al. Antenatal prediction of postpartum depression with blood DNA methylation biomarkers. *Mol Psychiatry* 2014;19:560-7.
- Mehta D, Rex-Haffner M, Søndergaard HB, et al. Evidence for oestrogen sensitivity in perinatal depression: pharmacological sex hormone manipulation study. *Br J Psychiatry* 2019;215:519-27.
- Dickens MJ, Pawluski JL. The HPA axis during the perinatal period: implications for perinatal depression. *Endocrinology* 2018;159:3737-46.
- Weigl T, Schneider N, Stein A, et al. Postpartal affective and endocrine differences between parents of preterm and full-term infants. *Front Psychiatry* 2020;11:251.
- Overgaard A, Lieblich SE, Richardson R, et al. Paroxetine blunts the corticosterone response to swim-induced stress and increases depressive-like behavior in a rat model of postpartum depression. *Psychoneuroendocrinology* 2018;89:223-8.
- Frokjaer VG, Erritzoe D, Holst KK, et al. Prefrontal serotonin transporter availability is positively associated with the cortisol awakening response. *Eur Neuropsychopharmacol* 2013;23:285-94.
- Jakobsen GR, Fisher PM, Dyssegaard A, et al. Brain serotonin 4 receptor binding is associated with the cortisol awakening response. *Psychoneuroendocrinology* 2016;67:124-32.
- Lu NZ, Eshleman AJ, Janowsky A, et al. Ovarian steroid regulation of serotonin reuptake transporter (SERT) binding, distribution, and function in female macaques. *Mol Psychiatry* 2003;8:353-60.
- Suda S, Segi-Nishida E, Newton SS, et al. A postpartum model in rat: behavioral and gene expression changes induced by ovarian steroid deprivation. *Biol Psychiatry* 2008;64:311-9.
- Bethea CL, Lu NZ, Gundlach C, et al. Diverse actions of ovarian steroids in the serotonin neural system. *Front Neuroendocrinol* 2002;23:41-100.
- Galea LAM, Frokjaer VG. Perinatal depression: embracing variability toward better treatment and outcomes. *Neuron* 2019;102:13-16.
- Dowlati Y, Ravindran AV, Segal ZV, et al. Selective dietary supplementation in early postpartum is associated with high resilience against depressed mood. *Proc Natl Acad Sci U S A* 2017;114:3509-14.
- Moses-Kolko EL, Berga SL, Kalro B, et al. Transdermal estradiol for postpartum depression: a promising treatment option. *Clin Obstet Gynecol* 2009;52:516-29.
- Gregoire AJ, Kumar R, Everitt B, et al. Transdermal oestrogen for treatment of severe postnatal depression. *Lancet* 1996;347:930-3.
- Li HJ, Martinez PE, Li X, et al. Transdermal estradiol for postpartum depression: results from a pilot randomized, double-blind, placebo-controlled study. *Arch Womens Ment Health* 2020;23:401-12.
- Wisner KL, Sit DKY, Moses-Kolko EL, et al. Transdermal estradiol treatment for postpartum depression: a pilot, randomized trial. *J Clin Psychopharmacol* 2015;35:389-95.
- Gordon JL, Rubinow DR, Eisenlohr-Moul TA, et al. Efficacy of transdermal estradiol and micronized progesterone in the prevention of depressive symptoms in the menopause transition: a randomized clinical trial. *JAMA Psychiatry* 2018;75:149-57.
- Munk-Olsen T, Laursen TM, Pedersen CB, et al. New parents and mental disorders: a population-based register study. *JAMA* 2006;296:2582-9.

- 36 Schulz KF, Altman DG, Moher D, *et al.* Consort 2010 statement: updated guidelines for reporting parallel group randomised trials. *Int J Surg* 2011;9:672–7.
- 37 Chan A-W, Tetzlaff JM, Altman DG, *et al.* Spirit 2013 statement: defining standard protocol items for clinical trials. *Rev Panam Salud Publica* 2015;38:506–14.
- 38 Danish Medical Birth Registry [Internet]. Available: <https://www.esundhed.dk/Registre/Det-medicinske-foedselsregister/Foedte-og-foedsler-1997-og-frem#tabpanel6119A72216248AC86DB508579760DED> [Accessed 10 Sep 2021].
- 39 Pomp ER, Lenselink AM, Rosendaal FR, *et al.* Pregnancy, the postpartum period and prothrombotic defects: risk of venous thrombosis in the MEGA study. *J Thromb Haemost* 2008;6:632–7.
- 40 Pinheiro E, Bogen DL, Hoxha D, *et al.* Transdermal estradiol treatment during breastfeeding: maternal and infant serum concentrations. *Arch Womens Ment Health* 2016;19:409–13.
- 41 Perheentupa A, Ruokonen A, Tapanainen JS. Transdermal estradiol treatment suppresses serum gonadotropins during lactation without transfer into breast milk. *Fertil Steril* 2004;82:903–7.
- 42 Hamilton M. Development of a rating scale for primary depressive illness. *Br J Soc Clin Psychol* 1967;6:278–96.
- 43 Topp CW, Østergaard SD, Søndergaard S, *et al.* The WHO-5 well-being index: a systematic review of the literature. *Psychother Psychosom* 2015;84:167–76.
- 44 Smith-Nielsen J, Matthey S, Lange T, *et al.* Validation of the Edinburgh postnatal depression scale against both DSM-5 and ICD-10 diagnostic criteria for depression. *BMC Psychiatry* 2018;18:393.
- 45 Spielberger CD, Gorsuch RL. *State-trait anxiety inventory for adults: sampler set: manual*. Tekst booklet and scoring key: Consulting Psychologists Press, 1983.
- 46 Condon JT. The assessment of antenatal emotional attachment: development of a questionnaire instrument. *Br J Med Psychol* 1993;66:167–83.
- 47 Buysse DJ, Reynolds CF, Monk TH, *et al.* The Pittsburgh sleep quality index: a new instrument for psychiatric practice and research. *Psychiatry Res* 1989;28:193–213.
- 48 Pontoppidan M, Nielsen T, Kristensen IH. Psychometric properties of the Danish parental stress scale: Rasch analysis in a sample of mothers with infants. *PLoS One* 2018;13:e0205662.
- 49 Luyten P, Mayes LC, Nijssens L, *et al.* The parental reflective functioning questionnaire: development and preliminary validation. *PLoS One* 2017;12:e0176218.
- 50 Gilmore L, Cuskelly M. Factor structure of the parenting sense of competence scale using a normative sample. *Child Care Health Dev* 2009;35:48–55.
- 51 Squires J, Bricker D, Potter L. Revision of a parent-completed development screening tool: ages and stages questionnaires. *J Pediatr Psychol* 1997;22:313–28.
- 52 Jensen CG, Hjordt LV, Stenbæk DS, *et al.* Development and psychometric validation of the verbal affective memory test. *Memory* 2016;24:1208–23.
- 53 Pezzuti L, Rossetti S. Letter-Number sequencing, figure weights, and cancellation subtests of WAIS-IV administered to elders. *Pers Individ Dif* 2017;104:352–6.
- 54 Dam VH, Thyrstrup CK, Jensen PS, *et al.* Psychometric properties and validation of the EMOTICOM test battery in a healthy Danish population. *Front Psychol* 2019;10:2660.
- 55 iMotions. *Facial expression analysis: the complete pocket guide* 2017, 2017.
- 56 Affectiva. *Emotion AI 101: All about emotion detection and Affectiva's Emotion Metrics*, 2017.
- 57 Young KS, Parsons CE, LeBeau RT, *et al.* Sensing emotion in voices: negativity bias and gender differences in a validation study of the Oxford vocal ('OxVoc') sounds database. *Psychol Assess* 2017;29:967–77.
- 58 Parsons CE, Young KS, Craske MG, *et al.* Introducing the Oxford vocal (OxVoc) sounds database: a validated set of non-acted affective sounds from human infants, adults, and domestic animals. *Front Psychol* 2014;5:562.
- 59 Bjertup AJ, Jensen MB, Schjødt MS, *et al.* Cognitive processing of infant stimuli in pregnant women with and without affective disorders and the association to postpartum depression. *Eur Neuropsychopharmacol* 2021;42:97–109.
- 60 Bayley N. *Bayley scales of infant and toddler development*. PsychCorp, Pearson, 2006.
- 61 Seth S, Lewis AJ, Galbally M. Perinatal maternal depression and cortisol function in pregnancy and the postpartum period: a systematic literature review. *BMC Pregnancy Childbirth* 2016;16:124.
- 62 Jairaj C, O'Leary N, Doolin K, *et al.* The hypothalamic-pituitary-adrenal axis in the perinatal period: its relationship with major depressive disorder and early life adversity. *World J Biol Psychiatry* 2020;21:552–63.
- 63 Staufenbiel SM, Penninx BWJH, Spijker AT, *et al.* Hair cortisol, stress exposure, and mental health in humans: a systematic review. *Psychoneuroendocrinology* 2013;38:1220–35.
- 64 Dahl J, Ormstad H, Aass HCD, *et al.* The plasma levels of various cytokines are increased during ongoing depression and are reduced to normal levels after recovery. *Psychoneuroendocrinology* 2014;45:77–86.
- 65 Frederiksen H, Johannsen TH, Andersen SE, *et al.* Sex-Specific estrogen levels and reference intervals from infancy to late adulthood determined by LC-MS/MS. *J Clin Endocrinol Metab* 2020;105:754–68.
- 66 Krontira AC, Cruceanu C, Binder EB. Glucocorticoids as mediators of adverse outcomes of prenatal stress. *Trends Neurosci* 2020;43:394–405.
- 67 Zannas AS, Wiechmann T, Gassen NC, *et al.* Gene-Stress-Epigenetic regulation of FKBP5: clinical and translational implications. *Neuropsychopharmacology* 2016;41:261–74.
- 68 Harris PA, Taylor R, Minor BL, *et al.* The REDCap Consortium: building an international community of software platform partners. *J Biomed Inform* 2019;95:103208.
- 69 Knudsen GM, Jensen PS, Erritzoe D, *et al.* The center for integrated molecular brain imaging (Cimbi) database. *Neuroimage* 2016;124:1213–9.
- 70 Fay MP, Proschan MA, Brittain E. Combining one-sample confidence procedures for inference in the two-sample case. *Biometrics* 2015;71:146–56.
- 71 Sjölander A, Martinussen T. Instrumental variable estimation with the R package ivtools. *Epidemiol Method* 2019;8.
- 72 Sussman JB, Hayward RA. An IV for the RCT: using instrumental variables to adjust for treatment contamination in randomised controlled trials. *BMJ* 2010;340:c2073.
- 73 Mansell G, Gorrie-Stone TJ, Bao Y, *et al.* Guidance for DNA methylation studies: statistical insights from the Illumina EPIC array. *BMC Genomics* 2019;20:366.
- 74 Hejblum BP, Skinner J, Thiébaud R. Time-Course gene set analysis for longitudinal gene expression data. *PLoS Comput Biol* 2015;11:e1004310.
- 75 World Medical Association, World Medical Association Declaration of Helsinki. *Ethical principles for medical research involving human subjects*. World Medical Association, 2018.