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## Exclusive breastfeeding at three months and infant sleep-wake behaviors at two weeks, three and six months

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## ABSTRACT

This study assessed infant sleep-wake behavior at two weeks, three and six months as function of feeding method at three months (exclusively breastfed, partially breastfed, and exclusively formula fed infants). Mothers of 163 first-born, full-term, normal birth weight, healthy infants completed socio-demographic, depression, anxiety, and infant sleep-wake behavior measures. No effects were found for sleep arrangements, depression or anxiety, on feeding methods and sleep-wake behavior at three months. At two weeks exclusively breastfed infants at three months spent more hours sleeping and less hours awake during the 24-h period than partially breastfed infants. At three months, exclusively breastfed infants had a shorter of the longest sleep period at night than exclusively formula fed infants. At six months, exclusively breastfed infants at three months spent more hours awake at night than partially breastfed infants, awake more at night than exclusively formula fed infants, and had a shorter sleep period at night than partially breastfed and exclusively formula fed infants. This study showed differences in sleep-wake behaviors at two weeks, three and six months, when exclusively breastfed infants are compared with partially breastfed and exclusively formula fed infants at three months, while no effects were found for sleep arrangements, depression or anxiety.

## 1. Introduction

Public health authorities recommend exclusive breastfeeding for the first six months of infancy (American Academy of Pediatrics, 2012; World Health Organization, 2009). Breastfed infants are commonly reported to sleep less, wake more and show less prolonged sleep at night when compared with formula fed infants. Infant sleep has been associated with other conditions (sleep arrangements, maternal depression, anxiety) related to breastfeeding. This study is unique in studying the effects of exclusive breastfeeding on infant sleep-wake behavior at two weeks, three and six months, comparing infants who were exclusively breastfed, partially breastfed and exclusively formula fed at three months.

Breastfed infants have usually been reported as sleeping less than formula fed infants (e.g., Hughes, Gallagher, & Hannigan, 2015; Nevarez, Rifas-Shiman, Kleinman, Gillman, & Taveras, 2010), but more hours of sleep per day (Ramamurthy et al., 2012). Breastfed infants were found to sleep less during the night (e.g., Schwichtenberg & Poehlmann, 2009; Quillin, 1997), although a trend for longer nocturnal sleep duration was noted in at least one study (Engler, Hadash, Shehadeh, & Pillar, 2012). Several studies have associated breastfeeding with more night wakings (Galbally, Lewis, McEgan, Scalzo, & Islam, 2013; Hysing et al., 2014) and with a shorter continuous nighttime sleep period (Ramamurthy et al., 2012). Moreover, infants were more likely to be sleeping through the

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night at nine months when they were weaned before 12 weeks in one study (Morgan, Lucas, & Fewtrell, 2004), while another study showed that early weaning does not facilitate continuous night sleep (Yilmaz, Gürakan, Cakir, & Tezcan, 2001).

Interestingly, in a recent study exclusively formula fed infants presented the greatest sleep percentage per day and fewer night wakings, followed by exclusively breastfed infants and partially breastfed infants, suggesting that partial breastfeeding may be associated with less sleep in infants (Huang et al., 2016). The inclusion of partially breastfed infants in this study suggested that infant sleep-wake behavior may not benefit from partial breastfeeding, as these infants sleep fewer hours and have more night wakings when compared with exclusively breastfed infants (Huang et al., 2016).

Additionally, short outcomes and long-term effects of breastfeeding appear to differ. The differences in sleep percentage and night wakings frequency between exclusively formula fed and exclusively breastfed infants seem to weaken over time (Huang et al., 2016). For example, despite more night wakings at six months, no significant differences were found at 24 and 36 months (Weinraub et al., 2012). Moreover, breastfeeding was associated with night wakings in infants at six months, but was not related to night wakings at 18 months (Mindell, Du Mond, Tanenbaum, & Gunn, 2012; Hysing et al., 2014).

Breastfed infants are more easily aroused from active sleep at two-three months of age than formula fed infants and this easy arousal from sleep is believed to be an important survival mechanism to prevent Sudden Infant Death Syndrome (SIDS; Horne, Parslow, Ferens, Watts, & Adamson, 2004). Although breastfeeding also had a protective effect on wheezing, coughing, snoring and breathing problems in another study (Galbally et al., 2013), breastfeeding was reported to increase the odds of sleep problems in at least two other studies (Hughes et al., 2015; Schmid, Schreier, Meyer, & Wolke, 2011).

Infant sleep-wake behaviors also differ across cultures (Field, 2017; Mindell, Sadeh, Wiegand, How, & Goh, 2010; Nevarez et al., 2010) and appear to be shaped by multiple variables. A recent literature identified the different risk and protective factors associated with infant sleep (Field, 2017). Most of these factors have not shown independent effects on infant sleep (e.g., Yilmaz, 2001). Infant sleep-wake behaviors have been notably associated with sleep arrangements, maternal depression and maternal anxiety. Bed-sharing infants were reported to sleep less and awaken more during the night than infants who sleep alone (Huang et al., 2016; Hughes et al., 2015). Infants of depressed and anxious mothers show more night wakings and more sleep problems (e.g., Petzoldt, Wittchen, Einsle, & Martini, 2016; Sharkey, Iko, Machan, Thompson-Westra, & Pearlstein, 2016).

Findings from prior research into the effect of feeding methods on infant sleep are inconsistent regarding breastfed infant sleeping less time per day and during the night and more reported sleep problems in breastfed compared to formula fed infants. Previous results are more consensual for a higher number of awakenings during the night and shorter continuous nighttime sleep period in breastfed compared to formula fed infants. Nevertheless, some studies found no significant differences in sleep patterns between breastfed and formula fed infants (e.g., Demirci, Braxter, & Chasens, 2012), and more recent studies provided more consistent results on differences between exclusive breastfeeding and exclusive formula feeding, and partial breastfeeding methods (e.g., Huang et al., 2016).

In this study infant sleep-wake behaviors at two weeks, three and six months according to the feeding method at three months were compared for the exclusively breastfed, partially breastfed, and exclusively formula fed infants.

## 2. Methods

### 2.1. Procedures

The Ethical Commissions of University of Minho and Centro Hospitalar do Porto approved the research protocol. Women ( $N = 583$ ) were contacted at the hospital during the third trimester of pregnancy, informed about the purposes and procedures of the study and invited to participate. Women who did not read or write Portuguese, non-caucasian, multiparas and multiple births were excluded (31%). Women who agreed to participate (89%) signed an informed consent and 43 participants were excluded: nine infants with gestational age less than 37 weeks, three infants with low birth weight ( $< 2500$ ), 24 infants admitted to the intensive care unit during the first two weeks of life, and six exclusively breast-fed infants at three months who were introduced formula during the first days or weeks.

At two weeks ( $M = 3.60$  weeks,  $SD = 2.22$ ), three ( $M = 14.32$  weeks,  $SD = 2.18$ ) and six months ( $M = 27.92$  weeks,  $SD = 3.05$ ) postpartum the mothers completed on-line an Infant Sleep Chronogram and a Socio-demographic Questionnaire and measures of depression and anxiety at three months postpartum. Exploratory analyses were conducted to examine outlier values on the Infant Sleep Chronogram leading to the exclusion of 13 (7.4%) infants. Of the 163 included infants, 148 (90.8%) had completed data on the Infant Sleep Chronogram at two weeks, 162 (99.4%) at three months, and 123 (75.5%) at six months.

### 2.2. Participants

The sample included 163 first-born, full-term ( $\geq 37$  weeks of gestation), of normal birth weight ( $\geq 2500$  g) and healthy infants (no hospitalization or admission to the intensive care unit in the first two weeks post-birth). All mothers were Caucasian and more than half were 26–34 years old (68.1%). The majority of the mothers were married and cohabiting (79.8%), and more than half were employed (71.2%), and had more than 12 years of schooling (64.4%). The majority of the infants were not resuscitated at birth (91.4%). More than half were male (54.6%) and born by vaginal delivery (68.1%). Mean gestational age was 39.23 weeks ( $SD = 1.10$ ), birth weight was 3262.29 g ( $SD = 381.38$ ) and birth length was 48.94 cm ( $SD = 1.76$ ). At three months, 17.6% of the infants was bed sharing daily with the mother. The majority of the infants (77.7%) were exclusively breast-fed at two weeks, but only 59.9% at three months, and 20.3% at six months; 19.6% were partially breast-fed at two weeks, 21.0% at three months, and 50.4% at

six months; and 2.7% were exclusively formula fed at two weeks, 19.1% at three months, and 29.3% at six months.

### 2.3. Measures

#### 2.3.1. Socio-demographic data

The Socio-demographic Questionnaire included the mother's demographic and obstetric information, gender, birth weight and length, gestational age, delivery mode, hospitalization, sleep arrangements, and feeding method at three months according to the Index of Breastfeeding Status (IBS) (Labbok & Coffin, 1997; Labbok & Krasovec, 1990). The feeding methods were defined: as (1) exclusive breastfeeding, that included Labbok & Krasovec's level one and two (full breastfeeding, when only breast milk is used, and almost exclusive breastfeeding, when water is also used); (2) partial breastfeeding, that included Labbok & Krasovec's levels three to seven (partial breastfeeding: high, medium, and low, when breast milk is combined with formula in different percentages, and token, that occurs when minimal breastfeeding is used to comfort the infant); and (3) exclusive formula feeding, that included infants fed exclusively on non-breast milk.

#### 2.3.2. Depressive symptoms

The Edinburgh Postpartum Depression Scale (EPDS; Cox, Holden, & Sagovsky, 1987) self-report scale assesses postpartum depression and is comprised of 10 items scored on a four-point Likert-type scale. Depressive symptoms are assessed within the previous seven days. The Portuguese version of the EPDS has shown good internal consistency (Cronbach's  $\alpha = 0.88$ ) and an optimal cutoff score of seven was proposed to screen for depression in postpartum women (Tendais, Costa, Conde, & Figueiredo, 2014). In the present sample EPDS also showed good internal consistency (Cronbach's  $\alpha = 0.84$ ).

#### 2.3.3. Anxiety symptoms

The State Anxiety Inventory (STAI-S) self-report scale measures current "state anxiety" and is comprised of 20 items scored on a four-point Likert-type scale (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). The Portuguese version of STAI-S showed good internal consistency (Cronbach's  $\alpha = 0.88$ ), and an optimal cutoff score of 34 was used to screen for high anxiety in postpartum women (Tendais et al., 2014). In the present sample the STAI-S also showed good internal consistency (Cronbach's  $\alpha = 0.91$ ).

#### 2.3.4. Sleep-wake behaviors

The Infant Sleep Chronogram (Figueiredo, Dias, Pinto, & Field, 2016) was used for the mothers to record infant sleep-wake behaviors over a 24-h period. It is a timeline comprised of 30-min periods from 8 a.m. one day to 8 a.m. the next day. "Daytime" was defined as 8 a.m. to 8 p.m. and "nighttime" as 8 p.m. to 8 a.m. Mothers recorded infant sleep-wake behavior on a typical day, and infants who presented health problems or other unusual circumstance were excluded. Based on the mothers' records, the following variables were coded for the 24-h period, day, and night: sleep hours, awake hours, wakings, latency to sleep, and longest sleep period. 24-h period sleep, awake and wakings were the sum of the day and night, while the longest sleep period during the 24-h period was the longest sleep period over the 24 h. Latency to sleep was the average number of hours the infant spent falling asleep for each sleep period during the 24-h period, the day and the night. The Infant Sleep Chronogram had acceptable internal consistency at two weeks (Cronbach's  $\alpha = 0.71$ ), at three months (Cronbach's  $\alpha = 0.62$ ), and at six months (Cronbach's  $\alpha = 0.63$ ) (Figueiredo et al., 2016). In the present study the Infant Sleep Chronogram also had acceptable internal consistency at six weeks (Cronbach's  $\alpha = 0.68$ ), at three months (Cronbach's  $\alpha = 0.60$ ), and at six months (Cronbach's  $\alpha = 0.65$ ).

### 2.4. Statistical analyses

Preliminary analyses were conducted to compare the three groups at three months on socio-demographic variables, sleep arrangements, maternal depression and anxiety at three months. Chi-square were used for nominal variables and Multivariate Analyses of Variance (MANOVAs) for continuous variables. The effects of sleep arrangements, maternal depression and anxiety at three months on infant sleep-wake behavior at three months were also analyzed, with two-way Multivariate Analyses of Variance (MANOVA). Sleep arrangements, maternal depression and anxiety at three months were included as independent variables and infant sleep-wake behaviors (day, night, and total) at three months were dependent variables (sleep hours, awake hours, wakings, latency to sleep, and longest sleep period).

In order to analyze infant sleep-wake behavior at two weeks, three and six months according to the feeding method at three months (exclusive breastfeeding, partial breastfeeding, and exclusive formula feeding), Multivariate Analyses of Covariance (MANCOVA) were performed. The first MANCOVA included feeding method at three months as the independent variable and infant sleep-wake behaviors (day and night) at two weeks as dependent variables (sleep hours, awake hours, awakenings, latency to sleep, and longest sleep period). The second MANCOVA included feeding method at three months as the independent variable and infant sleep-wake behaviors (24-h period) at two weeks as dependent variables (sleep hours, awake hours, awakenings, latency to sleep, and longest sleep period). The subsequent MANCOVAs followed the same model using as dependent variables infant sleep-wake behaviors at three months and at six months. Pair-wise comparisons were performed to determine the mean differences between the groups. The models included mother's age and marital status as covariates, given that associations were found between mother's age and marital status and exclusive breastfeeding, partial breastfeeding, and exclusive formula feeding at three months.

Statistical analyses were performed using the software SPSS version 22.0 (SPSS Inc., USA). The effect size measure partial eta squared ( $\eta_p^2$ ) was estimated for all the MANCOVAs. A priori power calculations, performed using the software G\*Power (Faul,

Erdfelder, Lang, & Buchner, 2007), demonstrated that the sample size ( $N = 163$ ) was adequate to detect small-to-medium effects on the MANCOVAs analyses (effect size  $f(V) = 0.25$ ,  $p < 0.05$ ,  $\alpha = 0.05$ , Power = 0.96, for three groups, and 10 response variables).

### 3. Results

#### 3.1. Preliminary results

The three feeding groups did not differ on: occupational status and years of schooling, infant’s resuscitation at birth, type of delivery, gestational age, birth weight, and birth length, gender, sleep arrangement at three months, depression at three months, and anxiety at three months. The three feeding groups significantly differed on mother’s age,  $\chi^2(4) = 10.34$ ,  $p = 0.035$ , and marital status,  $\chi^2(2) = 8.72$ ,  $p = 0.013$ . Older and single/divorced/widow mothers had higher rates of exclusive breastfeeding than younger and married/cohabiting mothers, so those variables were controlled in the analyses. Considering that the three feeding groups at three months differed on mother’s age and matrimonial status, these two variables entered as covariates.

Multivariate effects of sleep arrangements at three months on day and night, *Wilk’s Lambda* = 0.94,  $F(10,145) = 0.98$ ,  $p = 0.461$ ,  $\eta^2 = 0.06$ , and on 24-h period, *Wilk’s Lambda* = 0.95,  $F(5150) = 1.52$ ,  $p = 0.188$ ,  $\eta^2 = 0.05$ , infant sleep-wake behaviors were non-significant. Multivariate effects of mother’s depression at three months on day and night, *Wilk’s Lambda* = 0.94,  $F(10,145) = 0.99$ ,  $p = 0.459$ ,  $\eta^2 = 0.06$ , and on 24-h period, *Wilk’s Lambda* = 0.97,  $F(5150) = 1.01$ ,  $p = 0.414$ ,  $\eta^2 = 0.03$ , infant sleep-wake behaviors were non-significant. Multivariate effects of mother’s anxiety at three months on day and night, *Wilk’s Lambda* = 0.93,  $F(10,145) = 1.15$ ,  $p = 0.331$ ,  $\eta^2 = 0.07$ , and on 24-h period, *Wilk’s Lambda* = 0.98,  $F(5150) = 0.59$ ,  $p = 0.708$ ,  $\eta^2 = 0.02$ , infant sleep-wake behaviors were non-significant.

#### 3.2. Infant sleep-wake behavior at two weeks, three and six months according to the feeding method at three months

Non-significant multivariate effects of feeding method at three months were found on day and night infant-sleep wake behaviors at two weeks, *Wilk’s Lambda* = 0.82,  $F(20,268) = 1.36$ ,  $p = 0.142$ ,  $\eta^2 = 0.09$ . However, marginally significant multivariate effects of feeding method at three months were found on 24-h period infant-sleep wake behaviors at two weeks, *Wilk’s Lambda* = 0.89,  $F(10,278) = 1.75$ ,  $p = 0.070$ ,  $\eta^2 = 0.06$ . Results revealed significant univariate effects of feeding method on 24-h period sleep,  $F(2147) = 4.44$ ,  $p = 0.013$ ,  $\eta^2 = 0.06$ , and awake hours,  $F(2147) = 3.73$ ,  $p = 0.026$ ,  $\eta^2 = 0.05$ . Exclusively breastfed infants slept more hours ( $p = 0.032$ ) and spent fewer hours awake ( $p = 0.031$ ) during the 24-h period at two weeks than partially breastfed infants at three months. Non-significant univariate effects of feeding method were found on 24-h period awakenings, latency to sleep, and longest sleep period (Table 1).

Marginally significant multivariate effects of feeding method at three months were found on day and night infant-sleep wake behaviors at three months, *Wilk’s Lambda* = 0.83,  $F(20,296) = 1.45$ ,  $p = 0.098$ ,  $\eta^2 = 0.09$ . Results revealed significant univariate effects of feeding method on the longest sleep period during the night,  $F(2157) = 3.14$ ,  $p = 0.046$ ,  $\eta^2 = 0.04$ , and marginally

**Table 1**  
Infant sleep-wake behavior at two weeks according to the feeding method at three months.

Variables	Total sample ( $N = 148$ )	EB ( $n = 90$ )	PB ( $n = 30$ )	EF ( $n = 28$ )	$F$	
	$M (SD)$	$M (SD)$	$M (SD)$	$M (SD)$		
Two weeks						
Day and night						
Sleep day	6.09 (1.57)	6.36 (1.42)	5.42 (1.82)	5.95 (1.54)	4.46 <sup>†</sup>	EB > PB <sup>*</sup>
Sleep night	6.88 (1.38)	7.08 (1.33)	6.77 (1.55)	6.34 (1.21)	3.17 <sup>†</sup>	EB > EF <sup>†</sup>
Awake day	4.68 (1.52)	4.49 (1.42)	5.30 (1.86)	4.63 (1.29)	4.26 <sup>†</sup>	EB < PB <sup>*</sup>
Awake night	3.99 (1.15)	3.87 (1.13)	4.00 (1.11)	4.38 (1.18)	2.32 <sup>†</sup>	
Awakenings day	3.14 (0.85)	3.19 (0.85)	3.13 (1.01)	3.07 (0.78)	0.18	
Awakenings night	2.93 (0.92)	3.02 (0.83)	2.63 (0.67)	2.96 (0.88)	2.75 <sup>†</sup>	
Latency to sleep day	0.42 (0.40)	0.39 (0.39)	0.44 (0.40)	0.48 (0.43)	0.40	
Latency to sleep night	0.41 (0.46)	0.33 (0.31)	0.56 (0.75)	0.48 (0.40)	2.46 <sup>†</sup>	
Longest sleep period day	2.47 (0.79)	2.59 (0.86)	2.27 (0.72)	2.29 (0.55)	2.85 <sup>†</sup>	
Longest sleep period night	3.07 (1.02)	3.04 (1.00)	3.38 (1.12)	2.82 (0.90)	1.86	
24-h period						
Sleep hours	12.96 (2.53)	13.43 (2.34)	12.18 (3.02)	12.29 (2.27)	4.44 <sup>*</sup>	EB > PB <sup>*</sup>
Awake hours	8.67 (2.21)	8.36 (2.08)	9.30 (2.61)	9.00 (2.00)	3.73 <sup>†</sup>	EB < PB <sup>*</sup>
Awakenings	6.05 (1.30)	6.17 (1.24)	5.77 (1.38)	6.00 (1.39)	1.05	
Latency to sleep	0.41 (0.36)	0.36 (0.33)	0.48 (0.43)	0.48 (0.39)	1.25	
Longest sleep period	3.17 (1.03)	3.18 (1.02)	3.43 (1.11)	2.84 (0.89)	2.17	

Note. EB = Exclusive Breastfeeding; PB = Partial Breastfeeding; EF = Exclusive Formula Feeding;  $df = 2143$  in all the univariate tests.

<sup>†</sup>  $p > 0.05 < 0.10$ .

\*  $p < 0.05$ .

**Table 2**  
Infant sleep-wake behavior at three months according to the feeding method at three months.

Variables	Total sample (N = 162)	EB (n = 97)	PB (n = 34)	EF (n = 31)	F	
	M (SD)	M (SD)	M (SD)	M (SD)		
Three months						
Day and night						
Sleep day	4.77 (1.54)	4.98 (1.35)	4.29 (1.79)	4.60 (1.70)	2.60 <sup>†</sup>	
Sleep night	8.11 (1.29)	8.06 (1.30)	8.12 (1.22)	8.27 (1.35)	0.32	
Awake day	6.18 (1.72)	5.97 (1.50)	6.47 (2.02)	6.52 (1.98)	1.65	
Awake night	3.03 (1.16)	3.03 (1.16)	3.00 (1.16)	3.05 (1.20)	0.05	
Awakenings day	3.44 (1.09)	3.59 (1.03)	3.29 (1.14)	3.13 (1.18)	2.75 <sup>†</sup>	
Awakenings night	2.08 (1.17)	2.19 (1.07)	2.18 (1.36)	1.65 (1.17)	2.40 <sup>†</sup>	
Latency to sleep day	0.31 (0.31)	0.30 (0.28)	0.36 (0.27)	0.31 (0.41)	0.51	
Latency to sleep night	0.42 (0.45)	0.42 (0.45)	0.44 (0.41)	0.42 (0.52)	0.03	
Longest sleep period day	2.00 (0.74)	2.06 (0.73)	1.69 (0.66)	2.11 (0.81)	2.65 <sup>†</sup>	
Longest sleep period night	5.60 (2.28)	5.26 (2.15)	5.74 (2.31)	6.50 (2.44)	3.14 <sup>*</sup>	EB < EF <sup>*</sup>
24-h period						
Sleep hours	12.88 (2.06)	13.05 (1.87)	12.41 (2.21)	12.87 (2.44)	1.52	
Awake hours	9.21 (2.21)	9.01 (1.97)	9.47 (2.38)	9.56 (2.69)	1.23	
Awakenings	5.52 (1.70)	5.77 (1.56)	5.47 (2.05)	4.77 (1.50)	3.52 <sup>†</sup>	EB > EF <sup>*</sup>
Latency to sleep	0.36 (0.30)	0.36 (0.31)	0.39 (0.26)	0.33 (0.33)	0.60	
Longest sleep period	5.60 (2.27)	5.27 (2.14)	5.74 (2.31)	6.50 (2.44)	3.12 <sup>†</sup>	EB < EF <sup>*</sup>

Note. EB = Exclusive Breastfeeding; PB = Partial Breastfeeding; EF = Exclusive Formula Feeding; *df* = 2157 in all the univariate tests

<sup>†</sup>  $p > 0.05 < 0.10$ .

\*  $p < 0.05$ .

significant univariate effects on sleep hours,  $F(2157) = 2.59$ ,  $p = 0.078$ ,  $\eta^2 = 0.03$ , awakenings,  $F(2, 157) = 2.77$ ,  $p = 0.067$ ,  $\eta^2 = 0.03$ , and longest sleep period during the day,  $F(2157) = 2.65$ ,  $p = 0.074$ ,  $\eta^2 = 0.03$ , and on awakenings during the night,  $F(2157) = 2.40$ ,  $p = 0.095$ ,  $\eta^2 = 0.05$ . Exclusively breastfed infants had a shorter sleep period than exclusively formula fed infants at three months ( $p = 0.040$ ). Non-significant univariate effects of feeding method were found on sleep hours during the night, awake hours during the day and the night, and latency to sleep during the day and the night. Non-significant multivariate effects of feeding method at three months were found on 24-h period infant-sleep wake behaviors at three months, *Wilk's Lambda* = 0.91,  $F(10,306) = 1.51$ ,  $p = 0.133$ ,  $\eta^2 = 0.05$  Table 2.

Significant multivariate effects of feeding method at three months were found on day and night infant-sleep wake behaviors at six months, *Wilk's Lambda* = 0.75,  $F(20,218) = 1.69$ ,  $p = 0.037$ ,  $\eta^2 = 0.13$ . Results revealed significant univariate effects of feeding method on awake hours,  $F(2118) = 3.93$ ,  $p = 0.022$ ,  $\eta^2 = 0.06$ , awakenings,  $F(2118) = 6.02$ ,  $p = 0.003$ ,  $\eta^2 = 0.09$ , and longest sleep period during the night,  $F(2118) = 5.46$ ,  $p = 0.005$ ,  $\eta^2 = 0.09$ . Exclusively breastfed and exclusively formula fed infants at three months spent more hours awake during the night at six months than partially breastfed infants at three months ( $p = 0.024$  and  $p = 0.079$ ). Moreover, exclusively breastfed infants had more night wakings at six months than exclusively formula fed infants at three months ( $p = 0.006$ ) and had a shorter sleep period during the night at six months than partially breastfed ( $p = 0.034$ ) and exclusively formula fed infants ( $p = 0.033$ ) at three months. Results revealed non-significant univariate effects of feeding method on sleep hours during the day and the night, awake hours during the day, awakenings during the day, latency to sleep during the day and the night, and longest sleep period during the day. Marginally significant multivariate effects of feeding method at three months were found on 24-h period infant-sleep wake behaviors at six months, *Wilk's Lambda* = 0.87,  $F(10,228) = 1.71$ ,  $p = 0.079$ ,  $\eta^2 = 0.02$ . Results revealed significant univariate effects of feeding method on awakenings,  $F(2118) = 6.02$ ,  $p = 0.003$ ,  $\eta^2 = 0.09$ , and longest sleep period,  $F(2118) = 5.46$ ,  $p = 0.005$ ,  $\eta^2 = 0.09$ , during the 24-h period. Exclusively breastfed infants at three months had more night wakings and a shorter sleep period during the 24-h period at six months than partially breastfed infants at three months ( $p = 0.022$  and  $p = 0.034$ ), and exclusively formula fed infants at three months ( $p = 0.025$  and  $p = 0.033$ ). Results revealed non-significant univariate effects of feeding method on sleep hours, awake hours during the day, and latency to sleep during the 24-h period (Table 3).

#### 4. Discussion

Exclusively breastfed infants at three months spent more hours sleeping and fewer hours awake during the 24-h period at two weeks than partially breastfed infants. At three months, exclusively breastfed infants had a shorter sleep period during the night than exclusively formula fed infants. Exclusively breastfed and exclusively formula fed infants at three months spent more hours awake during the night at six months than partially breastfed infants. Moreover, exclusively breastfed infants at three months had more night wakings at six months than exclusively formula fed infants at three months and a shorter sleep period during the night at six months than partially breastfed and exclusively formula fed infants at three months.

These results are consistent with previous findings that showed that breastfed infants awaken more times during the night at six

**Table 3**  
Infant sleep-wake behavior at six months according to the feeding method at three months.

	Total sample (N = 123)	EB (n = 82)	PB (n = 22)	EF (n = 19)		
Six months						
Variables	M (SD)	M (SD)	M (SD)	M (SD)	F	
Day and night						
Sleep day	4.09 (1.28)	4.08 (1.28)	3.80 (1.32)	4.50 (1.18)	1.34	
Sleep night	8.40 (1.31)	8.29 (1.36)	8.93 (1.21)	8.29 (1.07)	1.92	
Awake day	7.00 (1.38)	7.09 (1.39)	7.20 (1.35)	6.39 (1.26)	2.06	
Awake night	2.73 (1.04)	2.86 (1.01)	2.14 (0.90)	2.87 (1.12)	3.93*	EB > PB*
Awakenings day	3.07 (0.84)	3.13 (0.83)	2.86 (0.89)	3.05 (0.85)	1.69	
Awakenings night	2.02 (1.01)	2.22 (1.01)	1.73 (0.94)	1.53 (0.90)	6.02**	EB > EF**
Latency to sleep day	0.31 (0.25)	0.28 (0.26)	0.35 (0.23)	0.37 (0.23)	1.00	
Latency to sleep night	0.44 (0.42)	0.39 (0.35)	0.51 (0.31)	0.57 (0.72)	1.53	
Longest sleep period day	1.90 (0.74)	1.88 (0.74)	1.89 (0.72)	2.00 (0.76)	0.23	
Longest sleep period night	5.88 (2.49)	5.38 (2.45)	6.98 (2.58)	6.76 (1.96)	5.46**	EB < PB,EF*
24-h period						
Sleep hours	12.50 (1.62)	12.37 (1.76)	12.73 (1.48)	12.79 (1.05)	0.73	
Awake hours	9.74 (1.54)	9.95 (1.57)	9.34 (1.29)	9.26 (1.50)	1.77	
Awakenings	5.09 (1.31)	5.34 (1.23)	4.59 (1.62)	4.58 (0.96)	6.01**	EB > PB,EF*
Latency to sleep	0.36 (0.26)	0.33 (0.26)	0.42 (0.22)	0.43 (0.29)	1.23	
Longest sleep period	5.89 (2.48)	5.39 (2.44)	6.98 (2.58)	6.76 (1.86)	5.46**	EB > PB,EF*

Note. EB = Exclusive Breastfeeding; PB = Partial Breastfeeding; EF = Exclusive Formula Feeding;  $df = 2118$  in all the univariate tests.

<sup>†</sup> $p > 0.05 < 0.10$ . \*  $p < 0.05$

\*\* $p < 0.01$ .

months (e.g., Galbally et al., 2013; Hysing et al., 2014; Hysing et al., 2014) and have a shorter sleep period during the nighttime period between 0 and 5 months (Ramamurthy et al., 2012) than formula fed infants. A previous study showed that breastfeeding at three months was associated with longer awake episodes during the night at six months (Tikotzky et al., 2015), but this study showed that partially breastfed infants at three months spent fewer hours awake during the night at six months than exclusively breastfed infants and marginally than exclusively formula fed infants. Moreover, contrary to a previous study that also considered three feeding groups, differences in sleep hours were not reported between exclusive and partial breastfeeding and exclusive formula feeding (Huang et al., 2016).

Breastfed infants showed more fragmented sleep during the night, with more awakenings and a shorter sleep period, consistent with previous studies (e.g., Galbally et al., 2013; Hysing et al., 2014; Hysing et al., 2014). Breast milk is more easily digested which could result in a shorter period of satiety and thus more fragmented sleep for breastfed infants (Burness, 1979). However, processes associated with breast milk, including a higher percentage of melatonin that has a hypnotic and relaxing effect may improve infant sleep and would suggest better sleep for breastfed infants (e.g., Cubero et al., 2005; Engler et al., 2012). The composition of breast milk varies over the course of 24 h and some components produced at night are likely to contribute to the infant's day/night entrainment (e.g., Cubero et al., 2005; McGuire, 2013). Melatonin, that is present in breast milk but in formula, could play a role in improving sleep and reducing colic in breastfed infants compared to formula fed infants (e.g., Cubero et al., 2005; Engler et al., 2012).

Sleep arrangements and maternal depression and anxiety did not affect infant feeding methods and infant sleep wake-behavior at three months. These results are inconsistent with data from previous studies, suggesting associations between sleep arrangements, maternal depression and anxiety and infant feeding methods (e.g., Adedinsewo, Fleming, Steiner, Meaney, & Girard, 2014; Figueiredo, Canário, & Field, 2014), and infant sleep-wake behavior (e.g., Huang et al., 2016; Petzoldt et al., 2016).

#### 4.1. Limitations

The results of this study are based on the mother's observation and report of the infant sleep-wake behavior and thus may be biased by their perceptions. However, good reliability has been noted between maternal report and other sleep measures, such as actigraphy (e.g., Werner, Molinari, Guyer, & Jenni, 2008). When compared with actigraphy, no differences were found in sleep-wake behaviors maternal report during the day and the 24-h period (Galland et al., 2016; So, Michael Adamson, & Horne, 2007). However, some differences were found in the measurement of sleep-wake behaviors during the night (Asaka & Takada, 2011; Galland et al., 2016; So et al., 2007). For example, mother's reports may not record night wakings not signaled by the infant (Asaka & Takada, 2011; So et al., 2007). But actigraphy may record night wakings that are not but rather episodes of self-soothing (Hall, Liva, Moynihan, & Saunders, 2015). Moreover, a comprehensive review pointed lack of consistency in the scoring rules and in the definition of the variables when using the actigraphy (Meltzer, Montgomery-Downs, Insana, & Walsh, 2012), although actigraphy has been widely used in infant sleep research. Our sample had different characteristics than other samples, namely only Caucasian mothers and first-born infants and a higher breastfeeding rate. Although the three groups differed on mother's age and marital status, these variables were controlled in the analyses.

## 5. Conclusion

This study contributed to the exclusive breastfeeding research by assessing infant sleep-wake behaviors at two weeks, three and six months, and infants who were exclusively breastfed, partially breastfed and exclusively formula fed at three months. Moreover, this study analyzed the effects of sleep arrangements and maternal depression and anxiety on feedings methods and infant sleep-wake behavior at three months. This study also covers infant sleep-wake behavior both during the day and the night. Given that exclusive breastfeeding is highly recommended by public authorities (American Academy of Pediatrics, 2012; World Health Organization, 2009), mothers should be informed about the differences in sleep-wake patterns in the first months. Moreover, they should receive support to help them deal with their infant sleep-wake patterns and to continue breastfeeding.

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