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)	PROTECT-Me: a parallel-group, triple blinded, placebo controlled randomised clinical trial protocol assessing antenatal maternal melatonin supplementation for fetal neuroprotection in early-onset fetal growth restriction.
AUTHORS	Palmer, Kirsten; Mockler, Joanne; Davies-Tuck, Miranda; Miller, Suzanne; Goergen, Stacy; Fahey, Michael; Anderson, Peter; Groom, KM; Wallace, Euan

VERSION 1 - REVIEW

REVIEWER	Ed Johnstone University of Manchester, UK
REVIEW RETURNED	28-Jan-2019

GENERAL COMMENTS	Please revisit line 70 - article summary Please bring second bullet point up to first and redo second sentence which doesn't make sense and is in wrong tense/mixed tenses. Second bullet should state number planned recruitment and primary and secondary outcomes. No need for study limitations this part of the study protocol. Otherwise well written and clear protocol. The only area I am very unclear on is the gestation split at 28 weeks between the 4 proposed groups and the reference used for justification. Looking at this referenced study, although it is difficult to estimate the exact gestation distribution of the cohort as this is incorrectly presented as mean and SD it seems likely from the weights that the majority of this cohort is >28 weeks at delivery (gestation at diagnosis is unknown). This makes it very difficult to estimate the effect size of being FGR diagnosed <28 weeks and therefore whether the sample size estimation is correct. Having thought about this and the fact that the majority of infants diagnosed before 28 weeks are going to be delivered after 28 weeks anyway I'm not convinced at present this is a logical or sensible split for the primary outcome. What would potentially more interesting is whether there is difference in outcome based on length of exposure to melatonin (e.g. </>2 weeks). Could the authors comment on this point?

REVIEWER	Viola Seravalli, MD Department of Health Sciences, University of Florence, Florence, Italy
REVIEW RETURNED	23-Feb-2019

GENERAL COMMENTS	The study design and methodology is appropriate. The rationale is well clarified and the methods are thoroughly described . I dont' see any major flaw in the study that would prevent a proper interpretation of the data, and I do not have any change to suggest.

VERSION 1 – AUTHOR RESPONSE

Response to Reviewer One's Comments:

- Please revisit line 70 - article summary. Please bring second bullet point up to first and redo second sentence which doesn't make sense and is in wrong tense/mixed tenses. Second bullet should state number planned recruitment and primary and secondary outcomes. No need for study limitations this part of the study protocol.

I thank the reviewer for highlighting a number of issues with this section. These have now been addressed, improving the communication of the trial's strengths. The limitation remains, as it is a requirement of the journal. This section now reads:

Strengths and limitations of this study

• The trial is powered to detect neurodevelopmental performance at 2-3 years of age from 332 pregnancies, which is a design strength, as assessments performed at an earlier age are less predictive of later neurodevelopmental functioning.

• The secondary outcomes, assessing maternal, fetal and neonatal safety and tolerability, will continue to add to the growing literature regarding the use of melatonin in pregnancy.

• The blinded, randomised, placebo-controlled nature of the trial with stratification to control for the impact of gestational age on neurodevelopmental outcomes strengthens the trial by reducing risks of bias on the results.

• The assessment of multiple developmental domains, including cognition, motor, language and behaviour, as well as structural development will further strengthen our understanding of the impact melatonin may have.

• A limitation of the trial is the current lack of longer-term follow-up, such as to 5-7 years of age, when improved assessment of executive function and other aspects of cognitive performance can be performed; though the trial group intend to maintain contact with the randomised cohort to enable later follow-up.

- The only area I am very unclear on is the gestation split at 28 weeks between the 4 proposed groups and the reference used for justification. Looking at this referenced study, although it is difficult to estimate the exact gestation distribution of the cohort as this is incorrectly presented as mean and SD it seems likely from the weights that the majority of this cohort is >28 weeks at delivery (gestation at diagnosis is unknown). This makes it very difficult to estimate the effect size of being FGR diagnosed <28 weeks and therefore whether the sample size estimation is correct. Having thought about this and the fact that the majority of infants diagnosed before 28 weeks are going to be delivered after 28

weeks anyway I'm not convinced at present this is a logical or sensible split for the primary outcome. What would potentially more interesting is whether there is difference in outcome based on length of exposure to melatonin (e.g. 2 weeks). Could the authors comment on this point?

I thank the reviewer for highlighting this point. From the available literature it is indeed challenging to exactly estimate the impact of gestation at diagnosis on subsequent neurodevelopmental performance. The power calculation has been based on results from a preterm cohort, however as you point out, these were generally delivered >28 weeks' gestation with no mention to timing of diagnosis. The trial is powered for those between 28-31+6 weeks gestation at recruitment. For those diagnosed with FGR <28 weeks' gestation findings from the TRUFFLE trial (Lees, UOG 2013;42(4):400-408), which reported on severe morbidity among survivors by gestational age at study entry, found that of those recruited at 26-27 weeks' gestation 37% had severe morbidity compared to 19% recruited between 28-31 weeks' gestation. While this was predominately in the form of sepsis and bronchopulmonary dysplasia, there was a 2.7-fold increased rate of germinal matrix and intraventricular haemorrhage in those diagnosed earlier. Of those recruited to the study <28 weeks, 67% were delivered >28 weeks' gestation. In addition, the work by Monier et al (BJOG 2017;124(12):1899-1906) looking at outcomes for fetuses identified as FGR between 21-27 weeks' gestation have shown significant differences in survival and outcome rates, of note substantial changes are seen between 23-27 weeks, which are the gestations to be included in our trial. They showed differences in clinician management preferences during this gestational window resulted in a livebirth rate of ~40% in those identified as FGR at 23-24 weeks' gestation, and >90% for those identified at 26-27 weeks' gestation. Due to these findings we felt it prudent to assess the impact of melatonin in both those with very early identification of FGR and those identified later in gestation. In regard to changing the comparison to duration of melatonin exposure, there is no available evidence regarding the impact of varying durations of treatment on neurodevelopmental outcomes to guide such a comparison. This information however will certainly be explored at the time of data analysis for the completed trial.

The sentence beginning at line 331 has been modified to further reflect this, so it now reads:

"There are no data to date to suggest melatonin will have a different effect size based on very early (<28 weeks) versus early-onset FGR (28-31+6 weeks), but we expect neurocognitive outcomes to differ by gestation at birth, as those identified with FGR before 28 weeks' gestation are known to have greater morbidity and mortality32,33." Two further references have been added to support this statement.