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Title Reference centiles for infant sleep parameters from 4 to 16 weeks of age: Findings from an Irish cohort

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Objectives To establish unconditional reference centiles for sleep parameters in infants 4 to 16 weeks after birth.

Design and Setting Secondary data analysis of sleep parameters recorded at 4-16 weeks after birth in a longitudinal randomised controlled trial (BabySMART).

Patients Healthy term infants assigned to the non-intervention arm of the RCT.

Main outcome measures Infants' sleep duration was recorded by parents/guardians daily, from week 2-16 after birth using a sleep diary. Reference centiles for total, daytime, night-time, and longest sleep episode duration were estimated using multi-level modelling.

Results One-hundred and six infants, mean (SD) gestational age 39.9 (1.2) weeks, and mean (SD) birth weight of 3.6 (0.5) kg, had sleep recorded contributing 1264 measurements for each sleep parameter. Between 4 and 16 weeks after birth total sleep duration in a 24-hour period, night-time sleep duration in a 12-hour period and infant's longest sleep episode duration increased while daytime sleep duration in a 12-hour period decreased.

Conclusions Reference centiles up to 4-months after birth in infants, highlight the gradual decrease in day-time sleep and large increases in night-time sleep, which occur in tandem with increasing lengths of sleep episodes. These reference centiles provide useful sleep values for infant sleep trajectory occurring in early life and may be helpful for parents and clinicians.

Keywords infant, sleep, development, sleep duration

Clinical Trial Registration BABY SMART (Study of Massage Therapy, Sleep And neurodevelopMenT) (BabySMART)

URL <https://clinicaltrials.gov/ct2/show/results/NCT03381027?view=results>

ClinicalTrials.gov Identifier NCT03381027

Introduction

Sleep is an essential part of early life and is crucial for early brain maturation. The formation of organised sleep patterns in infancy is considered a significant milestone in early development with an association between early infant sleep and construction of the sensory system of the brain ^{1,2}. In primate models, the circadian clock develops and starts to oscillate within the suprachiasmatic nucleus in early gestation, with day-night differences seen as early as the end of gestation ^{3,4}. Sleep evolves rapidly in the first years of life, with the first 6-months led by both consolidation of nocturnal sleep episodes and the lengthening of sleep episodes which occur in tandem ⁵⁻⁷. In the first weeks, infant sleep is divided equally between day and night, but patterns begin to develop as night-time sleep lengthens, and infant self-soothing habits emerge ^{8,9}. By 12 weeks of age, day-night rhythms in the production of melatonin can be observed ^{4,10}. Throughout this period, infants enter sleep in an active rapid-eye-movement (REM) stage and spend 50% of their sleep in an active REM stage; REM is considered to have a unique role in early brain development ^{8,11}.

What are considered as ‘normal’ sleep patterns in early life, such as frequent night-time awakenings and delayed sleep onset, can be perceived by parents as unhealthy infant sleep habits, with a quarter of parents reporting sleep problems in their infants in the first 4-months ^{5,11}. Further to this, high variability in sleep duration, within infants and between infants, in the first months of life has the potential to lead parents to consider common patterns that fall within the normative range as a sign of a more serious sleep disorder. Therefore, well-defined reference centile charts of infant sleep evolution in the first months after birth may be very helpful, for both parents and clinicians.

To date, many studies assessing infant sleep trends over time are based around sleep questionnaires, sleep diaries, and actigraphy measurements. Questionnaires only capture a

snapshot of infant sleep and technical difficulties with actigraphy devices mean that up to 28% of weekly recordings can be lost, and importantly most sleep diaries and actigraphy studies are limited due to short recording periods¹². This study aims to define the reference centiles of total sleep in the first 4 months of age, and secondly to define the reference centiles of night-time sleep, daytime sleep, and longest sleep episode as they evolve.

Methods

Study Sample

This study included healthy term infants assigned to the non-intervention arm of the BabySMART (Study of Massage Therapy, Sleep And neurodevelopMenT) randomised controlled trial, registered ID: NCT03381027. Infants were recruited from birth in Cork University Maternity Hospital, Cork, Ireland, from 2017 to 2018, based on the following inclusion criteria: ≥ 37 weeks gestation and singleton pregnancy, no NICU admission required, and no suspected congenital or metabolic anomalies. Infants in the non-intervention arm were randomised to standard parental care from 2 to 16 weeks of age by parents.

Ethical Approval

The study was approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals, Cork, Ireland (Ref: ECM 3 (ggg) 04/04/17 & ECM 3 (hhh) 06/06/17). Written informed consent was obtained from parents of all study participants.

Measurements and Questionnaires

At 2 weeks the parents were asked to respond to a study questionnaire including questions of sociodemographic and feeding status and were provided with a sleep diary in the form of either a paper diary or smartphone enabled sleep application (app) designed specifically for the study; the choice of recording medium was based on parental preference. Parents were requested to

record infant sleep episodes every 24-hours until the 4-month-appointment (approx. 16-weeks of age). Parental recordings in the paper diary involved filling in the duration of sleep in 30-minute (min) epochs across each 24-hour period every week. The recordings in the app involved entering the time, date, and duration of each sleep episode. After the sleep recording period was completed parents were asked to fill out additional questionnaires at the 4-month appointment (approx. 16-weeks of age) including the Brief Infant Sleep Questionnaire (BISQ), a questionnaire to evaluate sleep patterns, parent perception, and sleep behaviours in infancy which was used to gain further insight into parent perceived infant sleep duration and sleep quality ^{13,14}.

Infant Sleep Analysis

Recordings for each infant were split into 24-hour (hr) periods from 12:00 to 11:59. Day-time sleep was defined as sleep from 07:00 to 18:59 and night-time sleep from 19:00 to 06:59 ¹⁴. Sleep recordings that contained less than 4 hrs and more than 22 hrs of sleep in a 24-hr period, and those that did not have at least one day-time and one night-time recording in 24 hours were removed. ¹³ For each infant, total sleep time, day-time sleep, night-time sleep, and longest sleep episode within each 24-hour period were calculated and then averaged (across 7 days) for weeks 4 to 16 since birth. The 7-day average was used to adjust for day-to-day variability in parental reporting of their infant's sleep.

Statistical analysis

Descriptive statistics were used to describe the study sample. Continuous variables were described using mean and standard deviation (SD) for normal distributions, and median and interquartile range (IQR) for non-normal distributions. Categorical variables were described using frequency and percentage. Descriptive statistics were calculated using IBM SPSS Statistics (v26.0, IBM Corp., Armonk, NY, U.S.A.).

Unconditional reference centiles for total sleep time, day-time sleep, night-time sleep and longest sleep episode from 4 to 16 weeks after birth were constructed using a multilevel linear mixed modelling approach^{15,16}. A random coefficient model was used which accounts for variability in the sleep parameter at both the between-subject and within-subject levels, by incorporating subject-specific effects for the intercept and growth (slope) component. Prior to performing the linear mixed modelling, the sleep parameters (dependent variables) and age (independent variable) of the infant were transformed, if necessary, to meet the linear mixed model assumptions of normality of residuals, homoscedasticity and a linear relationship between the sleep parameter and age of the infant. Box–Cox regression was used to identify the power transformation (λ) of the sleep parameter that approximated a normal distribution and provided a homoscedastic relationship with age. The Box-Cox power transformation was a constant and was obtained using a Box-Cox transformation of the sleep parameter variable with age included as a third-degree polynomial. The choice of transformation took age into account because an apparent skewness in the sleep parameter variable may be due to skewness in age.¹⁷ After transformation of the sleep parameter, fractional polynomial regression was used to find the best-fitting linearising function of age¹⁷. A second-degree fractional polynomial was chosen if it fitted significantly better than a first-degree model; otherwise, a first-degree model was chosen. Let Z_{ij} denote the transformed sleep parameter and X_{ij} represent the fractional polynomial transformation of age. Then the mean (μ_{ij}) and variance (σ^2_{ij}) of Z_{ij} at transformed time X_{ij} are: $\mu_{ij} = E(Z_{ij}) = \beta_{0j} + \beta_{1j}(X_{ij})$; $\sigma^2_{ij} = \text{var}(Z_{ij}) = \sigma^2_{\beta_{0j}} + \sigma^2_{\beta_{1j}}(X_{ij}^2) + 2\sigma_{\beta_{0j},\beta_{1j}}(X_{ij}) + \sigma^2_{rij}$, where $\sigma^2_{\beta_{0j}}$ represents the (between-infant) variance of the random intercepts, $\sigma^2_{\beta_{1j}}$ represents the (between-infant) variance of the random slopes, $\sigma_{\beta_{0j},\beta_{1j}}$ is the covariance between them, and σ^2_{rij} is the estimated within-infant variance. The reference centiles for the sleep parameters are calculated from the mean and variance above as $[\mu_{ij} \pm \phi\sigma_{ij}]^{1/\lambda}$ if $\lambda \neq 0$ and $\exp[\mu_{ij} \pm \phi\sigma_{ij}]$ if $\lambda = 0$ where σ_{ij} is the standard error of Z_{ij} and ϕ is the standard normal deviation of the

distribution function (± 1.96 for the 2.5th and 97.5th centiles, ± 1.645 for the 5th and 95th centiles, ± 1.282 for the 10th and 90th centiles, ± 0.674 for the 25th and 75th centiles, and 0 for the 50th centile) ^{17,18}. Multi-level modelling was conducted in STATA (v15.1, StataCorp LP, College Station, TX, U.S.A), and figures were produced using the ggplot2 package in R (v1.2).

Results

Two-hundred and four term infants were initially recruited and from this one-hundred and six infants had recordings with minimum requirements documented in sleep diaries from week 4 to 16 after birth and were included in the analysis. The demographic characteristics of 106 infants, their feeding status and sleeping arrangement at 2 weeks of age, as well as the duration of sleep that infants should have in 24 hours as perceived by parents are presented in table 1. Almost half of the infants (49%) were exclusively breastfed and 25% had some form of co-sleeping at 2 weeks.

The number of infants with data available for each week is shown in supplementary table 1. The median(IQR) number of weeks (out of 13) with data available was 13(12-13) and almost 90% of infants (89%, 94/106) had data available for more than 10 of the weeks. For each sleep parameter, multi-level modelling results were based on 1,264 weekly summary measures for weeks 4-16 across 106 infants. The best fit models for the change in sleep durations can be seen in table 2. Projections of total sleep, daytime sleep, night-time sleep, and longest sleep trajectories based on centiles obtained from the best fit models can be seen in figure 1 and supplementary table 2.

Ninety-six parents completed a questionnaire at 16-weeks. At 16-weeks, 30 infants were exclusively breastfed (31%), 53 infants were formula-fed (55%) and 13 infants had mixed feeding (14%). Twenty-five infants (26%) had been introduced to solids.

Results of the BISQ are presented in table 3. The number of infants that always sleep in their parent's bed increased from 5% (5/105) at 4 weeks to 9% (9/96) at 16 weeks. Most parents (80%) did not consider their infant sleep as a problem. At 16-weeks the parental perception of their infant sleep from the BISQ was similar to sleep diary modelled estimates for daytime sleep, the BISQ daytime sleep was predicted to be on average 3.80 hrs, and the sleep diary model estimated daytime sleep at 16 weeks to be 3.72 hrs. A larger difference of 39 mins was found in night-time sleep, where the BISQ predicted 9.60 hrs, and sleep diary model estimated sleep to be 8.95 hrs.

Discussion

This is the first study to provide detailed sleep reference centiles for sleep parameters, using sleep diaries, from 4-16-weeks of age. The detailed recordings provided a rich source of information to investigate the intricate patterns occurring across the weeks in early infant sleep development, including detailed modelling of preference toward night-time sleep and longer sleep episodes ^{8,9,19}.

Total sleep, night-time sleep, and longest sleep duration gradually increase, and daytime sleep duration decreases over the first 16-weeks of age. Over the 13-week study period, total sleep increased by under 1-hour, daytime sleep decreased by nearly 2-hours, night-time sleep increased by over 2.5-hours, and longest sleep episodes increased by more than 3-hours. This study also shows how consolidation of night-time sleep occurs more rapidly than the decrease in daytime sleep, and so the reduction in total-sleep that becomes more prevalent after 6-months has yet to occur ¹³.

Iglowstein et al. 2003 has previously generated reference values from infancy to adolescence but the values generated for early infancy were limited as they were based on 1-, 3-, and 6-month questionnaires alone ^{14,20}. Reliance on sleep questionnaires can be limiting, as sleep

questionnaire studies are dependent on recalling sleep estimates at a later date, and so can be prone to recall bias ^{14,21}. Further to this, questionnaires are only a snapshot of an infant's sleep profile, the validated BISQ is considered the most popular assessment tool, however, parental questionnaires have been shown to estimate higher sleep durations when compared to more objective measures. This higher estimation in night-time sleep was also observed in our study when comparing the BISQ to our sleep diary entries ²¹. A strength of this study is the prospective and detailed nature of each individual sleep episode recorded daily by the infant's parents over a long study period of 13-weeks (4- to 16-weeks of age), in comparison to other sleep diaries studies which are limited to 6 to 7-days per month of sleep recordings ^{22,23 24}.

The BISQ at 16-weeks identified daytime sleep duration to be nearly identical, approximately 5-minutes longer, when compared with our reference estimates at 16-weeks, and this estimate was similar to previous questionnaire-based averages ^{13,25}. Night-time sleep was estimated to be higher in our BISQ results compared to reference estimates, this higher estimate was consistent with previous questionnaire-based averages ¹³. This might indicate that prospective sleep diary recordings are more accurate than estimates, but it is unclear if these night-time estimates are more accurate and the parents have failed to input all night-time sleep episodes in the sleep diaries, or if our parents had more difficulty in accurately estimating night-time sleep durations in the questionnaires, a questionnaire-based overestimation of sleep durations has been previously indicated when compared to polysomnography ²⁶. Mode of feeding was considered as an additional factor for the models, but no differences were observed when comparing those exclusively breastfed with those that received mixed or formula feeding (not reported).

Limitations to this study include the reliance on parental recordings, which may have led to under/over reporting or under/over estimating sleep durations. Sadeh et al., described a close relationship between objective measures such as actigraphy and sleep diaries, although sleep

diaries have a tendency for overestimation ²⁷. It could also be argued that in the later weeks there was a potential for over reporting sleep duration if the infants had begun to self-soothe and the parents were not alerted to the awakenings. Longitudinal continuous sleep diaries are considered a stronger method to retrospective questionnaires as they could become part of the bedtime routine and would not be affected by recall bias, leading to incorrect sleep duration estimates, where parents may be more likely to report on sleep durations commonly described for their infants age range. These problems can be more predominant in the questionnaire-based method ^{28,29}. Additionally, this study was based on a population of primarily white Irish infants, and it is unclear if the findings are generalisable to other ethnicities or societies. Furthermore, from the initial recruited population just over half of the parents completed the minimum requirements for the sleep diaries, $n = 106$, with mothers who remained in the study around 2 years older on average than mothers of infants that did not, and so this may limit the generalisability of our results to a general population. Another limitation to this study is that it remains unclear if the infants of parents that did not complete the diary had longer or shorter sleep episodes.

Conclusion

This study provides reference centiles for week-by-week development of sleep durations occurring from 4-weeks until 16-weeks after birth. Information on reference sleep ranges in early infancy is important for assessing infants with suspected sleep problems, but also for reassuring parents that variability and duration of early sleep projections in early sleep parameters are not a cause for concern and that periods of shorter sleep episodes and mixed circadian rhythm evolve to more rhythmic day-night trends and a more consolidated sleep over the first months after birth.

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Competing interests None declared.

Contributors' Statement Dr Marc O'Sullivan conceptualized the study, carried out the analyses, drafted the initial manuscript, and reviewed and revised the manuscript; Dr Vicki Livingstone conceptualised the study, carried out the analyses, and reviewed and revised the manuscript; Dr Irina Korotchikova conceptualised and reviewed and revised the manuscript; Prof Gene Dempsey and Prof. Deirdre Murray designed and conceptualised the study and reviewed and revised the manuscript; Prof. Geraldine Boylan designed, conceptualised, coordinated and provided study supervision, and reviewed and revised the manuscript; All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

What is already known on this topic

In the first postnatal weeks, infant sleep is divided equally between day and night, but over the early month's patterns begin to develop with the lengthening of night-time sleeps and the emergence of self-soothing habits.

What this study adds

Through parental recordings using detailed daily sleep diaries, this study provides reference centiles for infant sleep trajectory up to 4-months after birth, when early sleep patterns and sleep consolidation begins.

How this study might affect research, practice, or policy

The reference centiles for the sleep parameters provided in this study, can be informative to practitioners and researchers, and may help identify infants with sleep problems, in the first months after birth.

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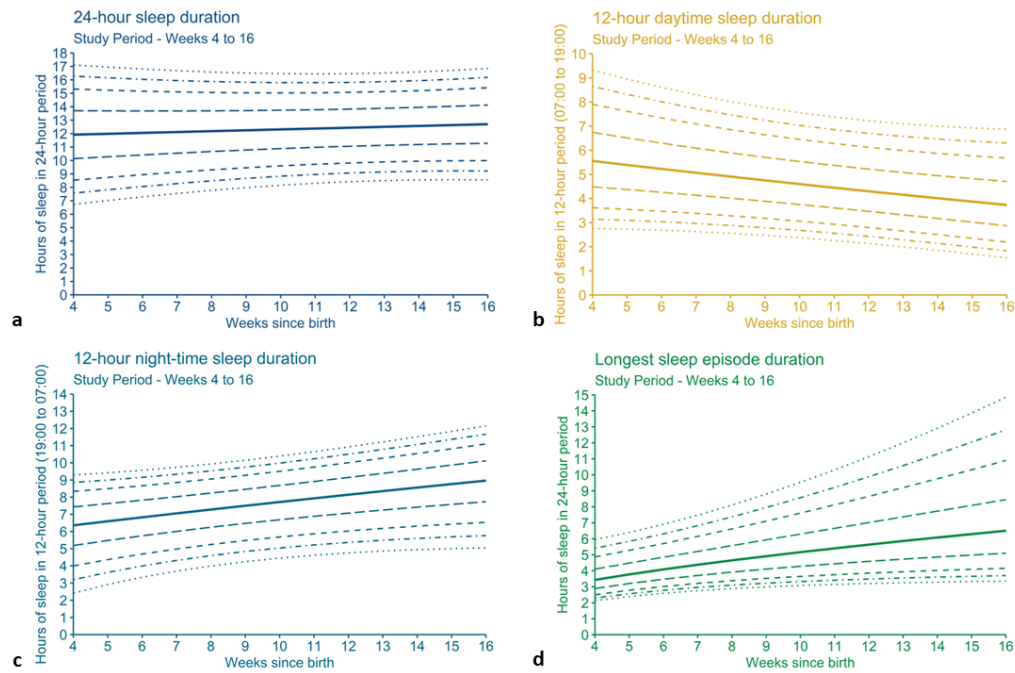


Figure 1. Reference centiles for (a) total sleep in 24-hours, (b) daytime sleep in 12-hours, (c) night-time sleep in 12-hours, and (d) longest sleep in 24-hours for weeks 4-16 since birth. The line type depicts different centiles: dotted lines indicate p2.5 and p97.5, dash-dotted lines indicate p5 and p95, short-dashed lines indicate p10 and p90, long-dashed lines indicate p25 and p75, and solid lines indicate p50.

Table 1. Demographics at registration and two-week questionnaire.

	<i>n</i>	
Registration		
Sex – male	106	61 (58)
Birth weight (kg)	106	3.6 (0.5)
Gestational age (weeks)	106	39.9 (1.2)
Maternal age (years)	106	34.6 (4.5)
<i>Mode of delivery</i>	106	
Spontaneous vaginal delivery		40 (38)
Instrumental		22 (21)
Caesarean		44 (42)
Two-week Questionnaire		
<i>Maternal ethnicity</i>	106	
White		99 (93)
Other		7 (7)
<i>Paternal ethnicity</i>	106	
White		101 (95)
Other		5 (5)
<i>Net income per annum</i>	105	
€30,000 or less		8 (8)
€30,001 to €60,000		25 (24)
€60,001 or above		40 (38)
Prefer not to say/do not know		32 (30)
<i>Feeding</i>	105	
Breastfed only		51 (49)
Formula only		43 (41)
Mixed		11 (10)
<i>Usual Infant sleep setting</i>	105	
Cot in parents’ room		100 (95)
In parents’ bed		5 (5)
Does infant ever co-sleep – yes *	105	26 (25)
How often should a newborn sleep in 24-hrs? (hours)	98	17.80 (16.00 to 18.10)

Mean (SD), median (Q₁ to Q₃) or *n* (%). SVD = spontaneous vaginal delivery. *Includes infants that usually sleep in parent's bed.

Table 2. Results of multilevel modelling for sleep measures in relation to age (weeks).^a

	Total sleep (TS)		Daily sleep (DS)		Nightly sleep (NS)		Longest sleep (LS)	
Transformations								
Dependent variable (Sleep measure)	-		DS ^{0.5}		NS ^{1.5}		LS ^{-0.3}	
Independent variable (Weeks)	-		-		-		ln(Weeks)	
Multilevel model								
Fixed part	Estimate	p-value	Estimate	p-value	Estimate	p-value	Estimate	p-value
Intercept	11.66	<0.001	2.499	<0.001	12.44	<0.001	0.8114	<0.001
Weeks ^b	0.06512	0.002	-0.03544	<0.001	0.8999	<0.001	-0.08697	<0.001
Random part	Variance		Variance		Variance		Variance	
Individual variance (Intercept)	8.594		0.1421		39.13		0.009080	
Individual variance (Weeks ^b)	0.03488		0.0008286		0.3653		0.002162	
Individual covariance (Intercept, Weeks ^b)	-0.4541		-0.008381		-2.687		-0.004063	
Residual variance	1.486		0.03777		15.87		0.0009163	
Model specifications								
Log restricted likelihood	-2292		38.34		-3765		2372	
AIC	4596		-64.69		7542		-4733	
BIC	4627		-33.83		7573		-4702	

^an=106 infants with 1264 measurements; ^bln(Weeks) for longest sleep model.

Table 3. Brief Infant Sleep Questionnaire at 16 weeks.

	<i>n</i>	
<i>Sleeping arrangement (%)</i>	96	
Cot in parents' room		72 (75)
Cot in a separate room		14 (15)
In parents' bed		9 (9)
Cot in a room with a sibling		1 (1)
<i>Infant sleeping position (%)</i>	96	
On back		79 (82)
On side		15 (16)
On belly		2 (2)
Daytime sleep duration (7 am to 7 pm), hours	96	3.80 (1.30)
Nocturnal sleep duration (7 pm to 7 am), hours	96	9.60 (1.30)
Nocturnal wakefulness (10 pm to 6 am), hours	95	1.00 (0.00 to 1.50)
Sleep-onset time, mins	96	15 (10 to 30)
<i>How does the infant fall asleep? (%)</i>	96	
In bed alone		30 (31)
While feeding		29 (30)
Being held		17 (18)
In bed near a parent		12 (13)
Being rocked		8 (8)
When does infant usually fall asleep for the night? hours:minutes	96	20:53 (20:00 to 21:30)
<i>Infant's sleep considered a problem (%)</i>	96	
Very serious problem		1 (1)
Small problem		18 (19)
No problem at all		77 (80)

Mean (SD), median (Q₁ to Q₃) or *n* (%).