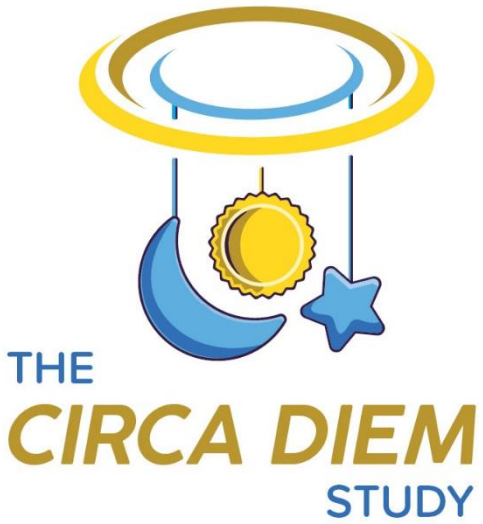


COGNITIVE IMPROVEMENT BY EARLY RESTORATION OF CIRCADIAN RHYTHMS IN VERY PRETERM INFANTS THROUGH ENVIRONMENTAL MODIFICATION:

THE CIRCA DIEM STUDY

Multicentre Clinical Trial Protocol

Chief investigators	Institute affiliations
Prof. Jane Pillow	The University of Western Australia and Telethon Kids Institute
Prof. Rod Hunt	Monash University
Prof. Peter Anderson	Monash University
Dr Peter Mark	The University of Western Australia
Prof. Alicia Spittle	The University of Melbourne and Murdoch Children's Research Institute
Prof. Andrew Whitehouse	The University of Western Australia and Telethon Kids Institute
Dr Julie Marsh	The University of Western Australia and Telethon Kids Institute
Prof. Catherine Elliott	The University of Western Australia and Telethon Kids Institute
Prof. Nadia Badawi	The University of Sydney

Associate investigators	Institute affiliations
Dr Elizabeth Geelhoed	The University of Western Australia
Mrs Catherine Gwyn	Lead consumer representative
Clin Assoc. Prof. Mary Sharp	King Edward Memorial Hospital
Dr Shailender Mehta	Fiona Stanley Hospital
Dr Calum Roberts	Monash Children's Hospital
Clin Prof. Adrienne Gordon	The University of Sydney
Assoc. Prof. Michael Stark	The University of Adelaide
Prof. Helen Liley	The University of Queensland
Dr Daphne D'Cruz	Westmead Hospital
Dr Andrew Wilson	Perth Children's Hospital and Telethon Kids Institute
Dr Thomas Iosifidis	Telethon Kids Institute
Dr Luke Garratt	The University of Western Australia and Telethon Kids Institute

Primary sites (recruiting)	Principal investigators
King Edward Memorial Hospital (WA, Australia)	Clin A/Prof. Mary Sharp
Fiona Stanley Hospital (WA, Australia)	Prof. Shailender Mehta
Monash Children's Hospital (VIC, Australia)	Dr Calum Roberts
Royal Prince Alfred Hospital (NSW, Australia)	Clin Prof. Adrienne Gordon
Women's and Children's Hospital (SA, Australia)	Assoc. Prof. Michael Stark
Westmead Hospital (NSW, Australia)	Dr Daphne D'Cruz
Gold Coast University Hospital (QLD, Australia)	Dr Peter Schmidt
Joan Kirner Women's and Children's Hospital (VIC, Australia)	Dr Niranjan Abraham
Mercy Hospital for Women (VIC, Australia)	Dr Melissa Weber
Nepean Hospital (NSW, Australia)	Dr Girish Deshpande
Wellington Hospital (New Zealand)	Dr Maria Saito-Benz
Auckland City Hospital (New Zealand)	Dr Gergely Toldi
Southmead Hospital (Bristol, UK)	Clin Assoc. Prof Charles Roehr
University College London Hospitals (UK)	Prof. Nikki Robertson
British Columbia Children's and Women's Hospitals (Vancouver, Canada)	Clin Assoc. Prof. Julia Charlton

Secondary sites (non recruiting)	Principal investigators
Rockingham General Hospital (WA, Australia)	Dr Rafiq Hemani
Armadale Health Service (WA, Australia)	Dr Cuila Gibson
Bunbury Hospital (WA, Australia)	Dr Andrea Drew
Joondalup Health Campus (WA, Australia)	Dr Ravisha Srinivas-Jois
Perth Children's Hospital (WA, Australia)	Dr Jonathan Davis
St John of God Midland (WA, Australia)	Dr Noel Friesen
St John of God Mount Lawley (WA, Australia)	Kylie Marston
St John of God Murdoch (WA, Australia)	Dr Leanne Monterosso
St John of God Subiaco (WA, Australia)	Dr Joanne Colvin
Casey Hospital (VIC, Australia)	Dr Paul Machet
Frankston Hospital (VIC, Australia)	Dr Simon Stokes
Jessie McPherson Private Hospital (VIC, Australia)	Dr Zanariah Jamal
Blacktown Mount Druitt Hospital (NSW, Australia)	Dr Anjali Dhawan
Grace Centre for Newborn Intensive Care at The Children's Hospital at Westmead (NSW, Australia)	Prof. Nadia Badawi
North Shore Hospital (New Zealand)	Dr Chris Peterson
Waitakere Hospital (New Zealand)	Dr Chris Peterson
Northland Hospital (New Zealand)	Dr Oliver Hainsworth
Middlemore Hospital (New Zealand)	Dr Michael Meyer
Hawkes Bay Hospital (New Zealand)	Daniel Riviere/ Oliver Grupp
Palmerston North Hospital (New Zealand)	Nigel Orr/ Nathalie de Vries
Lower Hutt Hospital (New Zealand)	Nick Reid/ Karla Fernandes
Nelson Marlborough Health (New Zealand)	Garth Smith/Wendy Hunter

Additional sites *(for substudy participant assessments and/or biological sample handling)*	**Principal investigators**
Telethon Kids Institute (WA, Australia)	Prof. Jane Pillow
PathWest at Perth Children's Hospital (WA, Australia)	Dr Bligh Berry

Sponsor
Telethon Kids Institute (WA, Australia)

Contents

Table of Figures

1 Abbreviations

ASD	autism spectrum disorder
ADOS-2	The Toddler Module of the Autism Diagnostic Observation Schedule - Second Edition
ASQ	Ages and Stages Questionnaire
ASQ-TRAK	Ages and Stages Questionnaire for indigenous study participants
Bayley-4	Bayley Scales of Infant Development Fourth Edition
BPD	Bronchopulmonary Dysplasia
CESD-R	Center for Epidemiologic Studies Depression Scale Revised
CI	confidence intervals
CIRCA DIEM	study acronym (meaning: “about a day”)
d	day
dB	decibel; measure of noise intensity
DSMC	Data Safety Monitoring Committee
EPDS	Edinburgh postnatal depression score
GAD7	general anxiety disorder scale (7 items)
GMA	general movements assessment
GMDN	Global Medical Device Nomenclature
HREC	Human Research Ethics Committee
ICROP3	International Classification of Retinopathy of Prematurity
IVH	intraventricular haemorrhage
ITSEA	Infant Toddler Social-Emotional Assessment
lux	measure of light intensity
m	month
MPAS	maternal postnatal attachment scale (19 items)
MRI	magnetic resonance imaging
NEC	necrotising enterocolitis
NHMRC	National Health and Medical Research Council
NICHD	National Institute of Child Health and Human Development
NICU	neonatal intensive care unit
NIDCAP	Neonatal Individualised Developmental Care and Assessment Program
NMA	National Mutual Agreement
P_IO_2	inspired partial pressure of oxygen
PMA	postmenstrual age
PVL	periventricular leukomalacia
cPNA	corrected postnatal age
PROBE	prospective randomised open blinded end-point
RCT	randomised controlled trial
REDCap	Research Electronic Data Capture; data capture tool
ROP	retinopathy of prematurity
SAE	serious adverse event
SD	standard deviation
SpO_2	peripheral oxygen saturation
w	week

2 Trial Details

Protocol/Clinical Trial Title:	Cognitive Improvement by Early Restoration of CirCADian Rhythms in Very Preterm Infants through Environmental Modification: The CIRCA DIEM Study		
Protocol Number (Version and Date):	CDv1.00 20-07-2018 CDv1.01 10-09-2018 CDv1.02 18-10-2018 CDv1.03 13-11-2018 CDv1.04 31-01-2019 CDv1.05 07-02-2019 CDv1.06 28-05-2019 CDv1.07 14-07-2020 CDv1.08 04-09-2020 CDv1.09 19-04-2021 CDv1.10 07-05-2021 CDv2.01 16-11-2021 CDv2.02 18-01-2022 CDv2.03 17-05-2022 CDv3.00 18-10-2022 CDv3.01 17-01-2023 CDv3.02 19-06-2023 (submitted, subject to approval)		
Amendment (Number and Date):	1. 08-02-2019 2. 09-05-2019 3. 08-01-2020 4. 21-01-2020 5. 22-03-2020 6. 30-06-2020 7. 17-07-2020 8. 20-04-2021 9. 14-05-2021 10. 28-09-2021 11. 16-11-2021 12. 18-01-2022 13. 09-02-2022 14. 22-03-2022 15. 17-05-2022 16. 21-06-2022 17. 20-07-2022 18. 16-08-2022 19. 18-10-2022 20. 15-11-2022 31. 17-01-2023 32. 19-06-2023 (submitted, subject to approval)		
Trial Start Date:	04/02/2019	Trial Finish Date:	30/6/2026
Coordinating Principal Investigator Name:	Professor Jane Pillow		
Coordinating Principal Investigator Contact Details:	Telethon Kids Institute 15 Hospital Road Nedlands, WA 6009 Ph: +61 8 6488 3318 Mobile: +61 (0)417 621960 Email: jane.pillow@telethonkids.org.au		
Sponsor Name (if applicable):	Telethon Kids Institute		

2.1 Trial Summary

Fetal circadian rhythms are vital to normal fetal development and are dependent on maternal circadian rhythms until near-term. Preterm infants spend the first months of postnatal life in the disruptive setting of constant environmental light and noise, without maternal circadian inputs. Disturbed circadian rhythms are associated with *in utero* programming for late-onset metabolic syndrome, neonatal morbidity, and prolonged initial hospitalisation. Importantly, circadian rhythm disruption compromises neurodevelopment in animals. Cognitive impairment remains the primary morbidity associated with extremely preterm birth and increases health care costs. Therefore, we aim to establish if individual diurnal cycling of environmental light and noise levels improves cognitive outcomes of very preterm infants compared to more constant background lighting and noise.

We propose a multicentre, prospective, randomised, open, blinded end-point (PROBE) controlled trial that assesses the effect of non-invasive application of eye-masks and ear-plugs for 10 hours per night on neurodevelopmental, social, psychological, physiological, and economic outcomes. We hypothesise that diurnal cycling of light and noise commenced from birth and continued until discharge home will improve the cognitive score (Bayley-4), in infants born at < 32 w gestation. Primary outcome will be a mean difference of 4 points in the cognitive score on Bayley-4 at 2 years corrected postnatal age. Sample size to achieve 90 % power and alpha 0.05 is 954 infants, assuming a standard deviation of 15 points, a 10 % loss to follow-up and a 30 % adjustment for multiple births. Secondary outcomes will target anthropometry and growth, key neonatal morbidities (BPD/NEC/ROP/Sepsis/IVH), length of hospitalisation, survival, Bayley-4 sub-scores, infant behaviour, hormonal and clock gene circadian profiles, health service use, caregiver mental well-being and health economics.

3 Trial Rationale / Background

3.1 Background Summary

Prematurity (birth before 37 completed weeks gestation) is one of the most pressing health problems: globally, preterm birth accounts for more than 10 % of births and over 1.1 million neonatal deaths annually.[1] Cognitive impairment is the major and most distressing disability arising from preterm birth and the primary concern for parents and carers of preterm infants after hospital discharge. Cognitive impairment reflects the vulnerability of the preterm brain to disrupted development: children born preterm perform at >0.8 SD below term peers on IQ tests.[2] Premature babies also exhibit higher rates of adverse motor, behavioural and psychiatric outcomes in late childhood compared to children born at term gestation, even in the absence of recognised perinatal brain injury.[3] Such life-long neurological deficits have enduring health and socio-economic consequences for these fragile infants, their family, and society. Rates of cognitive impairment after preterm birth have not improved in recent decades despite improved survival.[4,5]

Circadian rhythmicity is essential to neurogenesis, learning, memory, and thus vital to development of normal cognition, as well as the structural and function integrity of developing organs.[6]

Circadian rhythmicity in circulating melatonin promotes onset of sleep at night when melatonin peaks, whereas peaking of the circulating cortisol levels in the early morning promotes wakefulness. The fetus is dependent on maternal circadian physiological cues (e.g. temperature; melatonin and cortisol) to orchestrate developmental processes until near term.[7,8] Maternal circadian rhythm disruption (e.g. shift work), decreases fetal growth.[9] Restricted fetal growth impedes short- and long-term fetal brain development and brain function (including intellectual, behavioural and motor functioning).[10,11] In contrast, maternal melatonin improves neurodevelopment in the growth-restricted fetus.[12] Melatonin is neuroprotective for the developing brain: melatonin reduces inflammation, oxidative stress, lipid peroxidation, and cell death, and stabilises the blood-brain barrier after fetal asphyxia.[13-16] Early endogenous production of this hormone thus has scientific credibility for improving neurodevelopmental outcomes. Rhythmic release of glucocorticoids are similarly vital for regulation of neural cell differentiation and death.[17]

Very preterm infants (<32 w gestation) have not yet developed a primary endogenous circadian rhythm,[18] and are deprived abruptly of vital maternal circadian inputs after delivery. Furthermore, very preterm infants are cared for in neonatal intensive care units (NICUs) which have almost constant lighting and noise despite the efforts of staff. Bright light is a potent cue (zeitgeber) for regulation of circadian rhythms. Current

lighting guidelines suggest individual lighting of up to 2000 lux for specific procedures, where lux is a measure of light intensity.[19] Similarly, cochlear damage or disrupted growth and development of premature infants may result from noise above 45 decibels. Further, preterm infants are exposed to neuroactive medications such as caffeine, or glucocorticoids, given at non-physiological times. Disrupted cues for circadian rhythmicity likely disrupts development of coordinated circadian rhythms critical for neurogenesis, organ growth and development.[20]

Failure to deliver cyclical light/dark information to preterm babies delays development of autonomous circadian rhythms.[21] Stress imposed from the constant light and noise of the environment may disrupt white matter organisation in the early postnatal months:[10] white matter organisation at term is highly predictive for cognitive function at age 5 years,[22] whilst rodent studies show that the severity of neurobehavioural disturbances are increased when circadian disruption is experienced during earlier rather than later period of brain development.[10] Circadian rhythms reduce capacity for selective attention and executive function:[23] these complex cognitive tasks are frequently impaired in survivors of preterm birth.[24] Thus disrupted circadian rhythms may contribute to the increased incidence of subnormal academic achievement, attention-deficit/hyperactivity disorder and adolescent psychiatric disorders.[25] Impaired circadian rhythms may also increase vulnerability to autism spectrum disorder (ASD): birth before 34 weeks' gestation nearly doubles the risk of ASD compared to term infants;[26] further, the incidence and severity of ASD symptoms increase as gestation decreases.[27-29] Exposure of preterm infants to constant light and noise also impedes growth trajectories and consequently prolongs initial hospitalisation. Such circadian disruption exerts key programming effects on the developing clock, increasing risk for later metabolic disease.[30,31]

The American Academy of Pediatrics suggested introducing regular cycles of day-night lighting in the NICU in 1997,[32] however there is no substantive clinical evidence to support this practice and few units achieve adequate (300 lux) separation of artificial night and day. To the contrary, the widely implemented Neonatal Individualised Developmental Care and Assessment Program (NIDCAP), recommences continuous dim lighting (< 20 lux) for 24 hours/day, to emulate the darkened environment of the uterus.[33] However, this continuous dim lighting eliminates any environmental zeitgeber input to the suprachiasmatic nucleus (which the fetus normally receives from circadian maternal cues). Even low levels of white light inhibit melatonin secretion, and in animal studies results in increased anxiety and delayed growth.[34] Importantly, preterm infants who are exposed to cycled light from birth develop circadian rhythmicity in melatonin profiles as early as five days of age:[35] this observation indicates they can respond to cycled light and is consistent with the timing of functionality in the retinohypothalamic pathway.[36]

A 2016 Cochrane systematic review of cycled light versus a range of near darkness, or continuous bright light found likely benefit for cycled light for improved weight gain, oxygenation, and circadian activity patterns, and earlier hospital discharge.[37] Compared to continuous bright light, the weighted mean hospital stay for cycled light groups was significantly shorter (-16.5 d, CI -26.2 to -6.8). However, this 2016 review highlighted the poor quality of the seven randomised and quasi-randomised studies undertaken to date and inadequacy of sample size.[37] The Cochrane review findings of improved weight gain and earlier hospital discharge was reinforced by a 2017 trial evaluating the impact of cycled light commencing early (from 28 w PMA) or late (from 36 w PMA).[38] This most recent study is the only RCT to report the effect of cycled light on neurodevelopment: there were no neurodevelopmental differences observed between groups, however only 33 % (n = 40) of the cohort had neurodevelopmental assessments at 18 months postnatal age. The delay in starting cycled lighting until 28 w PMA meant some of extremely preterm infants spent up to 5 weeks without environmental cycling prior to commencing the intervention, despite no evidence of safety issues with light exposure on severity of retinopathy of prematurity (ROP)[39] or sensory development.[40] Further, night-time lighting protection only used cot-covers and dimmed ambient lighting, therefore preterm infants were exposed to light overnight for any medical procedures. Covering the eyes and protecting the babies from such inadvertent light exposure increases the duration of sleep per sleep epoch and total sleep per day.[41]

There are no studies of cycled noise exposure in preterm infants. The American Academy of Pediatrics recommends that NICU noise levels should not exceed 45 dB, but this is rarely achieved.[42,43] However, one very small (n=24) centre study evaluated the use of silicone earplugs worn throughout the admission in very low birthweight (< 1500 g) infants. A mean (95 %) increase in weight at 34 w post-menstrual age of 225 (45, 405) g was noted in the group assigned to earplugs compared to controls. An even smaller (n=12) subset followed up at 18-22 months had an increased Bayley MDI of 15.5 (3.0, 28.0) and head circumference 2.6

(1.0, 4.2) cm. These outcomes need to be validated in an adequately powered trial with a primary neurodevelopmental outcome.

In summary, animal studies show early circadian rhythm development is vital for neurogenesis and works systemically to promote normal organ development and reduce risk of late-onset metabolic disease. Underpowered and low-quality studies in preterm infants suggest short-term benefits of early cycling of environmental light for improved weight gain, earlier hospital discharge and more stable physiology: each are determinants of cognitive capacity. Current developmental care guidelines promote continuous dim NICU lighting, but the Cochrane review suggests this approach is inferior to cycled light for short-term outcomes. The effect of cycled light on cognitive outcomes is unknown. Few studies have addressed the issue of noise in the NICU.

3.2 Intervention

Very preterm infants will be randomised to standard environmental care (control) or a cycled environment (light and noise) from birth until discharge. The intervention group will receive cycled environmental light and noise to simulate day/night-time environments using a pragmatic 14 hour day (6 am – 8 pm), 10 hour night (8 pm – 6 am) cycle.

- Daytime intervention will include exposure to light including removal of cot-covers if present, to achieve lighting within the range of 300-600 lux,[44] whilst avoiding direct bright light to the infant's eyes.

- Nocturnal intervention will include repositioning of cot-covers, application of light-occlusive eye-masks and silicone ear-plugs. Eye-masks and ear-plugs will remain in position during any medical procedures or overnight cares unless removal is necessary for medical reasons.

Similar cycling of cot-blankets in combination with cycled light was used previously in a small trial without adverse effects.[45] Light meters positioned within the infant's cot will be used to ensure daylight lux targets are achieved and maintained (intervention group) or level of ambient lighting recorded (control group).

Infants in the intervention group may still participate in the routine practice of afternoon 'quiet time' if required. This includes 1-2 hours of time in the afternoon where the lights in the nursery may be dimmed. Eye-masks, ear-plugs or cot covers should **not** be used for infants in the intervention group during 'quiet time' periods.

Eye-masks will continue to be used for phototherapy and ear-protection for high-frequency ventilation as per normal nursery protocols (normally short-term). Respiratory stimulants (e.g. caffeine) will be regulated to 8am dosing for all infants, when prescribed, to avoid pharmacologic confounding on circadian rhythm development. Similarly, if postnatal glucocorticoids are prescribed for severe lung disease, they will be given at 6 am/6 pm to reduce nocturnal disruption.

The proposed interventions (eye-masks, ear-protection) are already used in Australian NICUs as short-term protection from either phototherapy light, or noisy ventilators (e.g. high-frequency oscillators), and thus medically recognised as safe for neonates and acceptable to NICU staff. Additionally, we surveyed 17 parents of preterm babies enrolled in a circadian rhythm observational study regarding their attitudes to the research and proposed interventions. Parents ranked the mean (SD) importance of circadian rhythm research in newborn infants at 93 (9) on a scale of 0 (not important) to 100 (extremely important). 100 % of the parents studied indicated they would enrol their baby in a prospective study such as the CIRCA DIEM RCT. Parent satisfaction scores with our proposed physiological monitoring techniques were high: saliva and cheek swabbing procedures to obtain information on hormonal and clock gene rhythms mean (SD) score was 96 (7) whilst wearing of actimeters to monitor activity was 97 (6). Parents were largely accepting of adjustment of neuroactive medication timing (mean score 88 (16)), and similarly prepared to allow infants to wear eye-masks (77 (15)) and ear-plugs (77 (22)) overnight to block out light and noise. These survey responses indicate a high willingness of parents to engage with our proposed trial, and acceptance of proposed study intervention and monitoring.

4 Trial Aims / Objectives / Hypotheses

4.1 Objectives

The overall aim of the proposed study is to establish whether targeted individual diurnal cycling of environmental light and noise levels results in improved neurodevelopmental social, psychological, physiological, and economic outcomes of very preterm infants compared to more constant background lighting and noise environments.

4.2 Primary Hypotheses and Outcome

Exposure to cycled light and noise from birth until discharge home will increase cognitive score on the Bayley-4 at around 2 years cPNA.[46]

4.3 Secondary Hypotheses and Outcomes

Exposure to cycled light and noise from birth until discharge home will: 1) improve neurodevelopmental sub-scores and neurobehaviour at 2 years cPNA; 2) not increase mortality or neonatal morbidities; 3) result in more rapid development of endogenous circadian rhythms; 4) reduce parental depression and anxiety and improve parent-infant interactions; and 5) decrease socio-economic costs of premature birth.

5 Trial Design

5.1 Study Endpoints

5.1.1 Primary outcome

- Cognitive scores on the Bayley-4 around 2 years cPNA[46]

The Bayley-4 is an individually administered instrument to assess developmental functioning in infants and young children from the age of 1 month to 42 months of age. The Bayley-4 is the current, universal national test of early development. The Bayley-4 sub-scores include cognitive, language (composite of receptive and expressive communication) and motor (composite of fine motor and gross motor). Scores are adjusted for prematurity, with a distribution centred on 100 with a standard deviation of 15. A 4-point change in cognitive score provides similar cognitive advantage as breast-feeding for at least 8 months and may be sufficient to obtain semi-skilled rather than unskilled work.[47] Expertise in Bayley-4 test administration is available within all Australian neonatal centres. A copy of the child's Bayley-4 results can be provided to parents upon request.

The trial will aim to conduct Bayley-4 assessments at 2 years cPNA. If participants are unable to undertake their Bayley-4 assessment at 2 years cPNA, a later Bayley-4 assessment will be undertaken as soon as reasonably possible and up to a maximum participant age of 3 years cPNA.

5.1.2 Secondary outcomes

Comprise short-term and longer-term neurodevelopmental and clinical outcomes as well as targeting physiological and molecular indicators of circadian rhythm development to inform mechanism and effectiveness of the intervention.

5.1.2.1 *Short term secondary outcomes*

- Survival to 36 w postmenstrual age (PMA)
- Weight, length, head circumference, and growth velocity (36 w PMA; 2 m, 6 m, 12 m cPNA)
- Duration of hospitalisation
  - Tertiary hospital
  - Total duration of hospitalisation prior to first discharge home
- Severity of respiratory disease

- BPD (defined by the NICHD and shift of the oxyhaemoglobin dissociation curve),
- right shift of the SpO_2/P_IO_2 curve at 36 w PMA relative to the fetal oxyhaemoglobin dissociation curve
- duration of mechanical ventilation,
- duration any respiratory support
- days of oxygen supplementation

- Other neonatal morbidities including:
  - NEC – stage II or more on Bell's classification
  - ROP – stage II or stage III ROP, and presence of 'plus' disease or greater on ICROP3
  - Brain - Grade 3 or 4 IVH, and presence of PVL
  - Sepsis - defined as number of confirmed (blood culture positive) episodes of sepsis
- Risk for childhood neuromotor deficit using the Prechtl General Movements Assessment at 36 w PMA, 3 m and 3.5 m cPNA[48-50]
- Disability risk: 2, 6, 12 and 24 months (Ages & Stages Questionnaire-3 or the ASQ-TRAK)[51]
- Mother-infant attachment using the Maternal Postnatal Attachment Scale (19 items) score (36 w PMA; 2, 6, 12 months cPNA)[52]
- Primary caregiver mental well-being (36 w PMA; 2, 6, 12 months cPNA).
  - Edinburgh Postnatal Depression Scale (EPDS)
  - Generalised Anxiety Disorder Scale (GAD-7).

5.1.2.2 *Long-term (2 y cPNA) secondary outcomes*

- Survival
- Proportion of scores < 1 SD and < 2 SD below mean, for the primary cognitive sub-score outcome at 2 y cPNA
- Individual components of the Bayley-4 at approximately 2 y cPNA
- Neurobehaviour using the Infant Toddler Social-Emotional Assessment (ITSEA)[53]
- Social communication using the Communication and Symbolic Behaviour Scales Infant-Toddler Checklist (CSBS-ITC) for early recognition of risk behaviours that may be predictive of later behavioural difficulties such as diagnosis of autism spectrum disorder
- Growth velocity
- Anthropometry including weight, height, BMI, fat-free mass
- Primary caregiver mental well-being
  - GAD-7
  - Center for Epidemiologic Studies Depression Scale Revised (CESD-R).

5.1.3 <u>Circadian rhythm biological sub-study (KEMH only, n=~70): confirming intervention effect on rhythms</u>

- Time to onset, mesor, amplitude, and acrophase of circadian rhythms in temperature, heart rate, oxygen saturation, salivary melatonin and cortisol and/or urinary melatonin, and buccal cell clock gene expression at study entry, 7 d, 14 d, 28 d and then 4 weekly until discharge home.

5.1.4 <u>Social Communication sub-study (n=infants identified at risk of ASD using the CSBS-ITC, in centres where ADOS-2 testing is available)</u>

- Assessment at 2 years corrected postnatal age with The Toddler Module of the Autism Diagnostic Observation Schedule - Second Edition (ADOS-2)[54]. Two outcomes will be reported from the ADOS-2.
  - the continuous scores of the ADOS-2 (i.e., autism behavioural severity)
  - the dichotomous outcome of whether or not children exceed the 'ASD' threshold (to be defined).

5.1.5 <u>Neonatal lung function and body composition sub-study (KEMH only, n=128)</u>

- Tidal volume
- Respiratory rate
- Area under the flow volume curve
- Body volume
- Body fat and fat free mass, and % body fat

5.1.6 Measurement of infant sleep quality and duration sub-study

- Parents will be asked to complete the Brief Infant Sleep Questionnaire (BISQ-R; 33 items) at 2 months and 6 months corrected postnatal age, to provide detail on their infants sleep behaviours over the previous 2 weeks.
- Parents will also be asked to keep a sleep diary of their own sleep behaviours over the same interval.

- Infants and parents recruited to the sleep sub-study from KEMH or from Fiona Stanley Hospital (FSH), will also be invited to participate in physiological studies to measure how the cycled light and noise intervention influences sleep during hospitalisation (phase 1) and after discharge (phase 2).
    - Phase 1 (Completed December 2021): sleep and wakefulness will be measured using actigraphy at baseline, 7 d, 14 d, 28 d, and then every 4 weeks until 36 w postmenstrual age in infants recruited from KEMH.
    - Phase 2: sleep and wakefulness will be measured in both the infant and the main caregiver (parent) using actigraphy at 36 w postmenstrual age (or prior to discharge home, whichever comes first), at 2 months corrected postnatal age and at 6 months corrected postnatal age. Saliva swabs will also be obtained from the infants before they go to sleep at night, and again when they wake in the morning, at each of these times.

Key reported measures will include:

- Total sleep time
- Total day (6AM-8PM) and night (8PM-6AM) sleep
- Sleep/activity ratio
- Number of sleep awakenings after sleep onset
- Longest continuous sleep during daytime and night-time
- Peak salivary melatonin
- Day:night salivary melatonin ratio

5.1.7 Magnetic Resonance Imaging sub-study

MRI outcomes will include a calculation of a global abnormality score, derived from metrics obtained from T1- and T2-weighted images. Two different scoring systems will be applied to the conventional sequences.[55,56]

Other MR measures of cerebral integrity will be derived from diffusion weighted imaging and include: Fractional anisotropy apparent diffusion coefficient in multiple areas of the brain.

An analysis of 208 MR images (n=104 from each group) will identify differences in the primary outcome of MRI Global Brain Abnormality Score (GBAS) of 2.85 assuming a standard deviation of 5 and adjusting for missing subjects (10 %) and 30 % multiple births.

5.1.8 Infection and Immunology sub-study

- Primary outcome will be hospitalisations for bacterial or viral infection in the first two years of life.
- Secondary outcomes will include:
    - Number of febrile episodes (> 38.5 ^{0}C) in the first 2 years of life
    - Number of courses of antibiotics
    - Number of hospitalisations for respiratory and/or gastrointestinal illnesses.
    - Number of respiratory viral infections
    - Doctor diagnosis of asthma or wheezy illness
    - Number of gastrointestinal viral infections
    - Indices of neutrophil and immune function
    - Indices of airway epithelial cell function

5.1.9 Health economic outcomes

Economic outcomes will be assessed at neonatal discharge and at 2 years cPNA, and will include estimation of long-term outcomes:

Given the low cost of the intervention and anticipated fewer days in the NICU (~$2 000-$3 000/d) for the intervention group, a cost-saving (with improved health) is expected in the very short-term if there is a significant difference in duration of hospitalisation. Net savings will be estimated by comparing the length of stay with the costs of the intervention. The intervention cost, at a single primary recruiting site, will be assessed by prospective resource unit tracking including materials and time required for application/removal of eye-masks and noise protection. Resources will be costed using standard sources.

- Comparison of all health resource use to 2 years cPNA will be monitored prospectively to assess cost savings. Resource use will include hospital readmissions, general practitioner and specialist appointments, and any other health resources. Cost effectiveness will compare costs with measured clinical outcomes.
- Outcomes such as the Bayley-4 scores will provide surrogate information to undertake modelling to estimate long-term outcomes resulting from the intervention. Improvements in cognitive ability can translate to increased professional opportunities, and increased contribution to the wider economy. Economic evaluations will be consistent with established economic evaluation methods.[57]

5.2 Study Design

The CIRCA DIEM trial is a multicentre, two-arm, parallel, Prospective, Randomised, Open, Blinded End-point (PROBE) controlled trial.

Very preterm infants are randomised to standard environmental care (control) or a cycled environment (light and noise) from birth until discharge. Participant flow charts are shown in

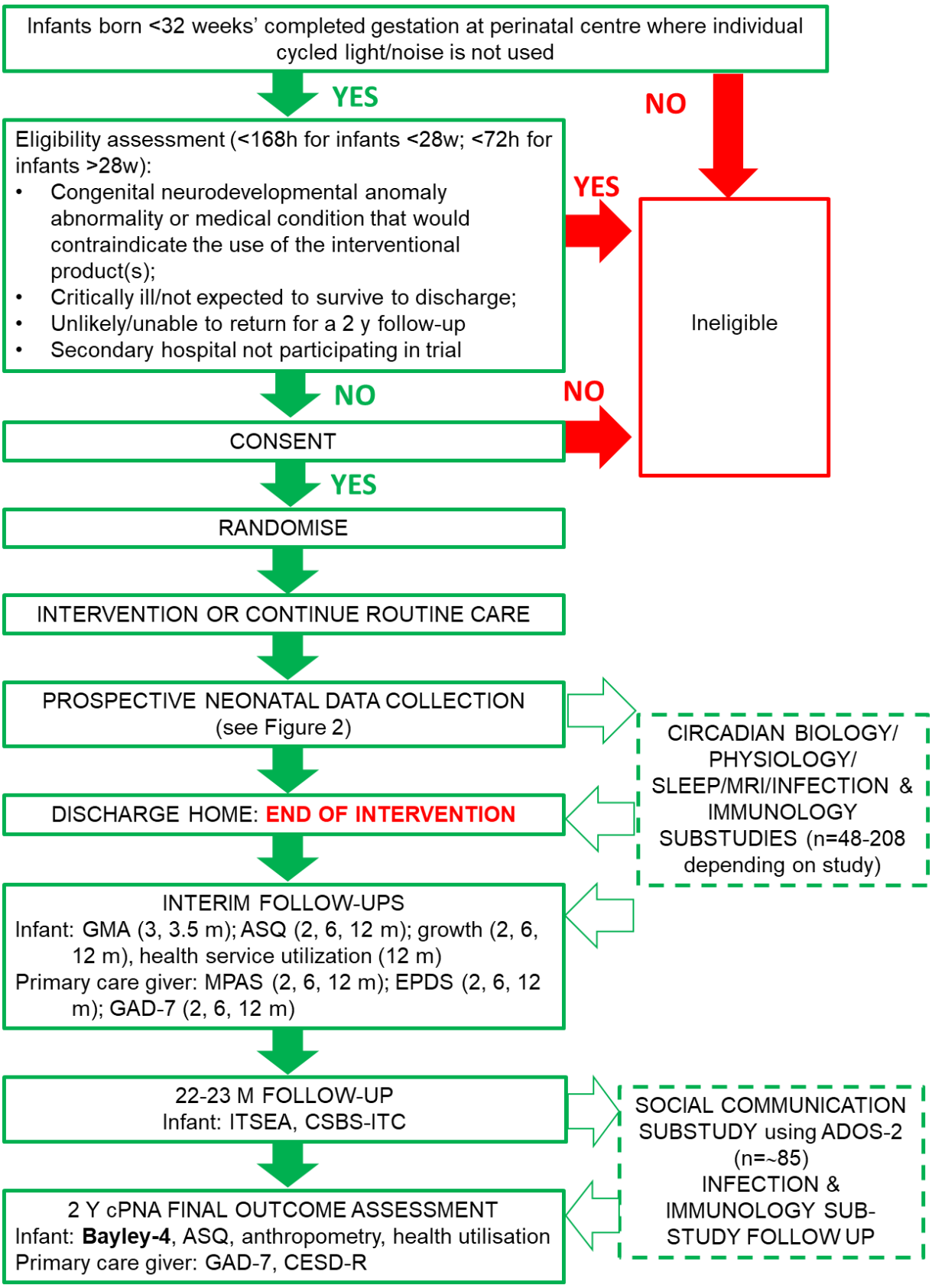


Figure 1 and Figure 2.

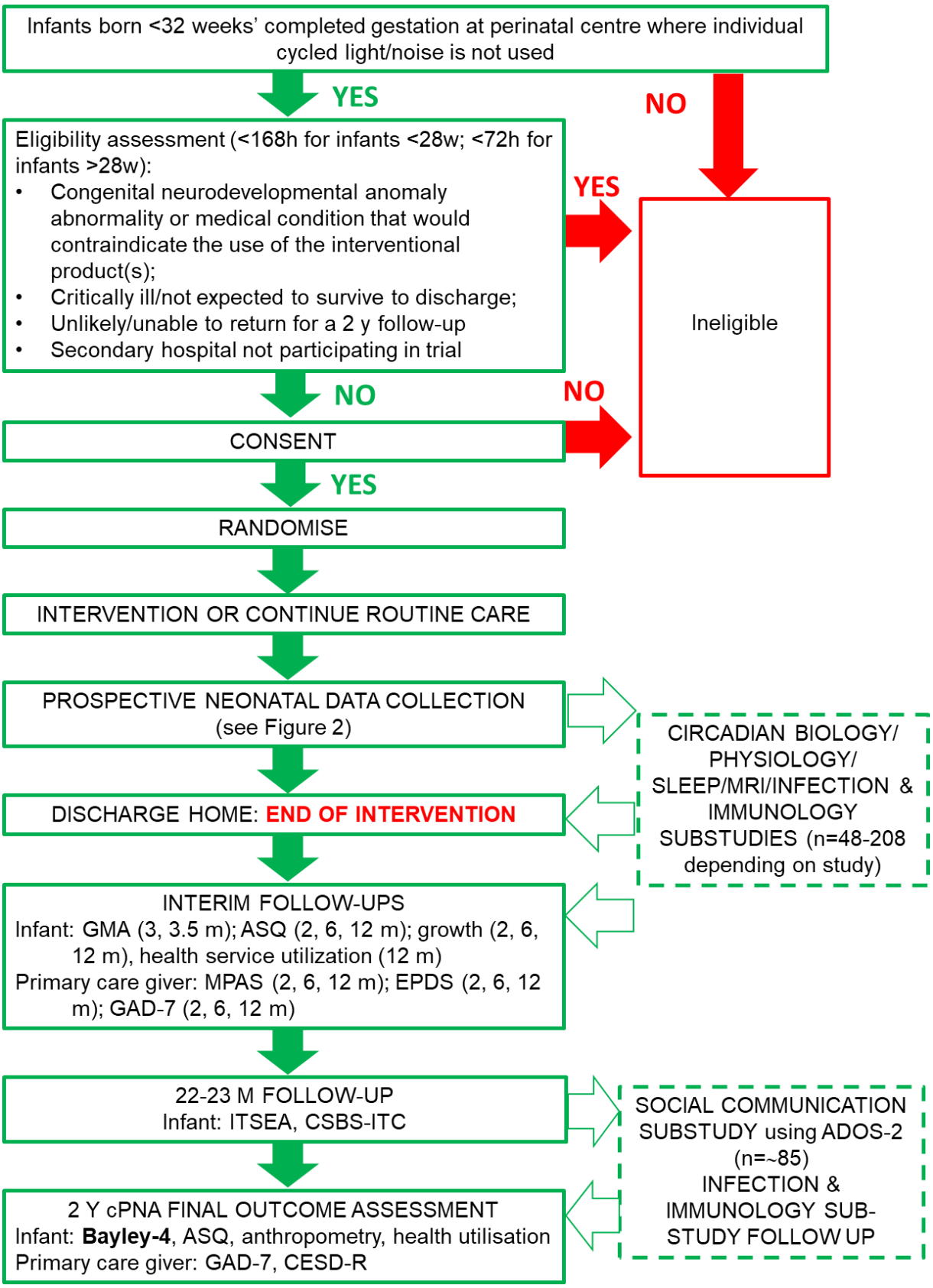


Figure 1 - Participant Flow Chart

cPNA – corrected postnatal age; ASQ – Ages and Stages Questionnaire; MPAS – Maternal Postnatal Attachment Scale; EPDS – Edinburgh Postnatal Depression Scale, GAD-7 – Generalised Anxiety Disorder Scale; ITSEA – Infant Toddler Social Emotional Assessment; CSBC-ITC - Communication and Symbolic Behaviour Scales Infant-Toddler Checklist; CESD-R - Center for Epidemiologic Studies Depression Scale Revised; ADOS-2 – The Toddler Module of the Autism Diagnostic Observation Schedule - Second Edition.

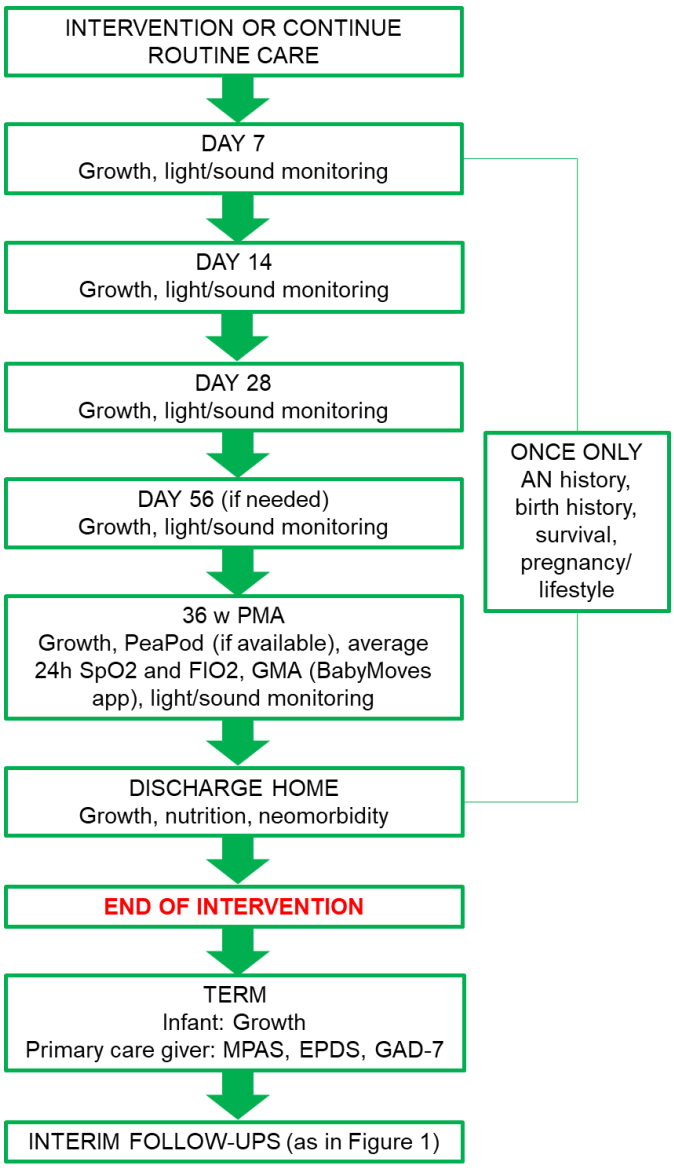


Figure 2 – Detailed Neonatal Data Collection
AN – antenatal; PNA – postnatal age; MPAS – Maternal Postnatal Attachment Scale; EPDS – Edinburgh Postnatal Depression Scale, GAD-7 – Generalised Anxiety Disorder Scale.

5.3 Minimisation of Bias

Infants meeting study eligibility criteria will be randomised to a control environment or a cycled environment (light and noise) from birth until discharge. Randomisation will use variable block sizes (block size 4 to 6) stratified by both gestation (<28 w, 28^0-31^6 w PMA) and study site, using the concealed randomisation facility built into the REDCap database via computer generated, randomisation tables developed and uploaded by the trial biostatistician (Dr Julie Marsh) and maintained by the Trial Management Team at the Telethon Kids Institute. Randomisation will maintain equal group sizes between the two study arms. Infants from multiple pregnancies will be assigned to the same group, to ensure planned secondary analyses on parent mental well-being and attachment are not confounded by conflicting infant circadian rhythms.

Blinding of families, healthcare providers during initial hospitalisation, and the co-ordinating study investigators to group allocation is not possible for this intervention. However, outcome assessors and data analysts will be blinded to allocation.

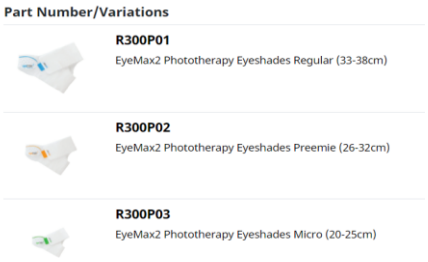
5.4 Randomisation Records and Codes

Infants will be given unique codes to protect their identity on all documentation utilised for recording and analysis purposes. Separate data records will be maintained for identifiable and de-identified data. As the intervention is not blinded, no procedures for breaking study code are anticipated that would alter an individual baby's care during hospitalisation.

5.5 Intervention/Product Description

Very preterm infants will be randomised to standard environmental care (control) or a cycled environment (light and noise) from birth until discharge home, including continuation of the intervention at any step-down secondary hospital.

The intervention group will receive cycled environmental light and noise to simulate day/night-time environments using a pragmatic 14 hour day (6 am – 8 pm), 10 hour night (8 pm – 6 am) cycle. Daytime intervention will include exposure to light including removal of cot-covers if present, to achieve lighting within the range of 300-600 lux,[44] whilst avoiding direct bright light to the infant's eyes. Nocturnal intervention will include repositioning of cot-covers, application of light-occlusive eye-masks and silicone ear-plugs. Eye-masks and ear-plugs will remain in position during any medical procedures or overnight cares unless removal is necessary for medical reasons. Similar cycling of cot-blankets in combination with cycled light was used previously in a small trial without adverse effects.[45]

Device Name	GMDN	Picture
Protection Equipment, Light Therapy, Eyewear (EyeMax2 Phototherapy Eyeshades; Micro, Preemie, Regular sizes)	30881	
Mack's Moldable Kids Size Silicone Ear-plugs	41230	

Handheld light meters positioned close to the infant will be used to ensure daylight lux targets are achieved and maintained (intervention group) or level of ambient lighting recorded (control group) at regular intervals throughout the day. Noise meters will also be positioned in the infant's cot to document the level of cyclicity in unit noise levels. Noise levels will be documented following an established schedule (see section 7.1.1).

5.6 Trial Duration/Schedule

The trial commenced in Western Australia in February 2019 with seed funding. NHMRC funding for the multicentre trial was obtained in 2021, commencing on 1st July. Each participant will remain enrolled until completion of the primary outcome assessment at 2 years cPNA. Figure 3 illustrates the anticipated timeline for the study. Allowing for recruitment of 954 infants in the multicentre trial, the study is expected to complete recruitment by December 2024, and will complete 2 year follow-up assessments by March 2027.

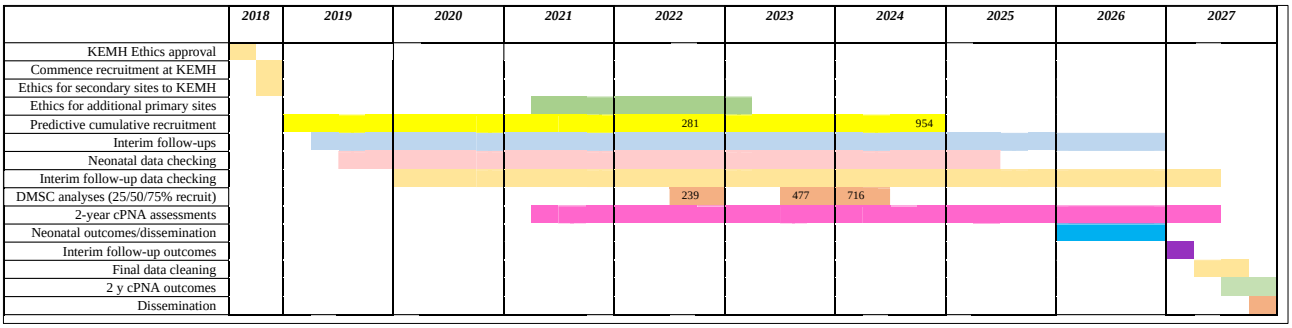


Figure 3 - CIRCA DIEM Timeline

cPNA – corrected postnatal age; DSMC – data safety monitoring committee

5.7 Trial Termination

A data safety monitoring committee (DSMC) will review the study data at 25 %, 50 %, 75 % recruitment to assess the safety of the intervention and to adapt the sample size as trial data accumulate. The DSMC may stop the trial if there are concerns that the study intervention is harmful to the patient, as defined prospectively in the charter. All adverse events will be reported to the respective Ethics Committee and the DSMC.

5.8 Data identification

Study data will be collected and entered into an ISO 27001/2 secure platform provided by the Biometrics team at the Telethon Kids Institute, hosted on Telethon Kids Institute infrastructure within the Perth Children's Hospital site. Following the development of a study data dictionary, the web-enabled trials database will be developed using a REDCap system platform. Identifiable and potentially identifiable data will be tagged to ensure placement of appropriate limitations of access to these data to a restricted group of approved research staff, and deidentification of downloaded data. Data access groups and user level security will be used to ensure site investigators can only access their own data. All data transactions will be recorded and any changes to data will be logged by data, time and operator.

5.9 Study Merchandise

Study merchandise (i.e. bibs) will be provided to the study families upon discharge from the NICU. Birthday cards will be sent to participants on their first and second birthday. Fridge magnets will be sent out to study families upon contacting them to arrange the 2-year follow-up assessment in the trial. These magnets will also act as a reminder for the follow up appointment.

5.10 Communications

The study will engage with participants' families and site teams through various channels to help ensure infants attend their final assessment appointment. This may include, but is not limited to, social media, websites, emails, eNews and newsletter stories, videos, digital screens, brochures, posters and media (print, TV and radio).

Communications may include site updates, study recruitment figures, progress through each of the milestones of the study, participant stories, and the importance of attending the final follow up appointment. Communications will be consistent with the study protocol, participant information sheets and informed consent forms and will be approved by the Coordinating Principal Investigator prior to use.

Any questions about the study raised on social media channels will be forwarded to the Coordinating Principal Investigator for clarification to ensure the correct information is communicated in a response where appropriate. An ad-lib, adaptable approach, based on the key messages, is required as the response must be tailored to ensure it is appropriate to the situation and context in which the question is asked.

Negative engagement on social media will be managed by the Telethon Kids Institute Communications team in collaboration with the Coordinating Principal Investigator. Inappropriate behaviour on social media will not be accepted, and any engagement of this nature will be hidden and user accounts will be blocked.

6 Source and Selection of Participants

6.1 Source of Participants

The trial will be co-ordinated by the sponsor Telethon Kids Institute. Participating centres will include tertiary NICUs admitting very preterm infants, and their secondary discharge hospitals. Participating hospitals will not use cycled lighting (defined as > 100 lux difference night/day) or cycled noise reduction (ear-plugs) for postnatal care. Clinicians in participating units will be in equipoise regarding environmental conditions in the trial context.

The primary sites currently include the King Edward Memorial Hospital (WA), Fiona Stanley Hospital (WA), Monash Newborn at Monash Children's Hospital (VIC), Women and Children's Hospital (SA), Royal Prince Alfred Hospital (NSW),Westmead Hospital (NSW), Gold Coast University Hospital (QLD), Joan Kirner Women's and Children's Hospital (VIC), Nepean Hospital (NSW), Wellington Hospital (New Zealand),Auckland City Hospital (New Zealand), Southmead Hospital (Bristol, UK), University College London Hospitals (UK) and British Columbia Children's and Women's Hospitals (Vancouver, Canada).

A sample size of 954 is required to detect a difference of at least 4 points on the cognitive sub-score at 2 years corrected postnatal age. This calculated sample size assumes 90 % power, 5 % significance level, standard deviation of 15 points, with a 2-sided t-test, 10 % loss to follow-up, and 30 % adjustment for multiple births. A blinded interim analysis will be performed after 50 % of participants have reached the 2 year endpoint to assess the assumptions in the sample size calculation.

The circadian biology/physiology sub-study (n=48/strata) has an 80 % power to detect a significant difference (at the 5 % level) in trend for the melatonin amplitude between control and intervention groups, using Wilks likelihood ratio test, assuming: 6 observations for each individual at time points 0, 1, 2, 4, 8, 12 weeks; an exponential increase in control means from 0 to 50 pg/mL and intervention means from 0 to 100 pg/mL; a standard deviation of 23.6 pg/mL;[58] a LEAR model for monotonically decreasing correlation between individual observations with a base correlation of 0.5 and a decay rate of 0.3, and; the open-source, web-based GLIMMPSE.[57]

Recruitment rates will be determined by the 477 infants required for the < 28 w gestation strata, who are most in demand for enrolment in neonatal clinical trials. Recruitment of an average of 18 infants/month in the < 28 w strata is achievable across 9-10 neonatal units (average 2 infants/month/site) to achieve our full study enrolment by 30 months after obtaining full study funding. The participation of up to an additional 10 study sites will be sought as necessary to ensure full study recruitment targets are achieved.

6.2 Participant Inclusion Criteria

Infants will be eligible for study inclusion if they meet all of the following inclusion criteria: 1) born at < 32 w PMA by best obstetric estimate (inborn or outborn); 2) initial care is at a perinatal centre where routine care does not include formal environmental light/noise cycling; 3) informed parental consent to trial participation, obtained either prior to or after birth. A register of potentially eligible infants will be documented by each study site to ensure transparent reporting of the study population.

6.2.1 Informed consent

Parent/s or guardian of an eligible infant will be approached by a CIRCA DIEM trial researcher prior to birth or within the first 48 h postpartum. Informed parental consent to trial participation is required within the first 168 h (7 days) postpartum for the < 28 w infants and within the first 72 h postpartum for the ≥ 28 w infants. This is so that the trial intervention and data collection can be initiated as quickly as possible postpartum however recognising that the parents of <28 w infants require more time to reach a decision regarding the inclusion of their child in the trial and typically have a longer stay in NICU where the study intervention would be maintained for longer compared to the ≥ 28 w infants. Circumstances surrounding the condition of both the mother and child will first be taken into consideration in the timing of study information and consent.

The CIRCA DIEM researcher will provide the parent/s or guardian with trial information in the form of a brochure, information sheet, video, and consent form. Trial information which will be discussed in detail

with the parent/s or guardian, and all questions by the parent/s or guardian will be answered in full. Should the parents approve the enrolment of their infant, the mother or primary caregiver will be required to sign either the paper informed consent form or the electronic consent form sent via REDCap surveys allowing us to take the measurements and perform samples collections that are outlined in the study design information.

The primary carer/parent will also be requested to complete a section of the informed consent form for their participation in the mental well-being and maternal/infant assessments. They will also be asked for their permission to be contacted after the final study assessment in relation to future studies.

6.3 Participant Exclusion Criteria

Infants will be excluded from participation in the trial if they meet any one or more of the following exclusion criteria: 1) have a congenital neurodevelopmental abnormality or medical condition that would contraindicate the use of the interventional product(s); 2) are critically ill and not expected to survive; 3) are unlikely to return for a 2 year follow-up; or 4) anticipated discharge to a non-participating neonatal unit prior to discharge home. Exclusion criteria will be documented for screening failures.

6.4 Participant Withdrawal Criteria

Participants may be withdrawn from the study at any time if requested by one or both parents. Participants may be withdrawn from the study by attending physicians if they develop a complication that may be reasonably attributed to the study. The reason for withdrawal will be documented. No further data will be obtained from the withdrawn infant, regardless of whether the infant still requires care in the NICU at any study site. Data obtained up until the point of withdrawal will still be used for data analysis.

Withdrawn participants will not be replaced by further recruitment of an infant for the respective age group.

7 Treatment of Participants

7.1 Description and Justification for Treatments, Interventions or Methods to be Utilised

7.1.1 Light and Noise Exposure

Infants assigned to the non-intervention group will be cared for in the normal neonatal intensive care environment. Difference in the intensity of light during the day and night (as measured in units of lux) will not be more than 100 lux and eye-masks will not be permitted unless required for phototherapy (see section 6.1). This change in light exposure for the non-intervention group is approximately the equivalent of walking from a dark room to the general low-level lighting that is found in a corridor, lift or stairwell. Infants who are in the non-intervention group may be cared for in continuous dim lighting, continuous bright light, or in an environment that changes in lighting level by not more than 100 lux.

In contrast, the light exposure for the intervention group will cycle between 0 lux and between 300-600 lux: hence, intervention group infants will have exposure during the day approximately equivalent to the minimum light level accepted in a general office or retail space, up to the brighter level of light required for reading or prolonged work on computers.

Nursing staff will have access to a portable light meter with a digital readout to ensure that prescribed lux levels are achieved and maintained as appropriate for infants in both arms of the study.

No background noise level is prescribed. There will be no formal hearing protection for non-intervention group infants, unless mandated by unit protocol for specific interventions (e.g. high-frequency ventilation; see section 6.2). The environmental sound is expected to be in the range of 63-72 decibels in most trial study units during the day, with the peak noise being around 80 dB. This range of background noise level is equivalent to the noise range of normal conversation through to the noise of a vacuum cleaner, peaking with a noise similar to an alarm clock (e.g., alarms from the monitor or ventilator). The background and peak noise is not expected to differ substantially between night and day. Infants in the intervention group will have a reduction in background and peak noise of approximately 22 dB when wearing the ear-plugs.

7.1.2 Other routine care

All other care and clinical decisions will be in accordance with the routine clinical protocols of each centre, with the exception that

- all trial babies will have neuroactive medications (e.g. caffeine) administered at 0800, when prescribed.
- postnatal steroids (e.g., dexamethasone) will be administered at 0600, and 1800, if prescribed by the attending physician for severe chronic lung disease.

Infants may still participate in the routine practice of afternoon 'quiet time' if required. This practice allows the dimming of light for 1-2 hours in the afternoon but does not permit the use of cot-covers, eye-masks or ear-plugs during this period.

7.1.3 Formal measurements of infant growth

- Formal measures of weight, length, head circumference, and growth velocity are obtained by study nurses at study entry, 36 w PMA, and 24 m cPNA and otherwise recorded from the maternal and child health record at intervals after birth.
- Infant body length is measured using a stadiometer/measurement board (e.g., SECA 210, Ecomed, NSW Australia) by one person placing the infant onto the measurement board, gently assuring that the infant is lying flat. Another person measures the body length on the stadiometer.
- Head circumference is measured using a flexible tape measure at the maximum diameter, through the supraorbital ridge to the occiput.
- Growth measurements obtained after discharge will otherwise be recorded from the infant's child health record.

7.1.4 Assessment of neonatal morbidities

- NEC severity will be defined by Bell's Criteria
- ROP severity will be defined by ICROP3

7.1.5 Measurement of infant neurological function

- Neurodevelopment is measured using Bayley-4 at approximately 2 y cPNA.[46]
- Neurobehaviour is measured on all infants at 22 months cPNA using the Infant Toddler Social-Emotional Assessment (ITSEA).[53] Neuromotor development is measured using Prechtl General Movements Assessment (GMA) at 36 w PMA and at 3 m and 3.5 m cPNA using the BabyMoves mobile phone app.[48-50] Movements will be recorded by parent/carer on the Baby Moves video smart phone app (app developed by CI Prof Spittle),[49] with blind scoring by two qualified independent assessors. A third assessor will be used if there is disagreement between the two primary assessors. If a child is identified as having an abnormal GMs trajectory, a member of the local research team will be contacted to discuss the outcome and whether the child already has a diagnosis/early intervention in place. Following these discussions, a member of the research team will contact the family to discuss the finding and if appropriate referral to clinical services made for a comprehensive developmental assessment if no services are currently in place.
- Disability risk is measured using Ages & Stages Questionnaire-3 (or ASQ-TRAK for indigenous study participants) at 2, 6,12 and 23 m cPNA.[51]

7.1.6 Measurement of maternal/primary caregiver mental well-being and maternal/infant attachment

Maternal/primary caregiver mental well-being and parent/infant attachment are assessed pre-hospital discharge, at 2 months cPNA (expected time of postnatal circadian rhythm establishment in control group), 6 months, 12 months and 24 months cPNA (as appropriate to the instrument) to establish if infant circadian rhythm and well-being at discharge home impact prevalence and severity of primary caregiver depression,

anxiety and attachment to the infant. These data are considered important as they may influence neurodevelopmental outcomes at 2 years. The instruments selected to obtain validated information with minimum parental effort include:

- Edinburgh postnatal depression scale (EPDS: 10 items) at discharge home, 2, 6 and 12 m cPNA;[51]
- Center for Epidemiologic Studies Depression Scale Revised (CESD-R: 20 items) at 2 y cPNA.[61,62]
- The Generalised Anxiety Disorder-7 (GAD-7: 7 items) score at discharge, 2, 6 and 12 m, and 2 y cPNA.[63]
- Maternal Postnatal Attachment Scale (MPAS: 19 items) score at discharge and 2, 6 and 12 m cPNA.[52]

7.1.7 Measurements of infant circadian biology (circadian rhythm biological sub-study – KEMH only)

The biological sub-study aims to identify if the intervention (cycled light and noise) results in earlier development of circadian rhythm after preterm birth. This sub study is vital to establishing if the mechanism of any differences in primary and secondary outcomes are related to differences in timing of circadian rhythms in preterm infants.

Saliva swabs, buccal swabs, temperature, heart rate, and peripheral oxygen saturation, will be obtained/measured at 4-hour intervals over a 24 hour period at study entry, 7 d, 14 d, 28 d and 4 weekly until discharge home.

- Heart rate and oxygen saturation will be determined from portable oximetry meters and data downloaded for analysis. We will make use of physiological data such as body temperature, respiratory rate, blood pressure collected routinely by nurses.
- Saliva swabbing (SalivaBio Infant's Swab by Salimetrics®) – An individually wrapped, soft synthetic swab of 90mm in length is held firmly at one end by the researcher/mother and the other end is placed into the infant's mouth, underneath the tongue for approximately 1 minute (can be done in shorter intervals) or until sufficiently saturated with saliva. The swab is then placed, saturated end of the swab first, into the tube insert of the swab storage tube and the lid is put securely fastened. The swab is then frozen at -20°C (temperature of a regular household freezer) until needed for analysis. Saliva is assayed for melatonin and cortisol levels using commercial EIA kits (Salimetrics).
- Buccal cells will be collected using buccal swabs (Catch-All™ Sample Collection Swabs by Gene TargetSolutions©) and buccal expression of key clock genes (Clock, Bmal1, Per2, Reverba) will be determined by qPCR.[60] (Clock genes are the genes that turn on and off in a cyclic (usually daily) rhythm, similar to the daily variations in our sleep wake cycle and variations in body temperature and blood pressure (amongst many other cyclic events). Clock genes are present in almost every cell of our body. The cyclic turning off and on of the clock genes in cells of our body is what drives our daily behaviours.) An individually wrapped, sterile, soft foam swab on a soft, flexible plastic handle is held firmly by the plastic handle and the swab is placed into the infant's mouth and gently wiped across the inside of the cheek for 20-30 seconds. The swab is then placed, swab side first, into the tube insert of the swab storage tube and the lid is securely fastened. The swab is then frozen at -20°C (temperature of a regular household freezer) until needed for analysis.

7.1.8 Assessment for autism (Social Communication sub-study, approved recruitment sites)

Infants with high CSBS-ITC scores will be eligible for a more formal evaluation of risk for ASD using the ADOS-2[54] at 2 y cPNA. The ADOS-2 will take approximately an hour to complete. The parent and child will be video recorded for scoring purposes. Once the videos have been scored, a single copy of the video will be retained on the Telethon Kids Institute server for the study data retention period with restricted access to the CIRCA DIEM study staff at the Telethon Kids Institute.

7.1.9 Measurement of infant lung function (neonatal lung function and body composition sub-study – KEMH only)

These measurements will tell us about the baby's normal breathing pattern and the size and function of the small airways. Tidal breathing, and respiratory mechanics will be obtained at 36 w PMA whilst the baby is in

quiet sleep. These measurements are minimally invasive and usually can be performed without disturbing the infant's sleep.

- We will record normal breathing volumes and respiratory rate, with a mask placed gently over the mouth and nose, while the baby is breathing spontaneously during quiet sleep using an ultrasonic flowmeter (Ecomedics Exhalyzer D).

7.1.10 Measurement of fat free mass (neonatal lung function and body composition sub-study)

- Infant fat-free mass is measured at 36 w cPNA using the PeaPod (Cosmed) non-invasive whole body densitometry device via air-displacement plethysmography technology for participants enrolled in the neonatal lung function and body composition study.
- Fat free mass will be obtained at 2 y cPNA using a compact & portable bioelectrical impedance monitor (Quantum III, RJL Systems) or similar.

7.1.11 Measurement of infant sleep quality and duration (infant sleep quality and duration sub-study)

Sleep is assessed from 24 hour actimeter (ActiGraph GT9X Link) recordings at study entry, 7 d, 14 d, 28 d and 4 weekly until discharge home in infant participants. Study personnel will administer the actigraphy devices to the infant's ankle for measurement and will be responsible for device removal after a 24 hour recording period.

A second group of infants will have quality and duration of day/night sleep at 36 w postmenstrual age (baseline) and again at 2 months and 6 months cPNA to determine if the intervention has a lasting impact on sleep quality and duration. Salivary melatonin will also be collected from infants and their main caregiver at each of these timepoints to determine if any differences in sleep behaviours are associated with a change in the amount of sleep hormone produced.

Parents of all study participants agreeing to be part of the sleep sub-study will be asked to complete sleep questionnaires (for infant) and sleep diaries (for parents) to provide a general understanding of the impact of the intervention on sleep behaviours after discharge.

7.1.12 MRI outcomes at term equivalent (MRI sub-study, approved recruitment sites)

MRIs are routinely performed at (near) term equivalent age in many of the participating recruitment sites for newborns deemed at increased risk of neurodevelopmental impairment, including infants < 29 w gestation. This study will be conducted at study primary recruitment sites that have access to a 3T scanner, and where MRI scans are performed routinely prior to discharge on high-risk infants (e.g. < 29 weeks). The MRI sub-study will obtain parental permission to review routine clinical MRI neuroimaging performed at (near) term equivalent age for analysis of key variables that may be predictive of adverse neurodevelopmental outcomes. Participants will not be subject to an MRI unless there is an independent clinical need established in advance of sub-study participation.

Typically, only the most advanced sequences available for infants will be applied, optimised to be robust to subject movement to ensure the least amount of time is taken for the procedure. The combined length of the below sequences will be approximately 45 minutes, which requires a 1 hour booking to allow for settling of the child and time between sequences. As many sequences will be obtained as possible, prior to the infant rousing, and will be collected in the following order:

1. Structural (T1, T2-weighted) image sequences are designed to maximise image resolution and contrast between tissue types in the infant brain. These sequences will undergo additional motion compensation during the acquisition, by using a volume navigator 'scout' echo-planar image (EPI). During this process, the field-of-view and slice positioning for the structural image is updated in real time whenever the subject moves. The following sequences will be acquired: 1) $0.8mm^3$ isotropic T2 Restore turbo spin echo sequence; 2) $0.8mm^3$ isotropic T1 multi-echo magnetization prepared rapid acquisition gradient echo (ME-MPRAGE).

2. Diffusion images are acquired using high resolution ($1.5mm^3$ isotropic) simultaneous multi-slice echo planar sequences with a twice refocused spin echo to minimize eddy current distortions, as well as multiband

excitation and multiple receivers for acceleration. Three gradient strength shells are acquired: b= 750 (25 directions), b=2000 (30 directions) and b=3000s/mm^2 (45 directions). Reverse phase encoding sequences are acquired to correct for image distortions.

7.1.13 Assessment of immune function and susceptibility to infection (Infection and Immunology substudy)

There is increasing awareness of the role that circadian rhythms play in maintaining a healthy immune system and preventing infections. The Infection and Immunology substudy has a broad aim to prospectively collect data on how the study intervention affects health and wellbeing in childhood and later life with a particular focus on the role of the circadian rhythmicity of the immune system and susceptibility to infection.

Parents will be invited to participate in prospective data collection to track their baby's general health including monitoring of temperatures, recording of symptoms of infection and any hospitalisation/GP visits, and doctor-diagnosed respiratory diseases such as asthma, using the smart phone MyCap App and/or a REDCap survey. Infants in Western Australia identified by the app to be at risk of a respiratory infection will be invited to have a diagnostic nasal swab during a home visit from a research nurse or at one of our clinical research centres.

Parents of babies enrolled in the trial at approved sites (KEMH, FSH) will also be invited to consent to collection of additional biological samples from their baby at study enrolment, prior to discharge home, and at 2 year follow-up visit. These additional biological samples (listed below) will allow detailed interrogation of the effect of the intervention on immune function, providing critical biological plausibility to explain any differences observed between the study groups in infection and immunological wellbeing over the first two years of life.

Routine samples for collection (approved KEMH and FSH sites only) on all infants will include the following samples.

- **retention of a small portion of the placenta for isolation of placental cells**. PathWest receives all placentas from births at KEMH and FSH and will receive automatic notification from the CIRCA DIEM REDCap database of any infant recruited to the trial with written permission for involvement in the CIRCA DIEM Infection and Immunology substudy, to facilitate the timely isolation of cells that will inform on an infant's baseline priming for later immunological disease.
- **a swab from inside the nose** (similar to a RAT test for COVID-19). Swabs will be obtained at study entry, prior to discharge (~ 34-36 w PMA) and at the 2 year follow-up. Each nostril will only be swabbed once on each occasion (either in the morning or in the evening) to minimise any risk of trauma.
- **collection of some saliva and buccal cells from the inside of the baby's cheek** morning and night at study entry, prior to discharge (~34-36 w PMA) and at 2 year follow-up appointment. Saliva is collected from a cotton swab placed (and held in place) under the baby's tongue for ~ 5 minutes. Buccal cells are collected with a gentle brushing against the inside of the infant's cheek for analysis of clock gene signals.
- **collection of samples of the baby's stools** (poo) from their nappy at the same time on two consecutive days at study entry, prior to discharge (~34-36 w PMA) and at 2 year follow-up appointment for analysis of microbiome rhythmicity.
- **collection of approximately 4 drops of blood** (~ 0.3 mL) in the morning and the evening at study entry, prior to discharge (~34-36 w PMA) and once only at the 2 year follow-up appointment. Blood collection will be timed to coincide with routine clinical blood collection whenever possible to avoid unnecessary additional blood draws. Blood will be placed in K3-EDTA tubes for testing of circadian gene expression, epigenetics, proteomics, metabolomics and immune development.

Optimal time of day for collection of biological samples (to observe maximum difference in daytime/night-time cell counts and immune function) will be established from the first 5 intervention babies enrolled in the substudy. These five babies will have biological samples obtained at 4 times across the day at 34-36 w PMA (~ 6 am, 11 am, 4 pm, 9 pm - timed with feeds/general cares). Prompt analysis of these samples will inform the definitive timing of morning/evening samples for the remainder of the Infection and Immunology substudy.

7.2 Permitted Medications/Treatments

- Eye-masks may be used 24/7 when phototherapy is being used in the first weeks of life.
- The use of eye-masks can be permanently or temporarily discontinued if a skin, eye or ear condition develops that precludes their use. All other interventions will be continued where practically possible to ensure the active intervention participant is still subjected to a cycled environment.
- Ear-plugs may be applied 24/7 for infants during high-frequency ventilation if this practice is in accordance with normal nursery protocols (normally short-term).
- Respiratory stimulants (e.g. caffeine) will be regulated to 8 am dosing for all infants, when prescribed, to avoid pharmacologic confounding on circadian rhythm development.
- If postnatal glucocorticoids are prescribed for severe lung disease, they will be given at 6 am/6 pm to reduce disruption to nocturnal sleep.

7.3 Monitoring of Participant Compliance

Noise levels will be documented for 24 hour periods on days 7, 14, 28 and 56, and at 36 w PMA at participating primary sites (see Figure 2 – Detailed Neonatal Data Collection). These records and appropriate placement of the intervention devices will be audited frequently by the trial site co-ordinator, and intermittently by the co-ordinating trial team.

8 The CIRCA DIEM Biobank (relevant to West Australian study participants and study sites)

Samples collected from Western Australian participants in the CIRCA DIEM Study who consent to participate in the Infection and Immunology sub-study will form the basis of the CIRCA DIEM biobank ("The Biobank"). The purpose of this biobank is to explore biological mechanisms that may contribute to neonatal outcomes of infants born prematurely as well as their well-being throughout childhood, adolescence and into adulthood. We anticipate that additional samples will be added to the CIRCA DIEM Biobank in subsequent years as follow-up studies of the CIRCA DIEM cohort are established. Samples may include saliva, buccal cells, nasal viral and epithelial swabs, epithelial cells, faeces, and blood. Whereas initial collection of the samples from CIRCA DIEM participants will require parental consent to sample collection and storage, CIRCA DIEM participants will be asked for consent for the Biobank to continue to retain their samples once they achieve their majority (18 years).

The Biobank custodian will be Telethon Kids Institute, and the Biobank will be managed on the Institute's behalf by a CIRCA DIEM Biobank Governance Committee chaired by Professor Jane Pillow, as the Principal Co-ordinating Investigator of the CIRCA DIEM study and two additional Biobank committee members with expertise in biobanking governance and management. The Founding Members of this committee will include Dr Luke Garratt and Dr Thomas Iosifidis in addition to Prof Pillow.

Biobank data will be managed within OpenSpecimen software, hosted on a secure server at Telethon Kids Institute and in accordance with relevant legislative requirements. Samples obtained and stored in the biobank will be labelled with a study number in addition to important information relating to the specific sample including date and time of sampling, as well as nature of the sample. Labelling will not include any identifiable information, but will be potentially reidentifiable as custodians of the biobank will retain a register linking study numbers to identifiable information. Parents (and participants when able to give informed consent) will be invited to give their permission for their infant/child's samples to be retained in the CIRCA DIEM biobank for future research studies relevant to understanding how prematurity impacts later wellbeing and/or susceptibility to illness.

Researchers wanting to access the Biobank will need to submit an application to the CIRCA DIEM Biobank Governance Committee and obtain ethical approval for their proposed study. Researchers will only be able to access de-identified data from the CIRCA DIEM study, and will be required to ensure the safety and security of data provided in accordance with Good Clinical Practice (GCP). The key to the code that permits reidentification of these samples will remain with the CIRCA DIEM Governance Committee.

9 Assessment of Safety

9.1 Risks and Benefits

9.1.1 Risks

This study is a low-risk research project. The eye-mask and ear-plugs are worn routinely by infants during periods of phototherapy and high-frequency ventilation respectively. The silicone ear-plugs are soft and mouldable to the ear canal. Routine cycled use of any of the interventional devices will not be a standard of care in any of the recruiting units. The placement of the eye-mask band over the ear acts as a safety measure against accidental dislodgement of the ear plug.

The circadian rhythm biological sub-study includes use of buccal and saliva swabs. These swab procedures involve negligible discomfort. There is a risk of aspiration/choking if the swabs are not used as directed i.e. was not held securely by the investigator/caregiver. Swabbing will be co-ordinated with feeds (obtained immediately pre-feed) to minimise impact on wakefulness on sampling days. The positioning of the actimeter on the ankle of the infant and wrist of the primary caregiver is anticipated to have negligible discomfort. The use of this wearable device on the infant participants is minimised through nurse checks every 3 to 4 hours while they are staying in hospital, the use of a neonatal posey (small strap designed specifically for pre-term infants), limited exposure (24 hours) and is limited to babies >1kg.

There is a risk that assessments of the mental well-being of primary caregivers may identify clinical conditions requiring medical assessment and care. All mental well-being questionnaires will be reviewed and graded promptly by the psychological assessment team to ensure prompt referral to medical care for individuals considered to be at risk.

9.1.2 Benefits

The potential benefits for infants assigned to the intervention include earlier development of circadian rhythm resulting in improved feed tolerance, growth, reduced illness severity, earlier discharge and improved long-term neurodevelopmental outcomes.

The potential benefits for the parents of infants assigned to the intervention include improved post-discharge sleep cycling, enhanced parent-infant attachment and improved mental well-being.

9.2 Safety

9.2.1 Data and Safety Monitoring Committee (DSMC)

The DSMC will review the study data at 25 %, 50 %, 75 % recruitment to assess the safety of the intervention and to adapt the sample size as trial data accumulate. The DSMC may stop the trial if there are concerns that the study intervention is harmful to the patient, as defined prospectively in the charter. Key safety outcomes will be assessed at 36 w PMA and will include:

A study-related adverse event (AE) will be:

1. Necrotising enterocolitis Stage IIb or greater (Bell's Criteria)
2. Retinopathy of prematurity with a Stage III or greater
3. Skin damage from use of wearables
4. Any perceived safety issue associated with the intervention devices

A study-related serious adverse event (SAE) will be:

1. Death before discharge home from hospital deemed potentially associated with the intervention.
2. Death after discharge and before 2 years of age associated with the intervention.
3. Any perceived life-threatening event during hospitalisation associated with the intervention devices.

9.2.2 Adverse event reporting

All adverse events will be documented in REDCap prospectively and reported to the respective Ethics Committee and the DSMC. The Telethon Kid's Institute will be responsible for providing the Annual Safety Report to the HREC.

9.2.3 Follow-up of adverse events

Type and duration of follow up following any adverse event will be subject to the discretion of the treating physician.

10 Data Management, Statistical Analysis and Record Keeping

10.1 Statistics and Interim Analysis

Data will be analysed in R software (open-source) on an intention to treat basis by the trial biostatistician (Dr Julie Marsh) and performed blinded to study group assignment.

Missing data will be handled via multiple imputation.

Children unable to be tested on Bayley-4 due to death, disability, severe delay or performance below threshold for individual composite scores will be assigned a score of 49.

Comparability of study groups at baseline will be based on patient demographic data (birth weight Z-score, gestation, exposure to chorioamnionitis and maternal steroids, maternal shift work) and baseline physiological circadian rhythmicity determined at randomisation.

Descriptive statistics will include mean (SD), median (IQR) for continuous variables with symmetric and asymmetric distributions, respectively, and counts/percentages for categorical variables.

Cognitive scores between the two groups at 2 years cPNA (primary outcome) will be analysed using (1^{o}) t-test and (2^{o}) linear mixed effects regression, adjusted for socioeconomic status, parental risk index, sex, birthweight Z score, gestation age, antenatal and postnatal steroid use, key neonatal morbidity count and including random effects for multiple birth ID and site. The false discovery rate employed for all secondary analyses will use the Benjamini-Hochberg algorithm.

10.2 Sample Size, Study Power, and Significance

A sample size of 954 is required to detect a difference of at least 4 points on the cognitive sub-score at 2 years corrected postnatal age.

This calculated sample size assumes 90 % power, 5 % significance level to detect a mean difference of 4 points in the primary outcome for a known population standard deviation of 15 points, with a 2-sided t-test, 10 % loss to follow-up, and 30 % adjustment for multiple births.

- A 2 sample t-test for mean difference of 4 points with SD of 15 points = 297 subjects/group resulting in a total unadjusted sample size of 594.
- The unadjusted sample size was rounded up to 600, then adjusted for expected 30 % prevalence of multiple births in the recruited population, and a 10 % loss to follow-up for the long-term outcome. 600/0.7/0.9 = 952.3 subjects
- The final sample size was rounded to 954, to ensure an even allocation of subjects to the two study groups.

A blinded interim analysis will be performed after 25 % and 50 % of participants have reached the 2 year endpoint to assess the assumptions in the sample size calculation.

Sample sizes for sub-studies target 80 % power to detect 0.5 SD mean difference with significance at the 5 % level.

Circadian rhythmicity will be assessed using cosinor analysis (non-linear regression) to determine mesor, amplitude, and acrophase of rhythms.

10.3 Statistical Plan Deviations

Any deviations to the statistical plan will be described and justified in updated versions of the protocol and in the final report, as appropriate.

10.4 Selection of Participants for Analyses

All infants recruited in the study will be evaluated statistically provided the participant is not withdrawn from the study before discharge from the hospital, and parents are satisfied for us to use the data.

10.5 Data Management

De-identified study data will be collected and entered into a secure online study database using REDCap (Research Electronic Database Capture) provided by the Biometrics team at the Telethon Kids Institute, hosted on Telethon Kids Institute infrastructure within the Perth Children's Hospital. REDCap has inbuilt capacity for eligibility confirmation, 24/7 randomisation/allocation within multi-stratified trial designs, and scheduling of follow-up, including timed survey distribution to participant families.

Randomisation tables will be generated by the trial biostatistician. We will use developed instruments for automatic de-identified import of routine pregnancy, birth, demographic and care during initial hospitalisation coded by the Australian New Zealand Neonatal Network. Additional study specific data fields will be collected and entered into the database prospectively.

Data collected will include primary and secondary outcomes, patient demographics (birth weight Z-score, gestation, exposure to chorioamnionitis and maternal steroids, maternal shift work) and postnatal confounders (e.g. duration of mechanical ventilation, exposure to therapeutic postnatal steroids, and episodes of confirmed sepsis (blood culture positive)).

10.6 Procedures for Missing, Unused and Spurious Data

Missing values will be imputed as appropriate and in consultation with a biostatistician. Data will be reviewed prior to analysis for extreme outliers and where reasonable these data will be removed from the analyses. All samples collected will otherwise be used in the study.

11 Monitoring / Audit

11.1 Monitoring, Audit and Regulatory Inspections Statement

Trial investigators/institutions will permit trial-related monitoring, audits, and regulatory inspections, providing direct access to source data/documents. This may include, but not limited to, review by external sponsors, Human Research Ethics Committees and institutional governance review bodies.

The CIRCA DIEM team will visit recruiting sites at least annually as permitted by travel restrictions to review procedures, data quality and intervention compliance and adherence.

12 Quality Control and Quality Assurance

12.1 Compliance Statement

The CIRCA DIEM trial will be conducted in compliance with the protocol, Good Clinical Practice, and the application regulatory requirements.

12.2 Quality Control

Adherence of study sites to the protocol and intervention will be monitored by:

- inspection of hospital and clinical record form logs
- direct observation by trials staff
- auditing of downloaded electronic recordings of environmental light and noise exposure (from light and noise exposure).

The CIRCA DIEM Trial Management Team at Telethon Kids Institute will provide routine auditing of REDCap study data quality and completeness in accordance with the CIRCA DIEM Data Monitoring Plan.

13 Ethics

13.1 Ethical Considerations

The proposed interventions are minimally invasive and have negligible ethical implications for the infants assigned to the intervention group. The interventions will not be permitted outside the study intervention arm for any infant admitted to a recruiting site.

13.2 Ethical Approvals

Ethics submission to the Child and Adolescent Health Service Human Research Ethics Committee in Western Australia via the National Mutual Agreement occurred in July 2018, and was approved in October 2018. Recruitment to the study commenced at the pilot trial site in February 2019. Several primary clinical sites started recruiting from mid-2022. Additional primary and secondary sites are planned into 2023 to ensure the full participant cohort is achieved.

Human Research Ethics Committees based in New Zealand, the UK and Canada will provide their approval for the study to be undertaken at international study sites that are relevant to their jurisdiction.

As infants and minors are unable to give consent, informed consent will be obtained prospectively from the parents/guardians of eligible preterm/term infants, for participation of their infant in the study, in addition to participation of the primary caregiver in relevant aspects of the mental well-being outcome assessments.

14 Budget, Financing, Indemnity and Insurance

14.1 Budget, Financing, Indemnity and Insurance

The pilot study (short term outcomes, infants recruited at KEMH only) was funded by the Telethon Perth Children's Hospital Research Fund and the NHMRC Preterm Infants Centre of Research Excellence.

A successful application to the WA Child Health Research Fund (2021-2023, $249,578) is supporting study follow-up of infants recruited to the study in the first 2 years.

The study was successful in achieving funding from NHMRC (2021-2026, $3.077 M) to complete the full main study protocol.

The study was successful in obtaining funding from a Channel 7 Telethon Grant in 2022 ($194,427.17) to initiate the post-discharge component of the sleep substudy (see 7.1.11).

The study was successful in obtaining funding from a Telethon Kids Collaboration Award in December 2022 ($125,000; Jan 2023-Dec 2023) to start collection of biosamples to underpin the Infection and Immunology substudy (see 7.1.13).

Recruiting sites will receive payment for recruitment and interim measurements. Study sites will also receive reimbursement for follow-up at 2 y cPNA. Reimbursement will be defined by standard Clinical Trial Research Agreements (CTRAs) established with each primary recruiting site. Equipment required for provision of the intervention devices (masks, ear plugs) and any measurement devices (light and sound meters, bioelectrical impedance, stadiometers, actimeters, software, assessment tools) will be provided by the trial team.

Indemnity and insurance will be provided by the sponsor, Telethon Kids Institute.

15 Publication and Knowledge Transfer

The CIRCA DIEM trial is registered by the Australian and New Zealand Clinical Trials Registry (ACTRN12618000371291).

Knowledge translation goals are identified for each knowledge user (clinicians, consumers, policy makers and researchers), and translation strategies developed including manuscript publication in leading international journals, inter-professional collaborations, educational outreach, mass media campaign, educational materials, champions and opinion leaders. Accelerated knowledge translation of CIRCA DIEM outcomes to clinical practice are expected, as clinicians are working in collaboration with the research team.

Follow-on studies of this valuable and unique large prospective study cohort will evaluate the children for evidence of ASD and early onset metabolic disorders as they attain the age of 5 y and 10 y, respectively, with potential later adult metabolic/cardiovascular studies. The CI/AI team includes expertise in long-term cognitive, neurobehavioural, neuropsychological and neuromotor outcomes, metabolic function, respiratory function, and circadian physiology and epigenetic biology.

16 References

1. March of Dimes, PMNCH, Save the Children, WHO. Born Too Soon: The Global Action Report on Preterm Birth. Geneva: World Health Organization, 2012.
2. Brydges CR, Landes JK, Reid CL, Campbell C, French N, Anderson M. Cognitive outcomes in children and adolescents born very preterm: a meta-analysis. *Dev Med Child Neurol* 2018.
3. Anderson PJ, Doyle LW. Cognitive and educational deficits in children born extremely preterm. *Semin Perinatol* 2008; **32**(1): 51-8.
4. Cheong JLY, Anderson PJ, Burnett AC, et al. Changing Neurodevelopment at 8 Years in Children Born Extremely Preterm Since the 1990s. *Pediatr* 2017; **139**(6).

5. Allotey J, Zamora J, Cheong-See F, et al. Cognitive, motor, behavioural and academic performances of children born preterm: a meta-analysis and systematic review involving 64 061 children. *BJOG* 2018; **125**(1): 16-25.
6. Malik A, Kondratov RV, Jamasbi RJ, Geusz ME. Circadian Clock Genes Are Essential for Normal Adult Neurogenesis, Differentiation, and Fate Determination. *PloS one* 2015; **10**(10): e0139655.
7. Weaver DR, Reppert SM. Maternal-fetal communication of circadian phase in a precocious rodent, the spiny mouse. *Am J Physiol* 1987; **253**(4 Pt 1): E401-9.
8. Mark PJ, Crew RC, Wharfe MD, Waddell BJ. Rhythmic Three-Part Harmony: The Complex Interaction of Maternal, Placental and Fetal Circadian Systems. *J Biol Rhythms* 2017; **32**(6): 534-49.
9. Armstrong BG, Nolin AD, McDonald AD. Work in pregnancy and birth weight for gestational age. *Br J Ind Med* 1989; **46**(3): 196-9.
10. Smarr BL, Grant AD, Perez L, Zucker I, Kriegsfeld LJ. Maternal and Early-Life Circadian Disruption Have Long-Lasting Negative Consequences on Offspring Development and Adult Behavior in Mice. *Sci Rep* 2017; **7**(1): 3326.
11. Murray E, Fernandes M, Fazel M, Kennedy SH, Villar J, Stein A. Differential effect of intrauterine growth restriction on childhood neurodevelopment: a systematic review. *BJOG* 2015; **122**(8): 1062-72.
12. Mendez N, Abarzua-Catalan L, Vilches N, et al. Timed maternal melatonin treatment reverses circadian disruption of the fetal adrenal clock imposed by exposure to constant light. *PloS one* 2012; **7**(8): e42713.
13. Welin AK, Svedin P, Lapatto R, et al. Melatonin reduces inflammation and cell death in white matter in the mid-gestation fetal sheep following umbilical cord occlusion. *Pediatr Res* 2007; **61**(2): 153-8.
14. Yawno T, Castillo-Melendez M, Jenkin G, Wallace EM, Walker DW, Miller SL. Mechanisms of melatonin-induced protection in the brain of late gestation fetal sheep in response to hypoxia. *Dev Neurosci* 2012; **34**(6): 543-51.
15. Gitto E, Marseglia L, Manti S, et al. Protective role of melatonin in neonatal diseases. *Oxid Med Cell Longev* 2013; **2013**: 980374.
16. Aridas JDS, Yawno T, Sutherland AE, et al. Systemic and transdermal melatonin administration prevents neuropathology in response to perinatal asphyxia in newborn lambs. *J Pineal Res* 2018.
17. Dickmeis T, Foulkes NS. Glucocorticoids and circadian clock control of cell proliferation: at the interface between three dynamic systems. *Mol Cell Endocrinol* 2011; **331**(1): 11-22.
18. Voiculescu SE, Zygouropoulos N, Zahiu CD, Zagrean AM. Role of melatonin in embryo fetal development. *J Med Life* 2014; **7**(4): 488-92.
19. Rodriguez RG, Pattini AE. Neonatal intensive care unit lighting: update and recommendations. *Arch Argent Pediatr* 2016; **114**(4): 361-7.
20. Fonken LK, Aubrecht TG, Melendez-Fernandez OH, Weil ZM, Nelson RJ. Dim light at night disrupts molecular circadian rhythms and increases body weight. *J Biol Rhythms* 2013; **28**(4): 262-71.
21. Watanabe S, Akiyama S, Hanita T, et al. Designing artificial environments for preterm infants based on circadian studies on pregnant uterus. *Front Endocrinol (Lausanne)* 2013; **4**: 113.
22. Keunen K, Benders MJ, Leemans A, et al. White matter maturation in the neonatal brain is predictive of school age cognitive capacities in children born very preterm. *Dev Med Child Neurol* 2017; **59**(9): 939-46.
23. Reid KJ, McGee-Koch LL, Zee PC. Cognition in circadian rhythm sleep disorders. *Prog Brain Res* 2011; **190**: 3-20.
24. Anderson PJ. Neuropsychological outcomes of children born very preterm. *Semin Fetal Neonatal Med* 2014; **19**(2): 90-6.
25. Perlman JM. Neurobehavioral deficits in premature graduates of intensive care--potential medical and neonatal environmental risk factors. *Pediatrics* 2001; **108**(6): 1339-48.
26. Darcy-Mahoney A, Minter B, Higgins M, et al. Probability of an autism diagnosis by gestational age. *Newborn Infant Nurs Rev* 2016; **16**(4): 322-6.
27. Kobayashi Y, Ye Z, Hensch TK. Clock genes control cortical critical period timing. *Neuron* 2015; **86**(1): 264-75.
28. Geoffray MM, Nicolas A, Speranza M, Georgieff N. Are circadian rhythms new pathways to understand Autism Spectrum Disorder? *J Physiol Paris* 2016; **110**(4 Pt B): 434-8.
29. Broring T, Oostrom KJ, van Dijk-Lokkart EM, Lafeber HN, Brugman A, Oosterlaan J. Attention deficit hyperactivity disorder and autism spectrum disorder symptoms in school-age children born very preterm. *Res Dev Disabil* 2018; **74**: 103-12.
30. Amaral FG, Castrucci AM, Cipolla-Neto J, et al. Environmental control of biological rhythms: effects on development, fertility and metabolism. *J Neuroendocrinol* 2014; **26**(9): 603-12.

31. Roggero P, Gianni ML, Garbarino F, Mosca F. Consequences of prematurity on adult morbidities. *Eur J Intern Med* 2013; **24**(7): 624-6.
32. AAP. Guidelines for perinatal care. Elk Grove, IL: American Academy of Pediatrics, 1997.
33. Als H, McAnulty G. Developmental care guidelines for use in the newborn intensive care unit (NICU): NIDCAP Federation International, 2015.
34. Borniger JC, McHenry ZD, Abi Salloum BA, Nelson RJ. Exposure to dim light at night during early development increases adult anxiety-like responses. *Physiology & behavior* 2014; **133**: 99-106.
35. Vasquez-Ruiz S, Maya-Barrios JA, Torres-Narvaez P, et al. A light/dark cycle in the NICU accelerates body weight gain and shortens time to discharge in preterm infants. *Early Hum Dev* 2014; **90**(9): 535-40.
36. Hao H, Rivkees SA. The biological clock of very premature primate infants is responsive to light. *Proc Natl Acad Sci U S A* 1999; **96**(5): 2426-9.
37. Morag I, Ohlsson A. Cycled light in the intensive care unit for preterm and low birth weight infants. *Cochrane Database Syst Rev* 2016; (8): CD006982.
38. Brandon DH, Silva SG, Park J, Malcolm W, Kamhawy H, Holditch-Davis D. Timing for the Introduction of Cycled Light for Extremely Preterm Infants: A Randomized Controlled Trial. *Res Nurs Health* 2017; **40**(4): 294-310.
39. Jorge EC, Jorge EN, El Dib RP. Early light reduction for preventing retinopathy of prematurity in very low birth weight infants. *Cochrane Database Syst Rev* 2013; (8): CD000122.
40. Brandon DH, Holditch-Davis D, Belyea M. Preterm infants born at less than 31 weeks' gestation have improved growth in cycled light compared with continuous near darkness. *J Pediatr* 2002; **140**(2): 192-9.
41. Valizadeh S, Hosseini M, Jafarabadi MA, Mirnia K, Saeidi F, Jabraeeli M. Comparison of 2 Methods of Light Reduction on Preterm Infants' Sleep Pattern in NICU: A Randomized Controlled Trial. *Crescent Journal of Medical and Biological Sciences* 2017; **4**(4): 211-6.
42. Noise: a hazard for the fetus and newborn. American Academy of Pediatrics. Committee on Environmental Health. *Pediatrics* 1997; **100**(4): 724-7.
43. Williams AL, van Drongelen W, Lasky RE. Noise in contemporary neonatal intensive care. *J Acoust Soc Am* 2007; **121**(5 Pt1): 2681-90.
44. Rivkees SA, Hofman PL, Fortman J. Newborn primate infants are entrained by low intensity lighting. *Proc Natl Acad Sci U S A* 1997; **94**(1): 292-7.
45. Mirmiran M, Baldwin RB, Ariagno RL. Circadian and sleep development in preterm infants occurs independently from the influences of environmental lighting. *Pediatr Res* 2003; **53**(6): 933-8.
46. Bayley N. Bayley Scales of Infant and Toddler Development. 3rd Edition ed. San Antonio, TX: Pearson PsychCorp; 2006.
47. Lee H, Park H, Ha E, et al. Effect of Breastfeeding Duration on Cognitive Development in Infants: 3-Year Follow-up Study. *J Korean Med Sci* 2016; **31**(4): 579-84.
48. Spittle AJ, McGinley JL, Thompson D, et al. Motor trajectories from birth to 5 years of children born at less than 30 weeks' gestation: early predictors and functional implications. Protocol for a prospective cohort study. *J Physiother* 2016; **62**(4): 222-3.
49. Spittle AJ, Olsen J, Kwong A, et al. The Baby Moves prospective cohort study protocol: using a smartphone application with the General Movements Assessment to predict neurodevelopmental outcomes at age 2 years for extremely preterm or extremely low birthweight infants. *BMJ Open* 2016; **6**(10): e013446.
50. Einspieler C, Bos AF, Libertus ME, Marschik PB. The General Movement Assessment helps us to identify preterm infants at risk for cognitive dysfunction. *Front Psychol* 2016; **7**: 406.
51. Squires J, Bricker D. Ages & Stages Questionnaires, Third Edition (ASQ-D). Baltimore, MD: Brookes Publishing; 2009.
52. Condon J, Corkindale C. The assessment of parent-to-infant attachment: Development of a self-report questionnaire instrument. . *J Reprod Infant Psychol* 1998; **16**(1): 57-76.
53. Carter AA, Briggs-Gowan M. Infant Toddler Social-Emotional Assessment. Australia & New Zealand: Pearson; 2005.
54. Lord C, Luyster RJ, Gotham K, Guthrie W. Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) Toddler Module. Australia & New Zealand: Pearson; 2012.
55. Kidokoro H, Neil JJ and Inder TE. New MR Imaging assessment tool to define brain abnormalities in very preterm infants at term. *American Journal of Neuroradiology* 2013; 34: 2208-14.
56. Nguyen ST, Anderson PJ, Hunt RW, Doyle LW, Shimony JS and Inder TE. A novel quantitative simple brain metric using MRI for preterm infants. *American Journal of Neuroradiology* 2009; 30 (1): 125-131.
57. Drummond MF, Sculpher MJ, Claxton K, Stoddart GL, Torrance GW. Methods for the Economic Evaluation of Health Care Programmes. 4th ed. Oxford, UK: Oxford University Press; 2015.

58. Novakova M, Paclt I, Ptacek R, Kuzelova H, Hajek I, Sumova A. Salivary melatonin rhythm as a marker of the circadian system in healthy children and those with attention-deficit/hyperactivity disorder. *Chronobiol Int* 2011; **28**(7): 630-7.
59. Kreidler SM, Muller KE, Grunwald GK, et al. GLIMMPSE: Online Power Computation for Linear Models with and without a Baseline Covariate. *J Stat Softw* 2013; **54**(10).
60. Kurbatova IV, Topchieva LV, Korneva VA, Kolomeichuk SN, Nemova NN. Expression of circadian rhythm genes CLOCK, BMAL1, and PER1 in buccal epithelial cells of patients with essential arterial hypertension in dependence on polymorphic variants of CLOCK and BMAL1 genes. *Bull Exp Biol Med* 2014; **157**(3): 360-3.
61. Cox JL, Holden JM, Sagovsky R. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. *Br J Psychiatry* 1987; **150**: 782-6.
62. Eaton WW, Muntaner C, Smith C, Tien A, Ybarra M. Center for Epidemiologic Studies Depression Scale: Review and revision (CESD and CESD-R). In: Maruish ME, ed. The Use of Psychological Testing for Treatment Planning and Outcomes Assessment. 3rd ed. Mahwah, NJ: Lawrence Erlbaum; 2004: 363-77.
63. Spitzer RL, Kroenke K, Williams JBW, Lowe B. A brief measure for assessing generalized anxiety disorder. *Arch Inern Med* 2006; **166**: 1092-7.