Title: A Randomised Controlled Trial of Antenatal Melatonin Supplementation in Fetal Growth Restriction for Fetal Neuroprotection.

Protocol V2.16 9 July 2024

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A Randomised Controlled Trial of Antenatal Melatonin Supplementation in Fetal Growth Restriction for Fetal Neuroprotection.

Complete Title:	A triple-blinded, randomized, parallel-group placebo-controlled trial (PROTECT Me trial) to assess the impact of maternal antenatal melatonin supplementation on early childhood neurodevelopmental outcomes in the setting of severe preterm fetal growth restriction.
Short Title:	A Randomised Controlled Trial of Antenatal Melatonin Supplementation in Fetal Growth Restriction for Fetal Neuroprotection**:** The PROTECT Me trial
Version & date:	Version 2.16, 09 July 2024
Phase of trial:	Phase III
HREC registration:	Monash Health HREC 17-0000-583A
ANZCTR registration:	ACTRN12617001515381
Universal Trial Number:	U1111-1203-6718
Lead Principal Investigator:	Dr Kirsten Palmer B.BioMed Sci, MBBS (Hons), PhD, FRANZCOG, CMFM Obstetrician, Monash Health Senior Research Fellow, Monash University
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Trial Sponsor:	Monash University Wellington Road Clayton VIC 3800

Protocol version control and change history

Version	Date from	Date to	Amendment
1.3	**18th October 2017**	**26th December 2017**	Ethics application, submission to Monash Health HREC
2.0	**27th December 2017**	**22nd May 2018**	HREC approval subject to conditions. Addition of DSMB Information. Inclusion of information regarding the treatment of tissue(s) following laboratory analysis, specifically, any future use.
2.1	**27th June 2018**		Following feedback from IMPACT clinical trials network.
2.2	**1st April 2019**		Addition of new trial personnel
2.3	**1st August 2019**	**12th November 2019**	Following peer review feedback and BMJ article publication
2.4	**13th November 2019**		Following finalisation of Baby Moves involvement for GMA assessment.
2.5	**15th January 2020**		Feedback from the trial steering committee to create a more generic protocol better suited to trial expansion to additional sites.
2.6	**24th July 2020**		Feedback from the trial steering committee to edit wording of MRI assessment
2.7	**7th August 2020**		Insertion of detailed information regarding MRI data sharing for post-processing analysis
2.8	**23rd October 2020**		Change 2-yr developmental outcome measure from the Bayley III to Bayley-4 to reflect newly updated version. Addition of Neonatal Cardiac and Cerebrovascular assessment sub-study
2.9	**16 June 2021**		Clarification for 48 hr assessment process to include fetal wellbeing assessment.
2.10	**21 July 2021**		Addition of the Fetal movement substudy
2.11	**18 February 2022**		Addition of new participating sites
2.11	**9th May 2022**		Change of timelines for initial ultrasound scans
2.12	**13 June 2022**		Combination of changes for two versions of 2.11
2.13	**12 July 2022**		Corrected date of protocol version on p.1
2.14	**23 August 2022**		Addition of new participating sites Update of proposed study timeline Amendment trial team members
2.15	**9 October 2023**	**08 July 2024**	Addition of data sharing process between research teams for participants enrolled in multiple studies
2.16	**09 July 2024**		Amendment to data sharing process to include consent to collect data performed for clinical care

Contents:

LIST OF ABBREVIATIONS

Abbreviation	Meaning
AC	Abdominal Circumference
AE	Adverse event
AFI	Amniotic Fluid Index
ALT	Alanine aminotransferase
AR	Adverse Reaction
BMI	Body Mass Index
BPD	Biparietal Diameter
BPPS	Biophysical profile score
Bayley-4	Bayley Scales of Infant and Toddler Development 4th edition
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DWI	Diffusion-Weighted Imaging
FGR	Fetal Growth Restriction
FL	Femur Length
GMA	General Movement Assessment
HC	Head Circumference
ID	Identification
ITSEA	Infant Toddler Social-Emotional Assessment
IUGR	Intrauterine growth restriction
NICU	Neonatal Intensive Care Unit
NOAEL	No observable adverse effect level
MFM	Maternal Fetal Medicine
PI	Perfusion Index
PSV	Peak systolic velocity
RI	Resistance Index
SAE	Serious Adverse Event
SCN	Special Care Nursery
SFH	Symphysio-fundal height
SUSAR	Suspected unexpected serious adverse reactions
TGA	Therapeutic Goods Administration
USS	Ultrasound Scan
VMIA	Victorian Managed Insurance Agency

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PRINCIPAL INVESTIGATOR STATEMENT OF COMPLIANCE

I have read and understand the contents of this clinical trial protocol. In my formal capacity as Principal Investigator, I agree to conduct the trial in compliance with all stipulations of this protocol, the conditions of Monash Health Human Research Ethics Committee (HREC) approval, The National Health and Medical Research Council (NHMRC), *National Statement on Ethical Conduct in Human Research* 2007 (Updated March 2014), the *NHMRC/ARC Code For The Responsible Conduct Of Research* (2007), International Conference of Harmonisation (ICH)/Good Clinical Practice, and other relevant local and national guidelines.

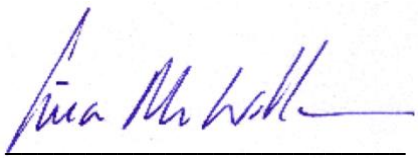
Furthermore, my duties also include ensuring the safety of the participants in this clinical trial and providing the sponsor, Monash Health, with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the trial will be strictly confidential, and this confidentiality requirement applies to all the research staff at this site.

Lead Principal Investigator

Signature: _____ **Date: 13th November 2019**

Dr Kirsten Palmer

Principal Investigator



Signature: _____ **Date: 13th November 2019**

Professor Euan Wallace

1. SUMMARY

Fetal growth restriction (FGR) is a significant health care issue, affecting 20,000 Australian pregnancies every year [1]. Undetected FGR is one of the key risk factors for stillbirth, but FGR can also cause significant impairments in short and long-term health outcomes for the child. It is a major risk factor for preterm birth and is a recognised causal pathway to the neurodevelopmental injury underlying cognitive and behavioural impairment and cerebral palsy.

Current obstetric care is focused on the detection of the growth restricted fetus and then ultrasound assessment of fetal wellbeing to guide timing of delivery. This approach seeks to maximize the gestational age of the fetus at delivery to minimise the risks of prematurity, while delivering the fetus in time to reduce the likelihood of stillbirth. Currently, *no therapies exist* that can maximize fetal wellbeing in the setting of growth restriction and minimise the frequency of antenatally acquired brain injury due to in-utero hypoxia.

This triple-blind, randomized, parallel group, placebo-controlled trial will administer maternal melatonin or placebo supplementation antenatally in the setting of early-onset severe FGR to determine whether melatonin can PROTECT the fetal brain and lead to improved neurodevelopmental outcomes.

2. BACKGROUND

2.1 Fetal Growth Restriction

In Australia, 1 in 16 babies are born with a low birthweight (<2500g) [1], however many more will have failed to reach their true growth potential. Identifying those babies that are genuinely growth restricted compared to those that are constitutionally small is an ongoing challenge in the field of obstetrics. Currently, many different definitions exist to try and improve our accuracy at identifying those babies unable to reach their growth potential. The definition is important as those babies that are small (traditionally classified at <10th centile) or those failing to achieve their growth potential are at significantly increased risk of stillbirth [2].

FGR can also cause significant impairments in short and long-term health outcomes for the child. For example, FGR is a major risk factor for preterm birth and is a recognised causal pathway to the neurodevelopmental injury underlying cognitive and behavioural impairment and cerebral palsy [3–6]. Indeed, premature growth restricted infants have a 4-6-fold increased risk of cerebral palsy [4] and are more likely to have persisting structural brain changes, as well as worse neurodevelopmental outcomes [3,5,6].

Not surprisingly, the impact of FGR extends beyond the individual child: potentially affecting the health and wellbeing of the parents, as well as having significant economic costs for the healthcare system and broader community. Indeed, stillbirth alone is estimated to cost the Australian economy $7.6 billion annually [7], and it is likely that the long-term financial repercussions of FGR are considerably higher.

While the complications of FGR are becoming increasingly understood, many challenges remain. Current methods for the clinical detection of the growth-restricted fetus are limited, particularly for FGR at term. In routine pregnancy care, fetal growth is still determined by measuring the symphyseal-fundal height through abdominal palpation. A measurement more than 2 cm smaller than expected for the gestation suggests FGR. Such an approach, which has been the foundation of pregnancy care for nearly a hundred years, detects, at best, less than half of all cases, and as few as 12-15% of cases in low-risk women [8]. Accurate detection is essential because undetected FGR babies are disproportionately represented in the stillborn population [8]. Where FGR is detected, the rate of stillbirth is halved because of timely intervention, including delivery [8].

In contrast, early-onset FGR is often more readily diagnosed through identification of growth restriction on the mid-trimester morphology ultrasound or through serial ultrasound assessments of fetal growth in women at high risk of FGR [9]. Therefore, the challenge with term FGR is its detection and diagnosis to enable timely delivery and minimise stillbirth, whereas with early-onset FGR the challenge is the prevention of impaired neurodevelopmental outcomes.

Early-onset FGR has multiple causes, such as underlying genetic abnormalities, environmental events, infections or placental insufficiency [10]. Placental insufficiency is the most common cause of early-onset FGR [10]. In the setting of impaired placentation, the fetus must make a number of compensatory adaptations in order to cope with the inadequate oxygen and nutrient supply. These adaptions serve to protect its vital organs, especially the heart and brain. It is now appreciated, however, that even with these physiological adaptions, significant structural brain changes can still occur.

Evidence derived from multiple animal models has demonstrated that severe FGR, is a significant antecedent to perinatal brain injury [11]. It is postulated that the cerebral insult is most likely attributable to the resultant hypoxia-ischaemia as a consequence of placental dysfunction [11,12]. Such prolonged compromise can give rise to intracranial haemorrhage (ICH) and periventricular leukomalacia (PVL) (both risk factors for cerebral palsy), as well as white matter hypomyelination and axonal injury [13]. It is these structural brain changes, which underpin the neurodevelopmental impairment seen in the newborn and infant [11,12]. Consequently, these children are at increased risk of cerebral palsy, learning difficulties at school, a lower IQ, as well as problems in executive function, behaviour, and language [5,9,14].

2.2 Pathophysiology

Inadequate placental implantation is a critical feature in early-onset FGR. In early pregnancy, the trophoblastic cells invade and remodel the maternal uterine spiral arteries within the inner third of the myometrial wall. In turn, this alters narrow, high-resistance vessels to lower resistance, dilated vessels capable of delivering the significantly larger volumes of oxygenated blood to the placental bed and developing fetus. If this process is impaired, the spiral arteries are only superficially or partially remodeled within the uterine decidua, blood flow to the placental bed is limited. This leads to ischaemia – reperfusion injuries within the placental bed, upregulation of hypoxia-induced genes and increased production of free radicals secondary to oxidative stress. Clinically, this process is associated with pregnancy loss, FGR and preeclampsia.

Indeed, pregnancy is a time of increased metabolic challenge for both mother and baby, and as such even normal pregnancy is associated with a state of oxidative stress compared with non-pregnancy [15], [16]. The placenta is a key source of this oxidative stress, due to its high metabolic rate and level of mitochondrial activity [17,18]. Oxidative stress occurs when the production of pro-oxidant free radical species exceeds the capacity of cells within an organ to neutralize or scavenge them. The capability of placental antioxidant defenses to ameliorate the effects of these potentially damaging free radicals is fundamental for healthy placental function and therefore optimal growth and development of the fetus. While a variety of fetal adaptations exist to allow the fetus to withstand acute or chronic changes in oxygenation, in the setting of impaired placentation, the developing fetus may be exposed to high levels of oxidative stress due to overproduction of oxygen free radicals and a decrease in the mitigating activity of available antioxidants.

Overproduction of reactive oxygen species from any cause leads to a well-described causal pathway of cellular damage through lipid and protein peroxidation, and cell death via apoptotic or necrotic pathways [19],[20]. The relationship between oxidative stress and proinflammatory processes is also noteworthy [2122]. When immune cells are activated, many produce oxygen free radicals, which in turn incite a further inflammatory response. The developing fetal brain is particularly vulnerable to both free radical-induced damage and systemic inflammatory stressors, and intuitively, in the context of low oxygen availability, the fetus may benefit from novel focused antioxidant neuroprotective strategies.

2.3 Current Clinical Management of Fetal Growth Restriction

Following detection of FGR, current goals in clinical care center on assessment of fetal wellbeing and evidence of a physiological adaption to placental insufficiency. This information guides the timing of steroids, if indicated, and planning of delivery to minimise the likelihood of stillbirth. Magnesium sulphate is the only available therapy shown to improve fetal brain development in the setting of placental insufficiency and hypoxia. Magnesium sulphate works through reducing glutamate release in a hypoxic environment, likely minimising hypoxic brain injury [23]. It appears to reduce the risk of subsequent cerebral palsy by approximately 30% [24]. However, magnesium sulphate is only used in the hours immediately before birth, while a significant proportion of underlying brain injury in FGR probably occurs over the preceding days to weeks. The use of a safe, maternally administered supplement commenced in the weeks prior to birth could provide further significant benefits in reducing the complications faced by premature infants in the setting of placental insufficiency.

2.4 Melatonin as an Anti-Oxidant

Melatonin (5-methoxy-N-acetyltryptamine) is an endogenous lipid-soluble hormone produced primarily by the pineal gland in humans. It provides circadian and seasonal timing cues due to neuroendocrine control in response to daylight. As such, melatonin secretion is relatively low during the daytime, with an exponential increase in synthesis and secretion occurring from mid-afternoon and peaking at midnight.

In addition to timing cues, melatonin is a powerful antioxidant, acting both as a direct scavenger of oxygen free radicals, especially the highly damaging hydroxyl radical, and indirectly via up-regulation of antioxidant enzymes including glutathione peroxidase, glutathione-reductase, superoxide dismutase and catalase [25,26]. The metabolites of melatonin provide further anti-oxidant effect [27].

2.5 Melatonin Use in Pregnancy

Melatonin is an appealing treatment for use as a fetal neuroprotectant in pregnancy, as it freely crosses the placenta [28] and blood-brain barrier [29]. It also has an excellent safety profile with no known adverse effects [30,31]. Placentae express receptors for melatonin[32], and thus melatonin may protect against oxidative stress generated by ischaemia-reperfusion injury of the placenta.

Melatonin has been studied in several clinical trials related to human reproduction and for different purposes. However, no randomized trial assessing the role of melatonin in fetal

neuroprotection has been completed [33]. Melatonin has been evaluated in assisted reproductive technology where the quality of oocytes is vital for the success of in-vitro fertilization (IVF). Melatonin and myo-inositol are two compounds found in the follicular fluid that are important for oocyte maturation and quality. Tamura et al. (2008) [34] and Rizzo et al. (2010) [35] conducted clinical studies where they co-treated patients with 2mg and 3mg melatonin respectively. The patients in the Tamura et al. (2008) study were given melatonin from the fifth day of the previous menstrual cycle until the day of oocyte retrieval. Both studies revealed improved oocyte quality, but the tendency to increase pregnancy rates failed to reach statistical significance. Unfer et al. (2011) administered 2g myo-inositol, 200µg folic acid plus 3mg melatonin per day for 3-months to women who failed to become pregnant in previous IVF cycles, at the commencement of a new IVF cycle[36]. This treatment resulted in a total of 13 pregnancies, 9 of which were confirmed ultrasonographically and 4 undergoing spontaneous abortion. Treatment continued after completion of the IVF cycle, throughout pregnancy until delivery. Treatment was associated with better quality oocytes and more successful pregnancies. All babies that were born from melatonin-treated pregnancies were in healthy condition with no abnormalities (Unfer, personal communication in 2012).

To evaluate the maternal-fetal transfer of melatonin Okatani et al. (1998) [37] administered a single oral dose of 3mg melatonin to 33 women at term (37-40 weeks gestation) 1- to 4-hours before a planned caesarean section. Levels of melatonin were evaluated in maternal venous blood and umbilical venous and arterial blood. A total of 12 healthy pregnant women delivered by vaginal birth served as controls. Administration of melatonin led to a rapid (<120 minutes) and marked (>20-fold) increase in the fetal serum levels. There were no differences between maternal and fetal serum levels of melatonin, suggesting a rapid and unrestricted transfer of melatonin from mother to fetus.

The same authors tested whether melatonin could up-regulate antioxidant enzymes [37]. No longer than 12 hours before voluntary termination of pregnancy (between 7- and 9-weeks gestation), an oral dose of 6mg melatonin was administered to 47 pregnant women. A significant increase of the antioxidant enzyme glutathione peroxidase was observed in chorionic homogenates derived after the procedure, leading to the conclusion that melatonin might provide an indirect protection against injury caused by reactive oxygen species as seen in preeclampsia, FGR and fetal hypoxia.

The dose used in this trial is based on data from a clinical trial of melatonin for preeclampsia [38] showing that 30mg per day was safe for mother and baby without any apparent adverse effects [39]. Venous cord blood concentrations of melatonin achieved were unchanged between a mother receiving 8mg and 30mg per day of melatonin (melatonin concentration ~2100pg/mL). This cord blood concentration would appear sufficient for neuroprotection according to information in sheep models. However, the degree of oxidative stress reduction achieved within the placental bed was less in mothers receiving 8mg melatonin per day. As such, it was felt that the higher dose of 30mg per day was more likely to achieve a clinically significant result.

2.6 Melatonin Use in FGR

Our group has shown that melatonin supplementation exerts multiple anti-oxidant and anti-inflammatory effects, leading to a significant reduction in oxidative stress and lipid peroxidation within the fetal brain in an ovine model of FGR [13]. In the absence of melatonin, this study showed that lipid peroxidation within the fetal brain led to significant white matter hypomyelination and axonal injury, causing impaired neurological performance in the lambs. Injury was ameliorated entirely in those exposed to melatonin supplementation, with no structural brain injury seen and neurodevelopmental outcomes normalised.

As a result, a small (n=12) phase 1 trial was conducted at Monash Health supplementing pregnancies affected by severe FGR with 8mg of melatonin per day. Melatonin use was well tolerated with no adverse effects seen. A reduction in the degree of placental lipid peroxidation was seen (n=6) [13].

3. TRIAL HYPOTHESIS AND AIMS

3.1 Hypothesis:

Improved neurodevelopmental outcomes will be observed in children affected by fetal growth restriction who receive antenatal maternal melatonin supplementation in comparison to those who receive placebo.

3.2 Primary Aim:

The primary aim is to determine whether neurodevelopment at two years of life is improved among survivors of FGR who receive melatonin antenatally compared with those who receive placebo.

3.3 Secondary Aims:

1. To determine the impact of melatonin supplementation on fetal growth and wellbeing.
2. To determine whether antenatal melatonin supplementation in fetal growth restriction is associated with less structural brain injuries at term corrected age.
3. To report the occurrence of any adverse and serious adverse events associated with melatonin use.

4. TRIAL AND DRUG SAFETY

4.1 Risks of FGR to the Fetus

Early-onset FGR carries significant fetal risks of premature birth. Following diagnosis, those babies requiring delivery <32 weeks gestation carry approximately an 8% risk of stillbirth or neonatal death, with those born <28 weeks gestation having a significantly higher perinatal mortality rate [40,41]. Around 30% of survivors will suffer serious neonatal morbidity [40]. Furthermore, 8% are found to have neurodevelopmental impairment at two years of life [42]. These numbers are likely to be an underrepresentation as they are from a trial population, which was closely surveyed compared to the general population.

4.2 Risks of FGR to the Mother

With approximately 97% of FGR infants born <32 weeks delivered by caesarean section, the mother of a preterm FGR fetus faces the risks associated with morbidity and mortality relating to caesarean birth [40]. Furthermore, they also face a significant risk of morbidity and mortality from pre-eclampsia, which develops among 15 - 40% of women who have a growth-restricted fetus [43,44].

4.3 Potential Risks of Melatonin Treatment

The most common side effects of melatonin are headache, dizziness, nausea and sleepiness [45]. Melatonin does not have any acute pharmacological effects on the nervous or vascular systems, apart from its benign but active impact on sleep mechanisms [46]. Extremely high doses of up to 800mg/kg of melatonin were safely administered to animals without deaths, meaning a median lethal dose could not be established. In humans, long-term treatment with high, daily doses of up to 10g melatonin did not cause any toxicity except for isolated cases of cutaneous flushing, abdominal cramps, diarrhoea, scotoma lucidum and migraine [47].

Prolonged ingestion of 1g melatonin per day caused only subjective drowsiness but did not provoke any toxicity in the eyes, liver, kidneys and bone marrow [48]. In a phase II clinical trial conducted in the Netherlands, 1400 women were given 75mg melatonin nightly over 4-years, with no side effects reported [49].

The safety of melatonin use in pregnancy was explored in early pregnant Sprague-Dawley rats, at doses ranging from 1 to 200mg/kg/day and did not affect antenatal mortality, fetal body weight or other measures of fetal wellbeing [50]. Maternal adverse effects seen at high doses, included mild sedation, reduced maternal weight gain and reduced food intake. This study sought to determine the maternal and fetal no adverse effect level (NOAEL). The NOAEL is the exposure level where a particular substance does not statistically or biologically significantly increase the frequency or severity of adverse effects in an exposed population compared to a suitable control population. The maternal NOAEL in this study was found to be 100mg/kg/day, the fetal NOAEL was established at ≥200mg/kg/day when administered to the mother. The maternal lowest observed adverse effect level toxicity was 200mg/kg/day. With the above information taken in context, the Australian Therapeutic Goods Administration (TGA) has assigned melatonin a Pregnancy Category B3 classification.

We have recently completed a phase 1 trial (NCT01695070) using melatonin supplementation in pregnancy, as well as a clinical trial in women with pre-eclampsia (ACTRN12613000476730) using the same dose as proposed for this trial, and to date no adverse effects have been identified in the mother, fetus or neonate [13,38,51,39].

5. TRIAL DESIGN

PROTECT Me aims to be a multicentre, triple-blinded, randomized, parallel group, placebo controlled trial.

5.1 Collaborating Centres

This trial will be undertaken and co-ordinated by Monash Health.

Other perinatal hospitals across Australia and New Zealand that have agreed to join the trial as additional recruitment sites include:

- Auckland City Hospital, Auckland, New Zealand
- Middlemore Hospital, Auckland, New Zealand
- Christchurch Hospital, Christchurch, New Zealand
- Palmerston North Hospital, Palmerston North, New Zealand
- Royal Women's Hospital, Melbourne, Australia
- Mercy Hospital for Women, Melbourne, Australia
- Royal Hobart Hospital, Hobart, Australia
- Royal Prince Alfred Hospital, Sydney, Australia
- Royal North Shore, Sydney, Australia
- John Hunter Hospital, Newcastle, Australia

- Mater Hospital, Brisbane, Australia
- Townsville University Hospital, Townsville, Australia
- Women's and Children's Hospital, Adelaide, Australia
- Sunshine Hospital, Melbourne, Australia
- St Vincent's Private Hospital, Melbourne, Australia
- Gold Coast Hospital and Health Services, Southport, Australia
- Wellington Regional Hospital, Wellington, New Zealand

Each centre will nominate a local investigator +/- a researcher to oversee local recruitment.

6. TRIAL POPULATION

Pregnant women with a singleton pregnancy complicated by severe fetal growth restriction identified at 23+0 – 31+6 weeks' gestation will be approached for recruitment.

6.1 - Inclusion Criteria

1. Singleton Pregnancy
2. Severe fetal growth restriction, defined as:
    - Abdominal circumference ≤3rd centile for gestational age according to charts supplied that have been adapted from Westerway et al [52]; or
    - Abdominal circumference <10th centile [52] in combination with at least one abnormal fetoplacental Doppler study, being:
        - Uterine artery (raised pulsatility index ≥95th centile)[53]
        - Umbilical artery (pulsatility index ≥95th centile[54] or absent/reversed end-diastolic flow)
3. Confirmed 23+0 – 31+6 weeks' gestation
4. Age ≥18 years
5. Understand English

6.2 - Exclusion Criteria

1. A fetus with a known chromosomal, major structural anomaly or non-placental cause of fetal growth restriction
2. Pregnancies requiring immediate delivery (e.g. absent A wave in ductus venosus, preterminal CTG or biophysical profile)
3. Co-recruitment in another clinical trial where a pharmaceutical product or nutritional supplement impacting on oxidative stress is the trial intervention.
4. Currently prescribed Fluvoxamine

6.3 - Withdrawal of Trial Participants

All participants are free to withdraw from the trial at any point if they wish. The study power calculation allows for a 5% drop-out rate. This drop-out rate is a conservative estimate of what has been observed in other clinical trials performed within our institution. Ongoing clinical care will continue as determined by the treating clinical team following best practice

guidelines. Data collected to the point of withdrawal will be retained unless the participant specifically advises against this.

Pregnancy, birth and postnatal outcome data will be collected from the participant's hard copy clinical notes, relevant Hospital databases and directly from the participant. Data related to the baby/child will similarly be collected from hard copy clinical notes and relevant Hospital databases. If the participant chooses to discontinue the trial intervention, participation in sample collection at birth and the neonatal outcome follow-up will still be offered.

7. SAMPLE SIZE AND POWER CALCULATIONS

The required sample size has been calculated to detect if melatonin supplementation affords a clinically relevant difference in neurodevelopmental outcomes among survivors. An increase of 4-5 quotient points in the Bayley-4 Cognitive scale has been deemed sufficiently clinically meaningful to drive changes in health policy previously [55]. Power analysis shows that 69 participants per group will allow the detection of a difference in the Bayley-4 cognitive score of 5 points between the two groups, with a power of 90% and an alpha level of 0.05, using 2sided T test for comparison. This assumes a standard deviation of 9 [6] and that, on average, the growth restricted infant has been shown to have a cognitive score 5 points lower than the healthy preterm infant and 8 points lower than the healthy term infant [6]. Typically, the Bayley-4 score has a standard deviation of 15, however reduced variability has been seen in the FGR population and this has informed the standard deviation used here. Among pregnancies complicated by early onset FGR a perinatal loss rate of ~15% is commonly observed [41,56]. Allowing for a perinatal loss rate of 15%, an extra 42 women will be recruited. Assuming an additional 5% loss to follow-up rate, we will aim to recruit an extra 14 participants.

This trial also aims to assess whether the impact of melatonin is different at different gestational ages. Therefore, a sub-analysis will be undertaken to compare those with early onset FGR identified <28 weeks' gestation to those with late-onset FGR identified between 28-31+6 weeks gestation. To ensure that this sub-analysis is adequately powered, participants recruited will be randomized to either melatonin or placebo based on their gestational age at diagnosis. Therefore, recruiting 83 participants per group will see the overall trial aiming to recruit 332 participants (see figure 1).

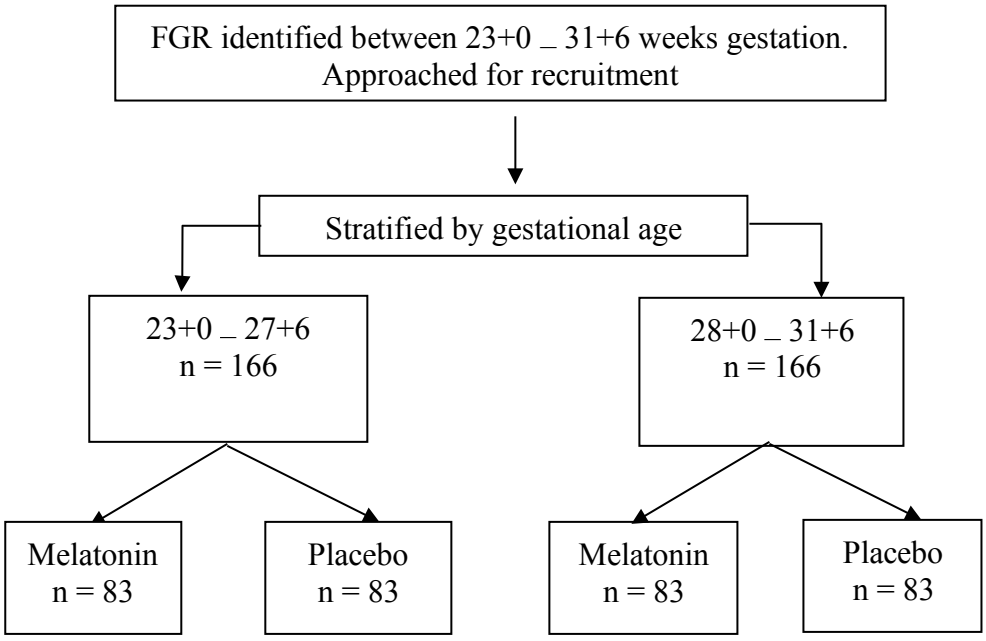


Figure 1: Flowchart of Participant Assignment to Trial Arms.

8. PARTICIPANT SELECTION AND RECRUITMENT

The proposed process for participant selection and recruitment to be adopted for the research study is:

a) The site trial coordinator/research nurse/midwife will identify women who may be eligible for trial participation.
b) Eligible women will be invited to participate by the site trial coordinator/research nurse/midwife (researcher). The researcher will introduce themselves and the research environment of the hospital and university. The researcher will explain that the mother is identified as potentially suitable for participation in this research project and seek the mother's permission to describe the research project to her
c) Provide the mother with the Participant Information and Consent Form (PI&CF) for the research project
d) Give a full verbal explanation of the purpose of the research project and a comprehensive description of what their participation entails, covering all elements that are stated in the Participant Information sheets
e) Inform the mother that participation in this, and any other, research project is voluntary, and her clinical care will not be affected if she chooses to decline
f) Encourage the mother to ask questions about the research project and to discuss it with their partner/family/doctor/midwife or any others of their choosing, before making a final decision
g) All women will be given as much time as they require to fully consider whether they enrol. Contact telephone numbers of a researcher(s) will be provided for any questions that the woman would like answered in the interim.

h) The researcher will meet the women again and, if she wishes to participate in the trial and remains eligible, written, informed consent will be obtained by the researcher(s) once the researcher is satisfied that the mother understands the implications of participating in the study
i) The participant's consent to share data between the research and clinical teams for neonatal and/or paediatric assessments that are commonly performed across multiple studies or for clinical care will be discussed and she will be asked to sign a separate consent form for this data sharing process.
j) Provide a copy of the signed Consent Form, together with the Participant Information sheets to the now participant
k) Place a signed copy of the Consent Form and Participant Information sheets in the medical records belonging to the participant
l) Keep the original signed Consent Form on file in the trial office for verification and audit purposes
m) No trial related procedure(s) will be undertaken until the consent process has been completed
n) Consent is an ongoing process throughout the trial. The researcher(s) will communicate with the participant throughout. Verbal consent will be obtained before the administration of the trial intervention (tablets) as well as for any other trial-related procedures, e.g. venepuncture and collection of, per protocol, blood samples
o) For those women who decline to take part in the trial, their routine antenatal care will not change. Melatonin is not available as part of this standard of care.

9. RANDOMISATION

Randomisation will be via an on-line computerised randomisation service. Randomisation will occur on a 1:1 ratio of melatonin to placebo with the goal of 83 participants randomized to each of the two trial arms stratified by gestational age at recruitment and the presence or absence of abnormal Dopplers. At the time of recruitment, each participant will be assigned a unique trial code. Subsequently, all data and tissue samples collected from the participant will be stored only with this associated code. Thus, all data and tissue samples are deidentified at the point of collection, but with the capability to re-identify them in the future for data verification, research audit or safety (e.g. needle-stick injury).

Furthermore, it will be possible to break the randomization code and reveal treatment group if necessary, such as in the event of a potential treatment-related serious adverse event occurring.

10. TRIAL STUDY GROUPS

10.1 – Trial Study Groups

Once informed, written consent has been obtained, participants will be stratified by gestational age. Hereafter, participants will subsequently be randomized into one of two groups to receive the trial intervention, either:

1. Melatonin
   10mg melatonin tablets, administered three times a day (tds) (a total daily dose of 30mg per day) **or**
2. Placebo
   Visually identical placebo tablets containing no active ingredient, administered three times a day (tds)

Participants, their healthcare providers and the research team will be blinded to the trial intervention the participant has received throughout the trial and during follow-up of the children until all participants have completed all the stages of the trial as per protocol.

10.2 – Drug Supply and Storage

Melatonin 10mg tablets with a Certificate of Analysis will be sourced by Akesa Pharma (Melbourne, Australia). A matched placebo will then be produced by the Pharmaceutical Packaging Professionals (Port Melbourne, Australia), who will repackage all trial medication to maintain blinding and manage its storage and distribution. Trial medication will be supplied in bottles containing 42 tablets to cover trial medication for a 2-week period. Trial medication will then be dispensed to participating sites designated trial pharmacies for dispensing to the individual trial participants on a fortnightly basis.

11. DURATION OF ADMINISTRATION OF THE TRIAL INTERVENTION

11.1 – Duration of Trial Intervention

The administration of the trial intervention i.e. melatonin or placebo tablets, will commence after informed, written consent has been obtained. Participants will continue to take the trial tablets, per protocol, three times daily (tds) until delivery at which time administration will immediately cease.

11.2 - Indications to cease administration of the trial intervention

1. Delivery of fetus
2. Request by the Principal Investigator responsible for trial-related medical decisions, for example, following a serious adverse event (SAE) or adverse event that is assessed by the PI, as being directly related to the administration of the trial intervention or as a consequence of trial participation.
3. Request by the Human Research Ethics Committee/Research Directorate
4. Recommendation by the Data Safety and Monitoring Committee (DSMB)

5. Participant request

12. PARTICIPANT TRIAL SCHEDULE

Participant flow through the trial is demonstrated in figure 2.

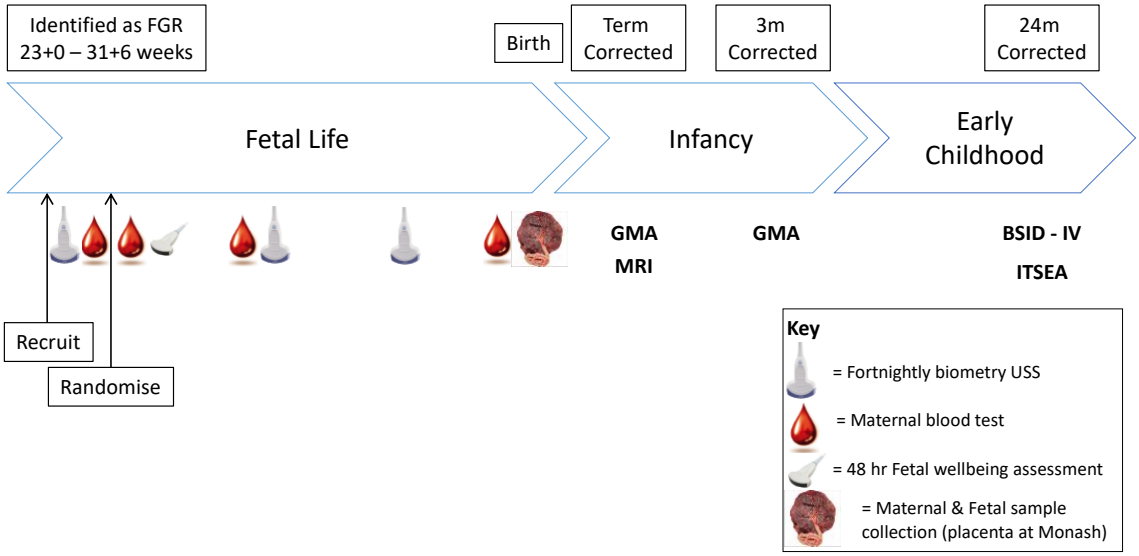


Figure 2: Individual Participant Trial Schedule. Investigations during fetal life will be performed as outlined until birth occurs. 3m = 3 months and 24 - 36m = 24-36 months corrected age. Note, some of the biological sample collections are optional.

12.1 Baseline data collection

Data for each participant will be collected, including details of age, ethnicity, height and weight, BMI, past obstetric history, past medical history, medication use, details of aneuploidy screening and invasive tests and fetal infection screen.

Previous USS data will be collected for growth, liquor volume, anatomy scan details and uteroplacental and fetal Doppler indices (where done).

12.2 Participant surveillance

Blood pressure measurement, maternal weight and urinalysis (dipstick for proteinuria) will be performed fortnightly. Ongoing monitoring for adverse effects will occur through the use of questionnaire and participant medication diary. Assessment of the newborn sleep patterns will occur over the first week after birth with a parent completed sleep-wake diary.

12.3 Participant blood tests and tissue samples

Full blood count, renal (urea, electrolytes, creatinine, urate) and liver (ALT, bilirubin, albumin) function will be tested before the commencement of treatment, 48 hours +/- 24 hours and 14 days (+/- 2 days) after commencement of trial medication and on the day of delivery. Results will be available to the treating clinical team.

Maternal and umbilical cord blood samples will be collected at the time of delivery for assessing melatonin concentrations within both the maternal and fetal circulations respectively. These results will not be available to the treating team.

12.4 Ultrasound scans

All of the following measurements will be made on the day of recruitment, or within 3 days prior, and then ongoing as described:

- Fetal wellbeing assessment at 48 hours (+/- 24 hours) including amniotic fluid index (AFI), single deepest vertical pocket, umbilical artery (PI and presence/absence or reversed end diastolic flow), umbilical vein (pulsatile or non-pulsatile), ductus venosus (PI, presence of positive a wave), middle cerebral artery (MCA) PI and uterine artery PI.
- Fetal growth measurements: abdominal circumference (AC), biparietal diameter (BPD), head circumference (HC) and femur length (FL). Growth parameters will then be repeated on a fortnightly basis.
- Amniotic fluid measurement: four quadrant AFI and single deepest vertical pocket will be performed fortnightly.
- Doppler waveforms: umbilical artery (PI and presence/absence or reversed end diastolic flow), umbilical vein (pulsatile or non-pulsatile), ductus venosus (PI, presence of positive a wave), middle cerebral artery (MCA) PI and uterine artery PI will be performed fortnightly.

Scans are performed in routine clinical practice at this frequency for assessment and surveillance of severe FGR. Standard operating procedures will be supplied for trial purposes. The timing of scans stated here are required for trial purposes; it is likely that scans may be performed more frequently.

These assessments will continue until delivery and be performed blinded to treatment group. The ultrasound images will be collected using standard approaches and images reviewed to ensure appropriate. The ultrasound results will be available to the treating team to guide clinical decision-making. However, the clinical team will also be blinded to treatment group.

12.5 Neurodevelopmental Assessments

Neurodevelopmental assessments will be performed using a General Movement Assessment (GMA), magnetic resonance imaging (MRI), Bayley Scales of Infant and Toddler Development 4th edition (Bayley-4) and the Infant Toddler Social-Emotional Assessment (ITSEA). GMA and MRI will be performed at term corrected, GMA will be repeated a further two times at 3 months corrected age ± 2 weeks, while Bayley-4 and ITSEA will be performed at 24-36 months corrected age. If participants are enrolled in concurrent studies where the same assessments are involved, or if the clinical team has flagged for this assessment to be complete, patient data will be shared between research and/or clinical teams to minimise the inconvenience for the participants. The research team will seek formal consent for this data sharing arrangements from these participants.

<u>12.5.1 - GMA</u>

GMA is a non-invasive measure of spontaneous infant movement. Episodes of spontaneous movements are assessed via video recordings using established methodology[57], with movements classified as normal, abnormal, or absent depending on the gestational age of the

infant and number of weeks post birth. While a simple measure that is easily learned, it has been shown to be a clinically useful indicator of an individual's likelihood of developing cerebral palsy with high positive and negative predictive values. This assessment is not currently in routine clinical use across all health services. All GMA's will be collected using the Baby Moves App (Murdoch Children's Research Institute) and assessed by assessors blinded to treatment group. One GMA will be collected at term equivalent, then two separate video's collected at 3 months corrected age a couple of weeks apart to assist the GMA assessors in reliably determining a reduction or absence of fidgety movements. For sites where the GMA is part of routine care and with established clinical follow-up pathways in place, GMA results will be available to the treating team and additional interventional care offered if indicated, as is usual practice at Monash Health. The research team will not be involved in this care or the decisions regarding the need for interventional therapy. However, at sites where the GMA is not clinically used, results will not be disclosed.

12.5.2 – MRI

MRI will be performed of the infant with a 'feed and wrap' technique. High-resolution axial imaging using T1, T2 and DWI sequences will be obtained. Diffusion tensor imaging (DTI) will be used to study white matter tract microstructure. This technique allows calculation of fractional anisotropy (FA), axial diffusion (AD) and radial diffusion (RD) of cerebral white matter tracts.

For sites contributing to the MRI specific study, a standard operating procedure will be provided including a scanning protocol. Each site will nominate a local radiologist for clinical reporting. All MRI studies should be reported by a single radiologist at each site blinded to treatment group. The MRI results will be available to the treating team to guide clinical decision making, the clinical team will also be blinded to treatment group. Each site will be responsible for the development of a local plan for the management of potential incidental findings and the local site team will take responsibility for sharing results with families and arranging any ongoing investigations and/or care.

To enable the detailed analysis of the white matter tract microstructure, acquired de-identified MRI data in digital form of DICOM files (there will be no hardcopy films) will be securely transferred to Commonwealth Scientific and Industrial Research Organisation (CSIRO) using CloudStor, the secure file transfer solution hosted on the AARNet network (Australia's national research and education network). Transferred data will be securely stored at CSIRO's servers which are directly managed by CSIRO's central Information Management and Technology department and ensures an extremely high level of data storage safety (multiple backups) and confidentiality (encryption, strong access controls). This will be for a term of 15 years, in keeping with the NHMRC's guidelines on responsible conduct of research. This is readily achievable thanks to the enormous storage capacity in CSIRO's servers. A copy will be stored in CSIRO's Australian E-Health Research Centre's (affiliation of the investigators of the project) Xnat and RedCap database. Similar very high levels of data storage safety and confidentiality are implemented for the Xnat and RedCap databases as for CSIRO's servers.

The results from the analysis of the white matter tract microstructure will not be made available to clinical treating teams, as this is not an analysis that is in clinical use.

12.5.3 - Bayley-4

The primary outcome is a clinically meaningful difference in cognitive development at 24-36 months corrected age identified by a change in the Bayley-4 Cognitive score of 5 points. We will administer the cognitive, language and motor domains of the Bayley-4. The Bayley-4 is a direct assessment of neurodevelopment and will be performed by an experienced paediatric developmental psychologist blinded to (i) treatment group and (ii) GMA scores. Any concerning results will be discussed with the family and when appropriate referral onto appropriate specialists/services made.

12.5.4 – ITSEA

The ITSEA assesses a wide array of social-emotional and behavioural problems and competencies. It is validated for use between 12-36 months of age and will be applied to our trial cohort at 24-36 months corrected age. It is a parent-reported assessment undertaken in the form of a questionnaire. Results will be analysed by an experienced paediatric developmental psychologist blinded to treatment group, GMA and Bayley-4 data.
Any concerning results will be discussed with the family and when appropriate referral onto appropriate specialists/services made.

12.6 Concomitant clinical management and co-interventions

Women with severe early-onset FGR are also at high risk of developing preeclampsia. In this occurrence, additional therapies, surveillance and possibly delivery indicated on maternal rather than fetal grounds.

All women with severe early-onset FGR, regardless of co-existing preeclampsia, will require intense fetal surveillance, possible in-patient stay and early delivery. Once the fetus has reached an appropriate gestational age and size (and is deemed viable) corticosteroids will be administered to improve fetal lung maturity and once delivery is planned magnesium sulphate therapy will be considered for perinatal neuroprotection per institutional guidelines. These additional therapies and management will be provided at the discretion of the clinician/trial centre caring for each woman. Trial investigators will collect data regarding these co-interventions.

12.7 Outcome data collection

The researcher will collect relevant data regarding pregnancy outcomes from the maternal clinical record (hard copy and online hospital databases). Neonatal and child (up to 3 years) outcome data will be collected from the hospital records (hard copy and online hospital records) from the time of birth up to 3 years corrected age. Consent will also be sought to access longer term childhood outcome data, such as educational performance. If participants are enrolled in concurrent studies where the same assessments are involved, or if the clinical team has flagged for particular common assessments to be complete, patient data will be shared between research and/or clinical teams to minimise the inconvenience for the

participants. The research team will seek formal consent for this data sharing arrangements from these participants.

12.8 Data collection and record

At the time of recruitment, each participant will be assigned a unique trial code. Hereafter, all data collected from the participant will be used and then stored only with this unique code associated with it. All electronic data, including GMA assessments using Baby Moves, will be stored on a RedCap database, that is password protected with restricted access limited only to the named principal investigators and the trial researchers. Likewise, any hard copies of material bearing participant information e.g. signed Consent Forms or Case Report Forms will be stored securely e.g. locked filing cabinet with restricted access, limited only to the named principal investigators and the trial researchers.

Upon trial completion, all data and records will be stored in the Department of Obstetrics and Gynaecology, Monash University, or another off-site secure location, and participating sites will also store their site specific trial related records for the duration of >25 years.

The principal investigator will be responsible for data storage, retention and destruction. Should the principal investigator leave the institution prior to the trial completion or the 25 years has been reached, the responsibility for record retention, storage and destruction will be passed to another individual nominated by the PI to assume responsibility.

12.9 Disposal of remaining tissue samples and optional future use

At the time of consent, participants will be given an option to agree to any remaining tissue(s) being stored and used, in any future related pregnancy/birth FGR related research project for which HREC approval is sought and given. The PI&CF clearly states that this is an optional component of the now proposed research project and does not impact upon (current) trial participation should the potential participant choose to decline this option for the future use of their tissue(s).

If a participant chooses to take part in the optional component of the research project and permit donation of any remaining tissues to any future related FGR research project, the PI&CF states that the future use may involve genetic testing. Specifically, "*The tissue(s) that you donate for any future, related FGR research could one day be used for genetic testing to learn about the role genes play in FGR. Genes are the basic "instruction book" for the cells that make up our bodies and may be passed on from generation to generation within families. Since the significance of any future genetic tests is not known for you, we will not release the results of any genetic testing nor will the results be used in the planning of your clinical care, or that of your child*".

For those participants who choose not to take part in the optional component and donate tissue(s) to any future, FGR related research projects, following the laboratory analyses of samples for this project, any remaining tissue(s) will be disposed of as clinical waste in line with site specific institutional policies on the handling and disposal of human tissues.

13. ASSESSMENT OF COMPLIANCE WITH THE TRIAL INTERVENTION

The trial intervention will be prescribed, as per protocol, on a fortnightly basis by the named investigators who hold medical doctor (MD) status, full registration with the relevant regulatory body e.g. AHPRA or the medical council of NZ and are employed at the trial institution. Subsequently, the prescription will be dispensed and stored according to the institution's policy for the handling and storage of tablets to be administered to participants in a clinical trial.

Participants will be asked to complete a trial intervention Compliance Log and return all containers including any remaining tablets, to the researcher to determine compliance with the administration of the trial intervention.

14. OUTCOME MEASURES

14.1 - Primary Outcome

Improved neurodevelopmental performance at 2 years of life among survivors of early onset FGR.

The ability of melatonin to protect the fetal brain in the setting of severe early-onset fetal growth restriction will be assessed by multiple assessments across the first 2 years of life. The trial is powered to detect a difference in neurodevelopmental performance at 24-36 months corrected age on the Bayley-4. Other tools of assessing brain development and neurocognitive performance will also include GMA and MRI at term, GMA again at 3-months corrected age and ITSEA at 24-36 months corrected age. These assessments will also be stratified depending on the fetal gestation and fetal Dopplers at the time of FGR diagnosis, with outcomes for those <28 weeks and between 28-31+6 weeks compared as a sub-analysis. Total mortality will be compared between melatonin and placebo.

14.2 - Secondary Outcomes

- Assessment of drug safety and tolerability: Maternal side effect profiles experienced, such as symptoms of drowsiness, abdominal cramps, flushing, migraines, gastrointestinal disturbance. This information will be collected from the participant medication diary.
- Maternal end-organ performance monitored through haematological and biochemical tests
- Fetal effects through assessing ultrasound parameters of fetal biometry (HC, BPD, AC and FL) and wellbeing (AFI, Doppler indices).

15. STATISTICAL ANALYSES

Outcome measures between the melatonin treatment and placebo groups will be analysed on an intention to treat basis. GMA, MRI, ITSEA and Bayley-4 scores will be compared between

groups using parametric or non-parametric testing, depending on their distribution. Simple regression analyses will be performed to explore relationships with functional outcomes.

16. SAFETY ISSUES AND MONITORING

There may be unexpected serious adverse reactions associated with melatonin when used in pregnancy. To date, clinical studies have not demonstrated any serious adverse reactions to melatonin, however metabolic changes during pregnancy may alter the pharmacological properties in unanticipated ways.

16.1 - Eliciting and documenting AE and SAE

The recording of adverse experiences is an important aspect of the trial's conduct. The lead PI responsible for trial-related medical decisions will be designated as the person responsible for the assessment, documentation and reporting of all events to the sponsor, Monash Health, that meet the definition of an AE, SAE or SUSAR. All site PI's are responsible for notifying the lead PI of SAE and SUSAR's as outlined below in section 16.5.

Assessment and reporting will be undertaken in line with the requirements of the Sponsor, Monash Health and the National Health Medical Research Council (NHMRC)[58]. For this trial, it is a stipulation of Monash Health Human Research Ethics Committee (HREC) that if a participant experiences a serious adverse event that is related/possibly related/unknown if related to the study drug, a report will be provided to the HREC and a copy will also be forwarded to the Pharmacy.

All AE/SAE will be recorded from the time of signing the consent form, until the participant has completed all trial related follow-up procedures i.e. until all follow-up visits and assessments of the child at approximately 2 years corrected age have been completed.

All observed or volunteered AE/SAE regardless of causal relationship will be recorded and reported. These events may be declared by the participant or legal guardian and/or identified in response to an open-ended question from the researchers or revealed by observation, physical examination, or while undertaking other trial related procedures.

The researchers are not obligated to actively seek AE/SAE in former trial participants. However, if the lead PI learns of any SAE, including a death, at any time after a participant or their child has been discharged from the trial, and the lead PI considers the event reasonably related to the trial participation, the PI would promptly notify the sponsor, Monash University. For all adverse events as detailed below, a notification email must be sent to scs-protectme@monash.edu, and enrolled patients will be alerted to this.

16.2 – Definitions

16.2.1 - Adverse Event (or Adverse Experience)

Any untoward medical occurrence in a participant or clinical investigation participant administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a pharmaceutical product, whether or not considered related to the pharmaceutical product.

16.2.2 - Adverse Drug Reaction (ADR)

For *marketed pharmaceutical products*, a definition of an adverse drug reaction in the post marketing setting is found in the Australian Code for the Responsible Conduct of Research [59]:

"A response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of disease or for modification of physiological function" p28.

The old term "side effect" has been used in various ways in the past, usually to describe negative (un-favourable) effects, but also positive (favourable) effects. It is recommended that this term no longer be used and particularly should not be regarded as synonymous with adverse event or adverse reaction.

16.2.3 - Serious Adverse Event (SAE) or Adverse Drug Reaction (ADR)

During the conduct of the trial, adverse events may occur which, if suspected to be related to the pharmaceutical product administered (adverse drug reactions), might be significant enough to lead to important changes in the way the pharmaceutical product is used (e.g. change in dose, population, monitoring required, consent forms). This is particularly true for reactions that, in their most severe forms, threaten life or function. Such reactions will be reported promptly to the sponsor, Monash Health and if necessary also the VMIA and TGA.

To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe," which are not synonymous, the following note of clarification is provided:

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as

"serious," which is based on participant/event outcome or action criteria usually associated with events that pose a threat to a participant's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

A serious adverse event (experience) or reaction is any untoward medical occurrence that at any dose:

1) Results in death
2) Is life-threatening
3) Requires hospitalisation or prolongation of hospitalisation
4) Results in persistent or significant disability/incapacity
5) Is a congenital anomaly/birth defect
6) Is an important medical event

The term "*life-threatening*" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe.

The term *disability* means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as: uncomplicated headache, nausea, vomiting, diarrhoea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

Important Medical Event: Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardize the participant or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalisation; or development of drug dependency or drug abuse.

16.2.4 - Suspected Unexpected Serious Adverse Reactions (SUSAR)

Melatonin is a marketed medical product thus an unexpected drug reaction will be defined as a response that is noxious and unintended and which occurs at doses normally used for prophylaxis, diagnosis or therapy of diseases or for modification of physiological function. The reaction will be considered unexpected in nature if the nature or severity of which is not consistent with the applicable scientific information e.g. Product Information document or similar for this product.

The named Lead principal investigator with responsibility for trial related medical decisions will be responsible for determining whether an adverse drug reaction is expected or unexpected.

16.3 - Assessment of Severity/Intensity

Severity and seriousness must be differentiated. Severity describes the *intensity* of an AE, while the term *seriousness* refers to an AE that has met the criteria for an SAE as described above.

The investigator will make an assessment of *intensity* for each AE and SAE reported during the trial and will assign it to one of the following categories:

- *Mild:* an event that is easily tolerated, requires minimal or no treatment, causes minimal discomfort and does not interfere with the participant's daily activities.
- *Moderate*: events result in a low level of inconvenience or concerns with the pharmaceutical product, minimal, local, or non-invasive intervention indicated. Moderate events may cause some interference with normal everyday activities/functioning.
- *Severe*: events interrupt a participant's usual daily activity and may require the participant to seek and receive medical attention, systemic drug therapy or other treatment. Severe events are usually incapacitating.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode if the severity of the intermittent event changes. An AE that is assessed as severe will not be confused with an SAE. *Severity* is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is defined as 'serious' when it meets at least one of the pre-defined outcomes as described in the definition of an SAE.

16.4 - Assessment of causality

All adverse events must have their relationship to trial intervention (melatonin) or trial participation assessed as either related or not related. Evaluation of relatedness must consider etiologies such as natural history of the underlying disease, concurrent illness, concomitant therapy, trial-related procedures, accidents, and other external factors.

To assess relationship of an event to the trial drug (melatonin), the following guidelines will be used:

- *Not related (unrelated*): This relationship suggests that there is no association between the trial drug and the reported event.
- *Unlikely related*: This relationship suggests that the clinical picture is highly consistent with a cause other than the trial drug but attribution cannot be made with absolute

certainty and a relationship between the trial drug and AE cannot be excluded with complete confidence.

- *Possibly related:* This relationship suggests that treatment with the trial drug may have caused or contributed to the AE, i.e., the event follows a reasonable temporal sequence from the time of drug administration and/or follows a known response pattern to the trial drug, but could also have been produced by other factors.
- *Probably related*: This relationship suggests that a reasonable temporal sequence of the event with the trial drug administration exists and the likely association of the event with the trial drug. This will be based upon the known pharmacological action of the trial drug, known or previously reported adverse reactions to the trial drug or class of drugs, or judgment based on the Principal Investigator's clinical experience.
- *Definitely related*: Temporal relationship to the trial drug. Other conditions (concurrent illness, concurrent medication reaction, or progression/expression of disease state) do not appear to explain event, corresponds with the known pharmaceutical profile, improvement on discontinuation, re-appearance on re-challenge.

16.5 - Recording and reporting adverse events and reactions to the Sponsor

16.5.1 - Suspected Unexpected Serious Adverse Reactions (SUSAR)

SUSAR (Suspected Unexpected Serious Adverse Reaction) that occur with a Monash Health participant or with a participant from a site that Monash Health has provided HREC Review through multi-site trial approval are to be reported to Research Support Services within 24 hours.

These events are reported on the SUSAR/USADE site report available at: http://www.health.vic.gov.au/clinicaltrials/application-instructions.htm

All SUSAR that are reported to Research Support Services will require an action plan from the investigator, specifying what action is being undertaken and a follow up report within 30 days of reporting. If Investigators suspect an adverse event may be a SUSAR, they should report it immediately to the TGA.

For SUSAR, it is mandatory to include the UR number, date of birth and participants initials as the SUSAR report is forwarded to the VMIA.

16.5.2 - Serious Adverse Events (SAEs)

All SAE that occur with a Monash Health participant or with a participant from a site that Monash Health has provided HREC Review through multi-site trial approval, which are considered by the investigator as being definitely related, probably related, possibly related and unknown are to be reported within 72 hours to Research Support Services.

These reports for AE and SAE are available at: http://www.health.vic.gov.au/clinicaltrials/application-instructions.htm

All SAE that are reported will require an action plan from the investigator, specifying what action is being undertaken and a follow up report is to be provided within 30 days of the initial report.

SAE should be reported immediately to the sponsor and the TGA, except for those SAE that the protocol identifies as not needing immediate reporting.

In the event of a death of a trial participant, sites should follow local site recommendations in addition to completing an SAE report. For example, for Monash Health participants, it is also mandatory for the death notification to be entered into the Riskman module.

16.5.3 - Adverse Events (AE)

The reporting of AE is at the discretion of the investigator based on whether the investigator is concerned that the adverse event poses a safety risk.

In addition, if the researchers or the sponsor is of the opinion that an adverse event, which is not graded 3 or above but poses a safety risk, should also be reported. Adverse events must be reported to the Research Support Services, Monash Health within 60 days of the date of the researcher learning of the event. These reports are available at:
http://www.health.vic.gov.au/clinicaltrials/application-instructions.htm

16.6 - Line Listings

A quarterly/six-monthly or annual line listing report which details all of the SUSAR and SAE that have occurred in that period must be submitted to Research Support Services accompanied with the Form 15 Summary Report. This form is to be completed by the sponsor.

16.7 – Data Safety Monitoring Committee

As this is a phase III trial, a Data Safety Monitoring Committee (DSMC) is required. The DSMC has been established and consists of:

Professor Paul Colditz (MBBS, FRACP, FRCPCH, MBiomedEng, DPhil (Oxon), GAICD)
Professor of Perinatal Medicine, The University of Queensland
Neonatal Paediatrician, RBWH
Director, Perinatal Research Centre, Faculty of Medicine
Deputy Director, University of Queensland Centre for Clinical Research
Group Head, Clinical Neurosciences Laboratory
Perinatal Research Centre, Faculty of Medicine
UQ Centre for Clinical Research, (Building 71/918)
Royal Brisbane and Women's Hospital
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Associate Professor Joanne Said (MBBS, PhD, FRANZCOG, CMFM, PDipEpid)
Maternal Fetal Medicine Subspecialist, Sunshine Hospital
Centre for Health, Research & Education NorthWest Academic Centre
The University of Melbourne
176 Furlong Rd,
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Dr Stella May Gwini
Biostatistician
Biostatistics Consulting Platform
Monash Health Translation Precinct, Monash University
27-31 Wright St, Clayton, Vic 3168
Phone: +61 3 8572 2458
Email: Stella.Gwini@monash.edu

16.7.1 - Purpose of the DSMC

Melatonin is not currently indicated as an antenatal fetal neuroprotective agent during pregnancies with FGR, therefore for reasons of participant and fetal safety a DSMC has been established. The DSMC will consist of two Australian Health Professional Regulatory Agency (AHPRA) registered medical doctors, being a neonatologist and an obstetrician, as well as a biostatistician. At least one member of the DSMC must have clinical trials experience and have participated as a member of a DSMC before.

16.7.2 - Rationale for the DSMC

The DSMC was established because Melatonin is not currently administered as an antenatal fetal neuroprotective agent during pregnancies with FGR, or for use in pregnancy.
For reasons of safety, the DSMC will review de-identified (but potentially re-identifiable) data on the following:

- Interim data for evidence of trial-related adverse events
- Data quality, completeness and timeliness
- Adherence to trial protocol
- Adequacy of compliance with goals for recruitment and retention
- Factors that might affect trial outcome:
    - Compromise of confidentiality of data, such as protocol violations, unblinding etc.
    - Scientific or therapeutic advances that may impact on participant safety or the ethics of the study

We would advise the DSMC to advocate complete cessation, or re-evaluation, of the trial conduct, if it is evident that either arm of the trial is associated with a statistically significant increase in or a 50% increased rate above the baseline ratio of any adverse events.

16.7.3 - Information presented to the DSMC

All data will be presented to the DSMC without any participant personal identifiers associated with it. The interim analysis results will not be available to anyone external to the DSMC unless the trial is required to cease prematurely.

16.7.4 - Frequency of DSMC reporting

We will provide information to the DSMB following the recruitment of 100 participants and again following recruitment of 200 participants. Information will be provided at other times if requested by the DSMC or the Sponsor, Monash Health Research Directorate and Human Research Ethics Committee. No interim statistical analyses of the primary or secondary outcomes are planned.

16.7.5 - Notification to the Monash Health Research Directorate and HREC of DSMC findings

The Lead PI will notify the sponsor, Monash Health Research Directorate and the Human Research Ethics Committee, of the DSMC's findings at each of the safety assessment time points.

The Lead PI will notify the DSMC of all SAE/AEs submitted to the sponsor, Monash Health.

16.8 - Reporting to Research Support Services

Forms will be emailed to Research Support Services at: Research@monashhealth.org

AE correspondence will be included in the next HREC agenda. Within a week of the meeting, acknowledgement will be forwarded to the Lead Principal Investigator and Study Coordinator. In order to respond to researchers in a timely manner, the Research Support Services will not send a formal letter. Instead, the cover letter/report form submitted by the researcher will be signed and dated by the HREC Chair or designee. A copy of the stamped letter will be scanned to the Lead Principal Investigator and Study Coordinator as verification.

16.9 - Events that will not be reported

It may be the events may occur that are not related to the trial or therapeutic intervention. Such as, prolongation of hospitalisation for a reason that is not related to trial participation e.g. social reasons. The details surrounding these events will be assessed by the Lead Principal Investigator and if deemed not-related, they will not be reported. The details of these events will however be stored.

17. MONITORING OF TRIAL PROGRESS

Regular progress reports will be circulated to all local investigators on a bimonthly basis. Reports will include information such as number of participants recruited, number of participants completing the trial and any AE or SAE that have been assessed as being directly attributed to trial participation.

18. PROPOSED TIMELINE

August 2017 – August 2018

Protocol preparation, Monash Health ethics, recruitment of further centres both nationally and internationally

October 2017 – April 2018

Grant applications to major funding bodies, recruitment of trial co-ordinator and research midwife, purchase and packaging of trial intervention.

Submission to Monash Health HREC

Set-up of trial database and randomisation service.

May 2019

Commence recruitment

Ongoing data collection

December 2023

End recruitment

January – June 2024

Perform laboratory analysis to determine the impact of melatonin on oxidative stress within the mother, fetus and placenta.

Analyse data regarding the safety and tolerability in mother and fetus

December 2025

Complete 2-year follow-up neurodevelopmental assessments and data collection.

January – April 2026

Complete data analysis

Publication preparation

19. ETHICS

Ethics approval for this trial has been obtained through both the Monash Health Research Ethics Committee (RES-17-0000-583A) and Monash University.

20. FUNDING

Funding has been obtained from local and national funding bodies.

This study is fully funded by funds secured from Equity trustee's, the Cerebral Palsy Alliance, as well as the National Health and Medical Research Council (NHMRC).

21. INSURANCE

The sponsor, Monash Health, is responsible for the provision of insurance for the trial through the Victorian Managed Insurance Agency (VMIA). This insurance provides cover for damage to research participants through injury or death.

22. DECLARATION OF INTERESTS

None declared for the authors listed.

23. DISSEMINATION AND PUBLICATION POLICY

Ownership of the data arising from this trial resides with the named PI(s) who will have the right to publish orally or in writing the results of the trial. On completion of the trial, it is intended that the aggregated data generated from it, will be used both for publication in scientific, peer-reviewed journals and presented at scientific meetings.

Authorship will be granted by the Principal Investigators to those who have contributed to the work in the capacities defined by: Monash University, NHMRC [59] and specific journal guidelines regarding the attribution of authorship.

24. APPENDIX 1 - ADDITIONAL RESEARCH COMPONENTS

In addition to the trial outlined above, there will be further research elements also undertaken at Monash Health. Other participating institutions are welcome to undertake these further components and contribute to data collection.

24.1 – Additional Secondary Aims

These are:

1. To assess biomarkers of oxidative stress and melatonin levels within the maternal, fetal and placental compartments.

These additional research components would be incorporated into the individual participant trial schedule (as outlined in figure 1).

24.2 – Additions to the Individual Participant Trial Schedule

24.2.1 - Maternal blood tests

Further blood samples will also be collected at the time of maternal sample collection as outlined in section 12.3 (recruitment, 48 hours and 14 days). The total blood sample volume collected will not exceed 20 ml. These samples will be used for assessment of circulating melatonin levels and markers of oxidative stress.
These results will not be available to the treating clinical team.

24.2.2 – Participant tissue samples.

Following birth (20 minutes), placental samples will be collected as described in section 12.3.

All tissue samples will be collected without compromising or obstructing a participant's clinical care, at all times participant well-being and treatment will remain the priority.

The researcher is responsible for the collection and de-identification of all tissue samples, as site storage at -80°C until recruitment has been completed. The lead trial team will be responsible for subsequent transportation to Monash Medical Centre for analysis.

The samples will be collected and processed with researchers blinded to the intervention that the participant has received and neurodevelopmental outcome assessments.

Using each participant's unique code, tissue samples will be reconciled with the intervention that the participant has received only at the time of data analysis.

The tissue samples will be used for measuring melatonin levels within the maternal and placental circulations. Markers of oxidative stress, such as malondialdehyde and 8hydroxyguanosine, will also be assessed to determine the potential action through which melatonin is exerting its effects.

24.2.3 – Neonatal Cardiac and Cerebrovascular Assessment

PROTECT Me trial participants delivering between 28-32 weeks' gestation will be invited to also take part in this additional sub-study. This sub-study seeks to assess whether melatonin supplementation antenatally can improve neonatal cardiac and cerebrovascular performance. This will be assessed using near infrared spectroscopy (NIRS) performed on day 2, 10, 17 of age for 2 hours each measuring the brain oxygenation, and a single functional echocardiography (fEcho) in the second postnatal week.

FGR neonates with in-utero haemodynamic redistribution and preferential perfusion of the brain (brain-sparing) have been associated with impaired cerebral autoregulation, predisposing to fluctuations in cerebral blood flow and oxygenation in the neonatal period (60). Evidence suggests that both postnatal cerebral hypo- and hyperoxia are associated with brain injury and neurodevelopmental delay (61). Therefore, we will use Near infrared spectroscopy (NIRS) to measure cerebral oxygenation postnatally. NIRS (NIRO 200NX, Hamamatsu Photonics, Japan) allows non-invasive monitoring of the cerebral oxygenation as tissue oxygenation index (TOI, %), using a self-adhesive infant-neonatal sensor placed on the neonatal head. Differences in near infrared-light absorption are detected by the sensor and used to calculate the concentrations of oxygenated (O2Hb) and deoxygenated (HHb) hemoglobin levels of the brain. The ratio between O2Hb and total Hb (O2Hb+HHb) is expressed as the tissue oxygenation index (TOI). Using TOI and the arterial oxygen saturation measured by pulse oximetry (SaO2), fractional oxygen extraction (FOE) will be calculated which indicates the ratio between tissue oxygen consumption and delivery. TOI and FOE have been shown to be altered in FGR infants in the first 1-2 weeks of life (62,63)

Studies have also shown that FGR neonates have altered cardiac function in the postnatal period (64,65). The functional echocardiography (fEcho) performed would be limited to haemodynamic assessment and would not include full anatomical assessment. The study would use the commonly used echocardiography views and will assess left ventricular function and cardiac output (superior vena cava flow, right ventricular and left Ventricular Output). The fEcho study would be limited to 10 minutes duration. Offline analysis that would be done from the bedside obtained data will give quantitative assessment of cardiac output from the left and right heart, systolic and diastolic left ventricle size and shortening fraction. The fEcho and NIRS data will be stored on password protected computer.

24.2.4 – Fetal Movements Questionnaire

Information on fetal movement patterns in women taking melatonin would be useful in the future, should melatonin therapy for fetal neuroprotection become a supported approach. Moreover, information on fetal movement patterns in early and late onset FGR may be informative for researchers attempting to understand fetal behavioural adaptations in the context of placental insufficiency

All women at participating centres enrolled in ProtectMe are eligible for this sub-study.

ProtectMe participants will be invited to complete a questionnaire about fetal movements at enrolment, 2 weeks after randomisation and at 34 weeks' gestation (if ongoing pregnancy and live fetus). The questionnaire is a modified version of a fetal movement tool previously used in two New Zealand pregnancy studies.[66,67] It takes around 5-10 minutes to complete.

The fetal movement questionnaire will be administered electronically, where possible, using the REDCap system. If women do not have email/internet access, a hard copy questionnaire will be available. All data will be entered and stored in the ProtectMe REDCap trial database, administered by the Clinical Data Research Hub, Liggins Institute, University of Auckland.

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