Maternal antibiotic exposure and the risk of developing antenatal or postpartum depression: The Maternal Experience Study protocol

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Abstract

Background

Limited epidemiological evidence suggests a link between antibiotic use and the development of depression. This study seeks to investigate this association in-depth, using a cohort of pregnant individuals. The primary aim is to explore any association between antibiotic use during pregnancy, delivery, and within 12 months postpartum, and the development of antenatal and/or postpartum depression.

Methods

A national prospective, observational, longitudinal cohort study has been designed to examine the relationship between the use of antibiotics during pregnancy and the development of antenatal depression up to the third trimester (32-42 weeks), as well as the use of antibiotics during pregnancy and within 12 months postpartum and the development of depression during the postpartum period. The development of depression is considered as either a diagnosis by a medical doctor and/or a scoring 13 or higher on the Edinburgh Postnatal Depression Scale. Data will be collected using online surveys, during the third trimester, and at 6 weeks, 6 months, and 12 months postpartum. These surveys include a wide range of variables previously identified as being associated with antenatal and postpartum depression (such as level of social support, history of depression, and intimate partner abuse), as well as antibiotic and probiotic use. The impact of the COVID-19 pandemic on both participants’ pregnancy experience and their mental health will also be explored. Recruitment began in August 2021, using a combination of online paid and unpaid advertisements, as well as distribution of the study flyer in relevant clinics and public spaces. It is anticipated that data collection will be completed in early 2024.

Discussion

This study will provide a much-needed update on the prevalence of depression during pregnancy and postpartum, and its associated factors. It will also, for the first time, comprehensively explore the potential association between antibiotic use during pregnancy and up to 12 months postpartum and the development of depression. Additionally, it will provide a better understanding of the mental health impacts of the COVID-19 pandemic on pregnant individuals in Australia.

Background

Depression is a leading cause of global disability, with an estimated 4.4% of the world’s population affected [1]. Several mechanisms are postulated to be involved in its pathophysiology. Of these, the gastrointestinal (GI) microbiota dysbiosis theory is increasingly garnering attention [2, 3]. Prior studies indicate that depression risk may increase by up to 20% following antibiotic exposure [4, 5]. This risk is increased further with the number of antibiotic courses and agents used and slowly reduces over the following ten years [4]. Although relatively consistent in their findings, there have been few studies exploring this association, each with inherent limitations related to their methodologies [6].
To explore the potential association between antibiotic exposure and the development of depression, we have designed a prospective cohort study in pregnant individuals. Adult pregnant individuals have been chosen for this study as they are a group who share some biological characteristics, who can be approached nationwide through targeted online media advertising and via relevant health professionals (e.g., obstetricians, general medical practitioners, midwives), and in whom approximately 1 in 2 individuals are exposed to antibiotics during their perinatal journey in Australia [7].

**Methods/Design**

**Aims**

The overarching aim of this project is to investigate the effect of exposure to antibiotics during pregnancy and the postpartum period (i.e., up to 12 months after giving birth) on the development of depression during pregnancy and up to 12 months postpartum.

**Study design and setting**

This study is an Australia-wide, prospective, longitudinal cohort study. Pregnant individuals will be recruited and complete four online surveys. Baseline data will be collected at the first survey, when individuals are at least 32 weeks pregnant. Follow-up data are collected at surveys completed around 6 weeks, 6 months, and 12 months postpartum. A 12-month follow-up period was chosen considering the World Health Organisation’s definition of postpartum depression (PPD) as being depression occurring within 12 months post-delivery [8].

**Inclusion and exclusion criteria**

Pregnant individuals who reside in Australia and are 18+ years will be eligible to participate. Participants will be excluded prospectively from the study if they are a surrogate, or if upon birth their child/ren will be cared for by someone else either permanently or semi-permanently. They will be excluded retrospectively if they have a stillbirth or lose their child for reasons such as miscarriage, prematurity, illness, or sudden infant death syndrome.

**Sample size calculation**

The Peduzzi formula for multivariate analyses [9] was used to estimate the required sample size for this study. In Australia, the point prevalence of depressive symptoms has been previously reported to be 9.5% at 12 months postpartum [10]. Calculations were made conservatively assuming 10 potential predictor variables, including antibiotic use, in the final multiple logistic regression model; this would require a sample size of 1,050 individuals. The recruitment target is 1,500, to account for a drop-out rate of up to 30%.

**Recruitment**
The study will be promoted both through online platforms (such as posts in pregnancy-related Facebook groups e.g., Pregnancy Support Group Australia) and targeted paid advertising (through Facebook, Google, and Instagram), as well as through strategic flyer placement in family doctors, midwife, and obstetrician practices, pharmacies, radiology clinics, supermarkets, and pregnancy-associated service areas (e.g., hypnobirthing classes, studios specialising in yoga in pregnancy). Individuals can find out more about the study and enrol if interested, by following the link on the advertisement/flyer or scanning the QR code. Participants will then be asked to read the electronic information sheet and provide consent through the electronic consent form. Prize draws will be used to encourage participation; there will be 41 chances to win a gift voucher for completing the study (with thirty AUD50 vouchers, ten AUD100 vouchers and one AUD500 voucher on offer). Participants can opt to be included in the draw by clicking on a link at the end of their final survey; this link will record their preference separate to their survey responses.

Data collection

A browser-based metadata driven capture system, Research Electronic Data Capture (REDCap), will be used for survey design and data collection. Data will be collected at four time points during an approximate 15-month period using online surveys. These time points will during the index pregnancy (at 32 or more weeks gestation), and at 6 weeks, 6 months, and 12 months postpartum. The first survey will be sent to participants when they are approximately 32 weeks pregnant, or as soon as possible thereafter, according to the information they provide in their consent form. In this first survey, the participant’s estimated due date (EDD) will be captured. They will then receive an email two weeks before their EDD, asking if they have delivered. If not, another email will be sent to them 4 weeks later. Only two time points have been chosen for these prompts, to avoid overburdening participants with correspondence.

The remaining surveys will be sent to participants based on their recorded delivery date; this ensures participants can complete the following survey if they have missed prior ones. They will receive up to 3 reminders to start any survey. Piloting data indicates the surveys take approximately 25 to 35 minutes to complete. Considering this, participants will be able to save their responses midway and resume later using a unique allocated password. They will then be sent up to 3 reminder prompts to complete each survey.

Data collected in the surveys will, where possible, be gathered using previously validated and/or used questions and scales. The data collected will include demographic and socio-economic variables (age, education, employment, income), medical and medication history (including history of personal and familial mental health disorders, and antibiotic use), and data relating to factors previously identified in studies as increasing the risk for developing depressive symptoms in general or specifically during and following pregnancy (Tables 1 and 2). Individuals who report having delivered in a public hospital in Tasmania, Australia (where the study team is based) will be asked to provide additional consent for the team to access their medical records at the hospital they delivered at, as well as from their local pharmacy(-ies). This additional step will be used to explore the accuracy of participants’ recall regarding antibiotic use.

In this study, the development of depression will be defined as a new diagnosis of depression made and/or confirmed by a medical doctor and/or obtaining a score of 13 or more on the Edinburgh Postnatal Depression Scale (EPDS) [11]. For example, in the first survey (which is completed in the third trimester), participants are
asked “During your pregnancy, were you at any point diagnosed with depression? This question relates only to a diagnosis made or confirmed by a medical doctor (e.g. GP, psychiatrist, obstetrician)” and in each follow-up survey (at 6 weeks, 6 months, and 12 months postpartum) individuals are asked “Have you been newly diagnosed with depression since you answered the previous survey?”. Additionally, participants will be asked to fill in the EPDS in each survey. A score of 13 or more is the standard cut-off score recommended for screening of major depression and has been used by prior Australian and international studies [11-18]. Furthermore, the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG) state that individuals with a score of 13 or more should be further reviewed, as this score may suggest a crisis [19].

In addition to the EPDS, all surveys will also include the Depression Anxiety Stress Scale (DASS-21) [20]. Unlike the EPDS, it assesses three aspects of mental health (depression, anxiety, and stress). Both validated tools are being used to allow collection of anxiety and stress symptoms, whilst also comparing the rate at which each tool identifies individuals requiring follow-up for depression symptoms.

Information regarding antibiotic use will be collected by asking participants about their antibiotic intake. For example, in the first survey the question is: “Have you taken any courses of antibiotics during this pregnancy?” and if they answer yes, then they will be asked for the name, number of courses, and the trimester that they used the antibiotics in.

To reduce the potential for illnesses requiring antibiotics to themselves bias the results, individuals will be asked prior to each survey whether they are using or have finished a course of antibiotics within the prior 14 days. If so, they will not be able to complete the survey until at least 14 days have passed following the self-reported expected date of completion for their antibiotics course; this will allow for recovery in the first week following antibiotics, and then assessment of their mental health state at the end of the second week (both the EPDS and DASS-21 ask individuals to respond reflecting on their experiences in the prior 7 days).

The consent form and the four surveys were piloted and extensively reviewed by four pregnant and parous individuals, with varying levels of English fluency and education, prior to their implementation. Participant recruitment began at the end of August 2021. Data collection is ongoing, and is anticipated to be completed in early 2024.

Table 1. Summary of proposed study variables at different time intervals.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Proposed tool</th>
<th>Description of tool</th>
<th>Pregnancy</th>
<th>Postpartum Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics and Socioeconomic (incl. participant +/- partner work) status</td>
<td>MPUQ</td>
<td>The Maternal Health Study conducted by Murdoch Children's Research Institute [21].</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Current medical history and medication use*, including antibiotic and probiotic use</td>
<td>IDQ</td>
<td>N/A</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Impact of COVID-19 pandemic on pregnancy experience</td>
<td>MPUQ</td>
<td>Modified questions from study by Durankuş &amp; Aksu [22].</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Antenatal class attendance</td>
<td>IDQ</td>
<td>N/A</td>
<td>✓</td>
<td>–</td>
</tr>
<tr>
<td>Physical activity</td>
<td>IDQ</td>
<td>N/A</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Social media usage (non-work related)</td>
<td>IDQ</td>
<td>N/A</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Pregnancy and fertility history</td>
<td>MPUQ</td>
<td>The Maternal Health Study conducted by Murdoch Children's Research Institute [21].</td>
<td>✓</td>
<td>–</td>
</tr>
<tr>
<td>Pregnancy expectation and experience</td>
<td>MPUQ and IDQ</td>
<td>The Maternal Health Study conducted by Murdoch Children's Research Institute [21].</td>
<td>✓</td>
<td>–</td>
</tr>
<tr>
<td>Delivery intention, expectation, and actual experience</td>
<td>MPUQ and IDQ</td>
<td>The Maternal Health Study conducted by Murdoch Children's Research Institute [21].</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Complications related to pregnancy/delivery</td>
<td>MPUQ</td>
<td>The Maternal Health Study conducted by Murdoch Children's Research Institute [21].</td>
<td>✓</td>
<td>–</td>
</tr>
<tr>
<td>Prior infant feeding experience</td>
<td>MPUQ and IDQ</td>
<td>The Maternal Health Study conducted by Murdoch Children's Research Institute [21].</td>
<td>✓</td>
<td>–</td>
</tr>
<tr>
<td>Intended and actual infant feeding method(s)</td>
<td>MPUQ and IDQ</td>
<td>The Maternal Health Study conducted by Murdoch Children's Research Institute [21].</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Previous contraception use</td>
<td>IDQ</td>
<td>N/A</td>
<td>✓</td>
<td>–</td>
</tr>
</tbody>
</table>
intended postpartum contraception use, and factors affecting use (e.g. personal +/- partner religion/cultural beliefs)

<table>
<thead>
<tr>
<th>Actual postpartum contraception use</th>
<th>IDQ</th>
<th>N/A</th>
<th>✓</th>
<th>✓</th>
<th>✓</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy within 12 months of index pregnancy</td>
<td>IDQ</td>
<td>N/A</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Knowledge about the postpartum period</td>
<td>IDQ</td>
<td>N/A</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Stressful life events</td>
<td>MPUQ</td>
<td>Modified questions from The Social Readjustment Rating Scale [23].</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior and current substance use (incl. alcohol, smoking, recreational drugs)</td>
<td>AUDIT-C, MPUQ and IDQ</td>
<td>AUDIT-C:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother-infant bonding</td>
<td>BPQ</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

AUDIT-C:
- Suggested by Australian Guide for the diagnosis of Fetal Alcohol Spectrum Disorder [24].
- Provides a standardised method for the assessment of maternal alcohol use.
- Allows categorisation of the level of fatal risk associated with maternal drinking by derivation of the AUDIT-C score.

The Maternal Health Study conducted by Murdoch Children's Research Institute [21].

Mother infant bonding
- Consists of a 25-item questionnaire [25].
- Designed to detect disorders of the mother-infant relationship.
<table>
<thead>
<tr>
<th>Measure</th>
<th>Description</th>
<th>CAS-Short</th>
<th>SQS</th>
<th>EPDS</th>
<th>DASS-21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current intimate partner abuse</td>
<td>• Consists of 18 items for assessing emotional or physical abuse by a partner or ex-partner [26].&lt;br&gt;• Explores history of abuse within the last 12 months. Developed and validated for use in general practices in Australia.&lt;br&gt;• Used by previous studies conducted in Australia exploring factors affecting PPD [10, 13, 27].</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleep quality</td>
<td>• Consists of a single item to measure overall sleep quality over the prior 7 nights [28].</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Depression</td>
<td>• Consists of a 10-item questionnaire [11].&lt;br&gt;• Widely used nationally and internationally.&lt;br&gt;• Recommended by RANZCOG for screening PPD.&lt;br&gt;• It has been used and validated for assessment of depression during pregnancy and postpartum.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Depression, anxiety, and life stress</td>
<td>• Consists of a 21-item questionnaire (7 questions for measuring each variable of depression, stress, and life stress).&lt;br&gt;• It is validated for its use in the general population, but not specifically for assessing</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
depression during pregnancy and postpartum [20, 29].

<table>
<thead>
<tr>
<th>Social support</th>
<th>OSSS-3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>

- Consists of a 3-item questionnaire.
- Measures level of social support [30].

IDQ = Investigator designed questionnaire, MPUQ = Modified previously used questionnaire, DASS-21 = Depression Anxiety Stress Scales; EPDS = Edinburgh Postnatal Depression Scale; OSSS-3 = Oslo Social Support Scale; SQS = Sleep Quality Scale; CAS-Short = The short version of the Composite Abuse Scale; PBQ = Postpartum Bonding Questionnaire; AUDIT-C = Alcohol Use Disorders Identification Test – Consumption; RANZCOG = the Royal Australian and New Zealand College of Obstetricians and Gynaecologists; PPD = Postpartum depression; N/A = Not applicable;

* At the time of completing a survey, if a participant is taking a course of oral or intravenous antibiotics or has completed a course in the previous two weeks, the survey will be delayed for two weeks following their expected date of completion. This is to minimise potential bias introduced by having an illness requiring antibiotic prescription on EPDS and DASS-21 scores. Upon returning to the survey after the delay, they will be asked again about their use of antibiotics in the prior two weeks; their survey commencement will be delayed again if their prior use continued past the expected date of completion.

† only smoking status will be explored at 12 months postpartum

**Ethics consideration**

There is no significant risk posed to the individual or their infant(s) from participating in this study. In acknowledgement, however, that some of the factors explored in this study may evoke feelings of discomfort (e.g., questions relating to intimate partner abuse), individuals will be repeatedly reminded throughout the study documents and surveys that participation is voluntary, responses are strictly confidential, and that none of the questions are compulsory. Furthermore, to encourage individuals to access services if required, a statement will preface all questions exploring mental health and experience of intimate partner abuse, with contact details for relevant nationwide counselling and support services. These individuals will be provided contact information for nationally available round-the-clock services that provide both counsellor and peer-support for individuals experiencing child loss.

Finally, the study platform will automatically identify and email individuals who either score 13 or more in the EPDS, or whose responses to specific questions indicate they, or their child, may be at risk of harm, informing them that their responses suggest they may benefit from a discussion with their preferred healthcare provider regarding their current circumstances. This email will also provide contact details for nationwide organisations
who provide support for individuals experiencing mental health crises, sexual assault, or family and domestic violence.

Data management

To protect participants’ privacy and identity, names and postal addresses will not be recorded for the main cohort; and any data derived from consenting participants’ medication information from Tasmanian hospitals and pharmacies in the sub-cohort, or address information for individuals who prefer to complete the survey using a hard copy, will be securely stored in a location separate to their survey data. Furthermore, all EDD and delivery date-related data will be removed prior to data analysis, and stored against participants’ study IDs in this same password-protected database located separately from the main database. All potentially identifying data will be permanently removed from all study databases following completion of the study and distribution of prize draws, unless participants provide consent for future follow-up in the final survey. The email addresses of individuals who provide this additional consent will be recorded against their allocated study ID, in a password-protected separate file stored in a separate location, accessible only to select members of the project team (MP, MT, MaW, TL, FV, and CM).

Data analysis and plan

The primary objective is to explore whether antibiotic use is associated with the development of depression. This will be explored during three time periods, as illustrated in Figure 1, and described below:

1. the antenatal period (from ~0 weeks gestation to mid-late third trimester);
2. perinatal period (from mid-late third trimester until 6 weeks postpartum); and
3. the postpartum period (from 6 weeks up to 6 months postpartum and from 6 months up to 12 months postpartum).

During the antenatal period, antibiotics used at any time during the index pregnancy will be explored as a potential predictor of depression. The outcome variable will be depression diagnosed during the index pregnancy and/or an EPDS score of 13+ at the time of completing the first survey during mid to late third trimester.

During the perinatal period, antibiotic use will be considered as any antibiotics that were received from ~0 weeks gestation until 6 weeks postpartum, including during caesarean section, labour, or hospitalisation. The outcome variable will be depression diagnosed after completion of the antenatal survey in the mid-late third trimester until 6 weeks postpartum and/or an EPDS score of 13+ at the time of completing the 6 weeks postpartum survey. Any individual diagnosed with depression during pregnancy in the antenatal survey will be marked as having had antenatal depression in the index pregnancy.

During the postpartum period at two intervals, from 6 weeks up to 6 months postpartum and from 6 months up to 12 months postpartum, antibiotic use will be defined as having used antibiotics at any time during the index pregnancy up until the end of 6 months postpartum and 12 months postpartum, respectively. The
outcome of interest will be defined as depressive symptoms (i.e., an EPDS score of 13+) at the time of completing the 6 months and 12 months postpartum survey and/or a new diagnosis of depression made or confirmed by a medical doctor in the period following the completion of the first postpartum survey (at approximately 6 weeks postpartum).

We acknowledge that collecting data at discrete time points, rather than continuously, creates some uncertainty regarding the relationship between the timing of antibiotic use and the timing of depressive symptoms. To address this issue, a sensitivity analysis will be conducted for each analysis using only an EPDS score of 13+ as the outcome measure, as the timing of the administration of the EPDS tool is clear in relation to antibiotic use that is disclosed in the same survey. In addition, the periodic nature of the surveys will allow us to exclude data from individuals whose depressive symptoms developed prior to any antibiotic use within the period of interest. For example, individuals diagnosed with depression during the antenatal period and then treated with antibiotics for the first time within the study period in the peripartum period (e.g., for mastitis) will be excluded from the logistic regression analysis of the perinatal period and postpartum period, as their diagnosis of depression preceded antibiotic use.

To explore potential predictors of depression, logistic regression analyses will be used. The primary outcome of interest in these analyses will be the proportion of individuals who develop depression, either as a new diagnosis made or confirmed by a medical doctor and/or an EPDS score of 13+.

Table 2 contains a list of all variables that will be used in the univariate analyses. De-identified data will be analysed using SPSS (Statistical Package for Social Sciences, IBM® Armonk, New York, USA). The Shapiro-Wilk Test of Normality and Normal Q-Q Plot will be used to test the data for normality numerically and graphically, respectively. If the data is approximately normally distributed, the following analyses will be conducted. Continuous variables will be summarised as means with standard deviations. The differences between groups will be tested using the chi-square test for categorical variables and independent t-test and one-way analysis of variance (ANOVA) for continuous variables (using Tukey’s HSD test post-hoc if the data meets the assumption of homogeneity of variances, and Games Howell if not). Pearson’s rank coefficient will be calculated for measuring correlations between continuous variables. All variables with p values ≤ 0.10 in the univariate analyses will be included in the logistic regression analyses, after checking for collinearity, where p value ≤ 0.05 will indicate statistical significance. Findings will be summarised as adjusted odds ratios with 95% confidence intervals.

There are additional secondary objectives that will be explored in the analyses. Firstly, the incidence and pattern of postpartum depression (diagnosis and symptoms) will be characterised. To account for participant withdrawal, person-years of follow-up will also be reported for incidence of depression symptoms.

Secondly, there will be a comparison of the rates (and degree of overlap) with which individuals are identified by the EPDS and DASS-21 tools as experiencing symptoms of depression (defined as scoring 13+ in the EPDS, and 21+ in the depression component of the DASS-21).
Table 2. List of potential risk factors being investigated for depression during the pregnancy and postpartum periods.
<table>
<thead>
<tr>
<th>Variables to be explored</th>
<th>Reference for association with depression in prior studies, where applicable</th>
<th>Variable investigated during mid-late third trimester</th>
<th>Variable investigated during postpartum time points (6 weeks, 6 months, and 12 months postpartum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
<td>[31-33]</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>[34-36]</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Marital status</td>
<td>[37, 38]</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Socioeconomic status, maternal leave, partner maternal leave</td>
<td>[32, 38-45]</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Level of physical activity during pregnancy and postpartum</td>
<td>[46, 47]</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Smoking status, alcohol consumption, recreational drug use</td>
<td>[32, 48-50]</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Personal and family medical history (incl. depression, anxiety, other mental illness)</td>
<td>[33, 38, 40, 43, 51-55] and NVI</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Nausea and vomiting during pregnancy</td>
<td>[56]</td>
<td>✓</td>
<td>N/A</td>
</tr>
<tr>
<td>Pre- and perinatal admission to hospital</td>
<td>[57]</td>
<td>✓</td>
<td>N/A</td>
</tr>
<tr>
<td>Whether the pregnancy was unplanned</td>
<td>[38, 45, 58-63]</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Use of reproductive technologies</td>
<td>[55, 64]</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Gravidity and parity (incl. previous miscarriages/termination of pregnancy)</td>
<td>[45, 58, 62, 63, 65, 66]</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Whether they delivered in a private or public hospital</td>
<td>NVI</td>
<td>N/A</td>
<td>✓</td>
</tr>
<tr>
<td>Peri- and postpartum complications for mother and baby (e.g., painful caesarean section wound)</td>
<td>[52, 57, 60, 67, 68]</td>
<td>N/A</td>
<td>✓</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td>[54, 69-71]</td>
<td>N/A</td>
<td>✓</td>
</tr>
<tr>
<td>If the birth plan/preferences were able to be followed</td>
<td>NVI</td>
<td>N/A</td>
<td>✓</td>
</tr>
<tr>
<td>Length of stay in hospital postpartum</td>
<td>[72, 73]</td>
<td>N/A</td>
<td>✓</td>
</tr>
</tbody>
</table>
Social support level | [40, 43, 45, 58, 61] | ✓ | ✓ |

Medication use (e.g., antibiotic and probiotic, hormonal contraceptive use, antidepressant/anxiolytic use) | [74-78] | ✓ | ✓ |

Number of other children to care for | [79] | ✓ | ✓ |

Current partner abuse | [13, 40, 45, 51, 60-62, 80] | ✓ | ✓ |

Antenatal depression | [38, 51] | - | ✓ |

Symptoms of stress and anxiety | [40, 53, 54] | ✓ | ✓ |

Stressful life events | [39, 53, 60] | - | ✓ |

COVID-19 stress and psychological impact of pandemic | [22, 81] | ✓ | ✓ |

Sleep quality | [82, 83] | ✓ | ✓ |

Social media use | [84, 85] | ✓ | ✓ |

Feeding plans and outcome | [86, 87] | ✓ | ✓ |

Contraception | [78, 88-90] | ✓ | ✓ |

N/A = Not applicable; NVI= New variable for investigation.

**Discussion**

Strengths and limitations of the study

One of the main strengths of this study is the scope and comprehensiveness of the data collected. There has been one prior study exploring the effect of antibiotic exposure on the risk of developing depressive symptoms in this population; however, it was small (n = 120), very limited in the variables collected and only considered antibiotics used during and within 14 days of delivery, despite reporting on their potential effect on mental health up to 6 months postpartum [74]. In contrast, our study is designed to be statistically powered to comprehensively investigate the potential association between antibiotic use during pregnancy, delivery, and up to 12 months postpartum and the development of depression, taking into account a large range of relevant variables.

There are, however, some limitations to this study. As discussed in the *Data Analysis and Plan* section, the collection of data at discrete time points introduces uncertainty when exploring a possible connection between antibiotic usage and depressive symptoms. To mitigate this limitation, a preliminary screening of the data will be conducted to exclude individuals who reported being diagnosed with depression and/or experiencing depressive symptoms prior to antibiotic use in previous surveys. Additionally, sensitivity analyses will be performed, focussing specifically on the EPDS as the outcome measure. This decision is based on the
relatively clear timing of the administration of the EPDS tool in relation to antibiotic use that is disclosed in the same survey.

Other limitations include the potential for individuals who are experiencing depression to be more likely to withdraw/drop out from the study, with the risk increasing with severity of depression. Additionally, participants who are experiencing abuse or similarly significant risk factors for depression may choose to not answer relevant questions. Due to the online and confidential nature of the study, we will be unable to explore the reasons for these decisions, or to identify if they are related to the development of depression, or other factors, such as time constraints. Another limitation is the sole reliance on individuals to provide accurate data, and the possibility of under-reporting antibiotic use due to the busy nature of parenthood and/or the long period between surveys. To explore this potential limitation, we will compare recalled antibiotic use to antibiotic prescription and dispensing data in a sub-cohort of Tasmanian participants.

If this study identifies associations between antibiotic use and changes in mental health outcomes for participants, future clinical trials could be conducted to evaluate the impact of probiotics and/or prebiotics on mitigating these effects, and on mental health in general. The results may also contribute to efforts already being made to reduce unnecessary antibiotic use worldwide.

**Abbreviations**

ANOVA  Analysis of variance

AUDIT-C  Alcohol Use Disorders Identification Test – Consumption

CAS-Short  The short version of the Composite Abuse Scale

DASS-21  Depression Anxiety Stress Scales

EPDS  Edinburgh Postnatal Depression Scale

EDD  estimated due date

IDQ  Investigator designed questionnaire

MPUQ  Modified previously used questionnaire

N/A  Not applicable

NVI  New variable for investigation

OSSS-3  Oslo Social Support Scale

PBQ  Postpartum Bonding Questionnaire

PPD  Postpartum depression

RANZCOG  the Royal Australian and New Zealand College of Obstetricians and Gynaecologists
Declarations

Ethic approval and consent to participate

Ethics approval for this study has been obtained from the University of Tasmania Human Research Ethics Committee (H0021790). All methods have been, and will continue to be, carried out in accordance with the Declaration of Helsinki. In this research, all the participants provide written online informed consent to participate in this study.

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The author(s) declare that they have no competing interests.

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Authors' contributions

MP, MT, CM, FV, GP, JH, TL, and MaW developed the study design, questionnaires, consent form, study flyer, participants information sheet, and study protocol. CM and MT completed and submitted the Ethics Application. MP, MT, TL, and CM designed and developed the online surveys on REDCap. CM, FV, and MaW piloted the study, alongside testers not within the team. KA and MeW provided clinical and practical opinions on the questionnaires. All the authors helped in the recruitment process. MP drafted this protocol paper. All authors read, provided comments on, and approved the final manuscript.
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References


Figures
Figure 1

The period of antibiotic exposure in relation to the diagnosis of depression and measured depressive symptoms (via the EPDS).

The different time periods where the exposure to antibiotic use was considered, as well as the diagnosis of depression was reported. The shows the point of time where the Edinburgh Postpartum depression Scale (EPDS) was used for screening of depressive symptoms.