PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Cannabis Use in Pregnancy and Downstream effects on maternal and infant health (CUPiD): A Protocol for a Birth Cohort Pilot Study
AUTHORS	Ramlawi, Serine; Murphy, Malia; Harvey, Alysha; White, Ruth; Gaudet, Laura; McGee, Amy; DeGrace, Amanda; Cantin, Christina; El-Chaar, Darine; Walker, Mark; Corsi, Daniel J.

VERSION 1 – REVIEW

REVIEWER	Lo, Jamie O. Oregon Health and Science University
REVIEW RETURNED	23-Jul-2022

GENERAL COMMENTS	This is a study protocol submission for a planned pilot prospective study to demonstrate feasibility for modern recruitment and data collection strategies adapted to the current cannabis environment to inform the design of a future full-scale prospective birth cohort. There are a few minor suggestions for the authors: - Consider not include pregnancies complicated by aneuploidy as this may confound the proposed study outcomes (e.g. apgar score, NICU admission, preterm birth, etc..) to assess the impact of prenatal cannabis exposure - In the analysis, consider also relevant maternal co-morbidities (e.g. Type 1 diabetes, chronic hypertension) that may also confound the primary outcomes studied - Currently authors have proposed to collect chorionic villous tissue biopsies from evenly spaced locations around the umbilical cord insertion site. There are different methodologies for placental tissue collection - it is very important that if any future RNA sequencing work is to be done that this is collected in <30min of delivery and that collection follows this paper for best representative results: https://pubmed.ncbi.nlm.nih.gov/31184493/

REVIEWER	Vyas , Arpita Kalla California Northstate University
REVIEW RETURNED	10-Aug-2022

GENERAL COMMENTS	In this manuscript, Ramlawi and colleagues consider an important topic, addressing the downstream effects of maternal cannabis use on maternal and infant health. They do a nice job of pointing out the need for more comprehensive prospective studies in this field addressing frequency, dose, model of consumption, chronicity of its use during pregnancy and other avenues for postpartum exposures including thru breast milk.

	Major comments: 1) Under exposure (Page 7 line 20-22): It is unclear why ± to 20 weeks of gestation was picked at a timepoint. Since the exposure measure is across pregnancy it may be better altering the statement …"The exposure of interest is consuming any cannabis related product in pregnancy starting in the first trimester" Most exposures are significantly harmful during the first trimester ±13 weeks. 2) Data collection (Page 9-Page 11) Table 2 (page 9 line 20-40) and Table 3 (page 11 line 1-26) : I have a concern with the 1st visit ± 20 weeks as this can include BOTH first and second trimesters and data will be collected in the second trimester at visit 2. I would recommend separating the visits into first trimester, second trimester and third trimesters as visits 1, 2 and 3 respectively to avoid overlaps which will occur if you leave visit 1 recruitment to anyone ± 20 weeks. Minor issues: 1) Under outcomes (Page 7 line 45-47) since some of the secondary outcomes the authors will measures have been measured in other studies it may be worth adding a line regarding association of these outcomes with frequency, route, timing, and duration of cannabis use during pregnancy and immediate postpartum period. 3) Table 1 (line 22-41) under exclusion criteria apart from those using illegal drugs, consider also either excluding or controlling for cigarette smoking and alcohol consumptions (as can be confounding factors). 4) Table 3 (page 11 line 1-26) under visit 5: infant samples "cord blood" I am presuming authors mean peripheral blood as not possible to collect cord blood at this age. 5) Page 13 line 12-22 under analysis of secondary outcome: consider controlling for gestational diabetes and alcohol consumption as well

REVIEWER	Sasso , Elizabeth B. University of Southern California, OBGYN
REVIEW RETURNED	16-Aug-2022

GENERAL COMMENTS	This is a pilot study establishing the feasibility of establishing a multi-center prospective cohort for the study of the effect of cannabis exposure prior to 20 weeks on the mother, fetus and child. Secondary outcomes of this study will include perinatal and neonatal outcomes. 50 cannabis users and 50 non-users will be recruited for the initial study. Participants will be excluded if they are <16 years old, cannot provide informed consent, not delivering at participating site, surrogate or giving child up for adoption, >20 weeks, using non-prescription controlled and illegal drugs other than cannabis or using prescription opioids. I recommend including alcohol use as an exclusion criteria as this may impact the quality of the data being collected. Participants will have 5 visits with the study team. During each visit, a survey will be performed, anthropometric measurements will be taken and blood/tissue samples will be taken and stored for analysis of cannabinoid metabolites. Participants will be reimbursed for travel and given a gift card at each study appointment. With regard to the methodology - there is not enough

	detailed information on the surveys or plan for laboratory analysis of biological samples for this portion of the study to be replicated. The plan for sample analysis includes immunoassay screening, liquid chromotography-tandem mass spectrometry by validated methods that are not referenced in the protocol. I suggest adding an attachment of the proposed patient survey and adding a reference to these validated methods for the purpose of replication. This will likely be modified once the study has begun, however the details are too vague at this time for replication. Data analysis will be reported using STROBE guidelines. Descriptive statistics will be reported and summarized for each group using frequency, means and medians. Distributions of characteristics will be reported based on non-exposure or tertile of exposure (low vs moderate vs heavy). I suggest operationalizing how cutoffs for low/moderate/heavy will be determined. The primary outcome will be assessing participant recruitment, reason for exclusion, engagement and rate/reason for withdrawal, summarized descriptively. Secondary outcomes will be compared based on reported cannabis use (rather than from metabolites collected in blood/tissue samples). I recommend including the rationale for not aligning metabolite levels to assessment of secondary outcomes in favor or reported use, as this may negatively impact results by reporting bias. Overall this is an important proposal for a pilot study that will determine feasibility of a multi-site cohort study of cannabis users with the potential to yield long term data on the effects of cannabis use in the pregnant population and downstream effects on the exposed neonate.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1:
1. Consider not include pregnancies complicated by aneuploidy as this may confound the proposed study outcomes (e.g. apgar score, NICU admission, preterm birth, etc..) to assess the impact of prenatal cannabis exposure

Response: Thank you for your comment. We have decided not to explicitly list fetal anomalies as exclusion criteria for study entry. We are planning to recruit patients early in pregnancy. Therefore, these individuals may not have had their diagnostic ultrasound, where anomalies may be identified. For this pilot study, we will not exclude those with anomalies; however, any anomaly identified on ultrasound or at birth will be documented with the date of diagnosis. These patients may be later excluded at the analytical stage to address possible confounding. Further, based on analysis of the pilot study data, we may consider an exclusion for fetal anomalies in the main study, we have discussed this possibility in a new section titled “considerations for the main study” on page 12.

2. In the analysis, consider also relevant maternal co-morbidities (e.g. Type 1 diabetes, chronic hypertension) that may also confound the primary outcomes studied

Response: Thank you for your comment. Relevant co-morbidities and existing health conditions will be collected by reviewing the participant's medical chart as well as documented by the participant in the surveys. All co-morbidities will be considered for multivariable adjustment in the analyses for the secondary outcomes.

3. Currently authors have proposed to collect chorionic villous tissue biopsies from evenly spaced locations around the umbilical cord insertion site. There are different methodologies for placental tissue collection - it is very important that if any future RNA sequencing work is to be done that this is collected in <30min of delivery and that collection follows this paper for best representative results: https://can01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fpubmed.ncbi.nlm.nih.gov%2F31184493%2F&data=05%7C01%7Csramlawi%40ohri.ca%7Cff6507d832aa4a9514b608da86b0d105%7C859b41b6130f4d13a6931ffec4e7cb5a%7C0%7C0%7C637970391226607010%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=7lal7IQjeAfpj4S9%2Fl5WcLrf2EaVqyj9VkwbuFcx6fQ%3D&reserved=0

Response: Thank you for sharing this publication with us. We will be following similar methodologies for placental tissue collection. We anticipate completing placenta sample collection within 30 minutes of delivery, we include this citation and clarify these methods on page 10.

Reviewer 2:
1. Under exposure (Page 7 line 20-22): It is unclear why ± to 20 weeks of gestation was picked at a timepoint. Since the exposure measure is across pregnancy it may be better altering the statement …"The exposure of interest is consuming any cannabis related product in pregnancy starting in the first trimester"
Most exposures are significantly harmful during the first trimester ±13 weeks.
Response: Thank you for the insight regarding the chosen time points. We appreciate that exposures may be most harmful during the first trimester, and in this pilot study, we aim to recruit patients as early as eight weeks' gestation. However, although efforts will be made to recruit patients early in pregnancy, from our previous prospective studies, we experienced some challenges in recruiting patients in the first trimester. Therefore, for this pilot, we will allow recruitment for up to 20 weeks' in order to maximize recruitment and testing of our study procedures. In addition, we will document the gestational age at recruitment to plan the main study. Based on these data and the rate of first-trimester recruitment, we will consider changing the inclusion criteria and updating the visit timepoints to better align with each trimester. (see page 12)
2. Data collection (Page 9-Page 11)
Table 2 (page 9 line 20-40) and Table 3 (page 11 line 1-26) : I have a concern with the 1st visit ± 20 weeks as this can include BOTH first and second trimesters and data will be collected in the second trimester at visit 2. I would recommend separating the visits into first trimester, second trimester and third trimesters as visits 1, 2 and 3 respectively to avoid overlaps which will occur if you leave visit 1 recruitment to anyone ± 20 weeks.
Response: Thank you for the comment. Ideally, we will aim to recruit patients in the first trimester, with visit one conducted before 13 weeks' gestation. Recruitment before 13 weeks will allow the remaining pregnancy visits to align with each trimester. However, as described above, because we anticipate some challenges to first-trimester recruitment, we allow recruitment for up to 20 weeks for the pilot study. Therefore, the remaining pregnancy visits will correspond to the intervals given in table 2. Gestational age at recruitment and each study visit will be recorded and analyzed to see how many patients we recruit before 13 weeks. Based on this, we will determine if recruiting patients efficiently within the first trimester is feasible. If so, we will update the protocol for the main study to specify that visit one will occur in the first trimester. We describe this in the 'considerations for the main study on page 12'
3. Under outcomes (Page 7 line 45-47) since some of the secondary outcomes the authors will measures have been measured in other studies it may be worth adding a line regarding association of these outcomes with frequency, route, timing, and duration of cannabis use during pregnancy and immediate postpartum period.
Response: We agree with this suggestion and plan to investigate these associations. We have added the following line to page 12 under "Analysis of secondary outcomes". "Although we will have a limited sample size, we will conduct exploratory analyses to estimate differences in the risk of perinatal

outcomes between classes of cannabis exposure (i.e., frequency and secondhand exposure), route of cannabis consumption, timing in pregnancy and postpartum, and duration of cannabis use in pregnancy and postpartum."

4. Table 1 (line 22-41) under exclusion criteria apart from those using illegal drugs, consider also either excluding or controlling for cigarette smoking and alcohol consumptions (as can be confounding factors).
Response: We agree that cigarette smoking and alcohol may be confounding factors, and we plan to control these in all analyses. We will not explicitly exclude patients using tobacco or alcohol in the pilot study. Still, we will analyze the use rates of these substances in the study population. In addition, we will consider alcohol and tobacco use for later modification of the exclusion criteria for the main study (see page 12, considerations for the main study).
5. Table 3 (page 11 line 1-26) under visit 5: infant samples "cord blood" I am presuming authors mean peripheral blood as not possible to collect cord blood at this age.
Response: Thank you for the clarification. We have updated Table 3 to reflect that at visit 5 we will be collecting infant blood. It is discussed in the text below Table 3 that infant blood will be collected as a dried blood spot. We will not be collecting peripheral blood from infants.
6. Page 13 line 12-22 under analysis of secondary outcome: consider controlling for gestational diabetes and alcohol consumption as well
Response: We will collect data on gestational diabetes and alcohol use from the survey questionnaire and medical records. We will control for any medical conditions (i.e., gestational diabetes), tobacco and alcohol consumption, and other confounding factors in the analysis of the secondary outcomes.
Reviewer 3:
1. 50 cannabis users and 50 non-users will be recruited for the initial study. Participants will be excluded if they are <16 years old, cannot provide informed consent, not delivering at participating site, surrogate or giving child up for adoption, >20 weeks, using non-prescription controlled and illegal drugs other than cannabis or using prescription opioids. I recommend including alcohol use as an exclusion criteria as this may impact the quality of the data being collected.
Response: We agree that alcohol use may be a confounder with implications for cannabis-outcome associations. For the pilot study, we will collect data on alcohol use in pregnancy, although we will not explicitly exclude patients for alcohol use. Analyses with outcomes will control for potential confounding factors, including alcohol consumption. As part of the current study, we will analyze the data on alcohol and substance use, and for the main study, we may revise the exclusion criteria. We discuss this on page 12 under 'considerations for the main study.'
2. Participants will have 5 visits with the study team. During each visit, a survey will be performed, anthropometric measurements will be taken and blood/tissue samples will be taken and stored for analysis of cannabinoid metabolites. Participants will be reimbursed for travel and given a gift card at each study appointment. With regard to the methodology - there is not enough detailed information on the surveys or plan for laboratory analysis of biological samples for this portion of the study to be replicated. The plan for sample analysis includes immunoassay screening, liquid chromotography-tandem mass spectrometry by validated methods that are not referenced in the protocol. I suggest adding an attachment of the proposed patient survey and adding a reference to these validated methods for the purpose of replication. This will likely be modified once the study has begun, however the details are too vague at this time for replication.
Response: Thank you for your recommendations, in our revised submission, we include all surveys, cannabis diary, and case report forms as an appendix (Appendix A).
For the sample analysis, we will work with a clinical diagnostic laboratory with experience and expertise in measuring and testing for the presence of drugs in various matrices. The lab is proficient and experienced in mass spectrometry for cannabis metabolite detection. Utilizing high-performance liquid chromatography coupled with mass spectrometry is a well-established and well-accepted analytical method to detect and measure cannabinoids and metabolites in clinical and research laboratories. We include additional publications on the topic in the protocol for reference (page 10,

references 38-41).
3. Data analysis will be reported using STROBE guidelines. Descriptive statistics will be reported and summarized for each group using frequency, means and medians. Distributions of characteristics will be reported based on non-exposure or tertile of exposure (low vs moderate vs heavy). I suggest operationalizing how cutoffs for low/moderate/heavy will be determined.
Response: Thank you for your comment. At this time, we have not specifically determined the cutoffs for low, moderate and heavy users, and we will adapt this based on our final population and analysis of the frequency of use. We anticipate low, moderate and heavy users to represent occasional, weekly (2-3 times per week), and daily users, respectively. Final tertiles will be decided after recruitment and evaluation of the use of the participants in our cohort. We described this on page 11 and page 12.
4. The primary outcome will be assessing participant recruitment, reason for exclusion, engagement and rate/reason for withdrawal, summarized descriptively. Secondary outcomes will be compared based on reported cannabis use (rather than from metabolites collected in blood/tissue samples). I recommend including the rationale for not aligning metabolite levels to assessment of secondary outcomes in favor or reported use, as this may negatively impact results by reporting bias.
Response: We agree with this suggestion. We now incorporate that the secondary outcomes will be assessed using reported cannabis use and detected metabolites from the collected biological samples (page 12)
Thank you again for your careful review of our manuscript. We look forward to hearing your response to the revised version.

VERSION 2 – REVIEW

REVIEWER	Lo, Jamie O. Oregon Health and Science University
REVIEW RETURNED	21-Sep-2022

GENERAL COMMENTS	No further suggestions.

REVIEWER	Vyas , Arpita Kalla California Northstate University
REVIEW RETURNED	26-Sep-2022

GENERAL COMMENTS	No further comments.

REVIEWER	Sasso , Elizabeth B. University of Southern California, OBGYN
REVIEW RETURNED	24-Oct-2022

GENERAL COMMENTS	The previous revisions suggested have been addressed. One further point of clarification is requested with regard to the analysis regarding Group A and Group B. It is not explicitly stated whether Group B will be screened for cannabis use using biological samples in addition to using subject self-report. Please address whether biological samples will be screened in Group B. In addition, in the analysis portion, please address how secondary analysis will be performed on participants who cross-over into Group A.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1
Dr. Jamie O. Lo, Oregon Health and Science University Comments to the Author:
No further suggestions.
Reviewer: 2
Dr. Arpita Kalla Vyas , California Northstate University Comments to the Author:
No further comments.
Reviewer: 3
Dr. Elizabeth B. Sasso, University of Southern California Comments to the Author:
The previous revisions suggested have been addressed. One further point of clarification is requested with regard to the analysis regarding Group A and Group B. It is not explicitly stated whether Group B will be screened for cannabis use using biological samples in addition to using subject self-report. Please address whether biological samples will be screened in Group B. In addition, in the analysis portion, please address how secondary analysis will be performed on participants who cross-over into Group A.

Response: Thank you for your comment. We plan to analyze the biological samples from both groups. We clarify this on page 10 of the revised manuscript. In addition, we will analyze participants according to their cannabis use, as determined through biological analysis of samples or self-report, and not explicitly according to the group at recruitment. This will allow us to analyze Group B participants crossing into Group A as cannabis users. We indicate this on Page 12 of the revised manuscript.