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Article

Maternal Humming during Kangaroo Care: Effects on Preterm Dyads' Physiological Parameters

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Abstract: Humming is probably more effective than speech improving mothers' cardiorespiratory function and infants' self-regulation. We intend to understand the effects of: 1) maternal humming vs. speech on preterm infants' physiological parameters; 2) maternal humming vs. speech on mothers' physiological parameters; 3) humming melodic contours and of the lengthening of the final note on preterm infants' physiological parameters. This study was designed as a single group repeated measure, using microanalytical methodology (ELAN software), with a protocol (silent baseline / speech or humming / silence / humming or speech / silence) applied to preterm dyads (N = 36). Audio and video observations were recorded. Infants' and mothers' heart rate (HR) and O2 saturation were observed once a minute. Proportion of O2 saturation relative to HR (Prop. O2 saturation/HR) was estimated for both partners during protocol. We found an infants' HR mean significantly lower during humming ($p = .028$), a significantly higher Prop. O2 saturation/HR during humming for infants ($p = .027$) and for mothers ($p = .029$). The duration of sinusoidal contours together with the lengthening of the final note predict infants' Prop. O2 saturation/HR. Musical features of humming seem to improve the physiological stability of preterm infants during kangaroo care.

Keywords: preterm dyads; physiological parameters; kangaroo care; maternal humming; melodic contours

1. Background

During the transition between prenatal and neonatal life, the autonomic nervous system (ANS) plays a crucial role for cardiovascular and respiratory functioning [1]. A primary goal of neonatal care for preterm infants is to ensure the stability of ANS [1]. This is achieved through neuroprotective individualized neonatal care centered on infants' neurodevelopmental needs. NIDCAP (Newborn Individualized Developmental Care and Assessment Program) focused on a detailed reading of each infant's behavioral cues helping its' self-regulation. According NIDCAP, environment and care are adapted to enhance each infant's strengths and self-regulation collaboratively with parents that play the primary role for infants' development. [2].

In NIDCAP approach, Kangaroo mother care is one of the multimodal sensory experiences provided in NICU through the early skin-to-skin contact between mothers and preterm infants [2]. Kangaroo mother care has shown benefits for preterm infants' self-regulation as well as for maintaining the intimate connection between mother and infant [3]. This is achieved through the ANS balance having impacts on infants' physiological stability [2].

Comparatively to the original vertical positioning, "kangaroo supported flexion diagonal positioning" protocol [4] replaces the previous vertical position of the preterm infant by a diagonal positioning improving mother-infant gaze contact. Furthermore, it ensures preterm infants' physiological stability improving the contingent interaction between mother and infant [5] and so it has been underlined as a promissory method to the understanding of the innate basis of

intersubjectivity in the context of very preterm birth [5]. Also, using this protocol, a significant decrease in maternal stress and a lower risk of postpartum depression were found [6].

When combined with kangaroo care, maternal singing shows a positive and significant impact in autonomic stability of preterm infants [7]. Parental “Early Vocal Contact” [8] using parental singing and parental speech has also been highlighted as a promising approach in NICU to promote preterm infants’ autonomic balance [8,9]. Live maternal singing enhances preterm infants’ vagal activity in the short term, thus improving neonatal autonomic stability [10].

Benefits of live music therapy using humming in a contingent way – synchronizing musical features with infants’ signals - were observed on early preterm infants’ self-regulation in NICU [11–17].

Music therapy and sound healing based in vibroacoustic effects of sounds are increasingly utilizing vocal toning including humming in clinical practice to promote a deep relaxation and a change in the state of consciousness [18]. Vocal toning is a type of vocalizing that utilizes the natural voice to express sounds including humming on the full exhalation of the breath and open vowel sounds [18] as well as “OM” *chanting* [19]. A study with adults observed that vocal toning can improve attention, awareness, and consciousness [18]. Also “meditative”, “calm”, and “relaxed” states, which are associated with the parasympathetic nervous system and with a decrease of HR, were the best descriptors of vocal toning. On the contrary, singing evoked emotions that were stronger than the emotions evoked by vocal toning; among those the three most common descriptors included “nostalgia”, “tenderness”, and “joyful activation” [18].

After preterm birth, maternal emotional state can make it difficult to communicate verbally with the infant and therefore, singing (with words) can evoke very intense emotions while humming can facilitate the connection with the preterm infant in a safer way.

According NIDCAP approach [2], preterm infants’ vocal responsiveness can be considered as an indicator of behavioral self-regulation if a balance is present in the relationship among the autonomic, the motor, and the states of consciousness subsystems. Otherwise, it may be interpreted as a behavior that may require too much effort and energy expenditure for preterm infants.

Vocal toning, including humming improves cardiovascular function, respiratory flow, and diaphragmatic breathing [19–21]. These improvements have been identified as good psychophysiological indicators of decreased anxiety and increased relaxation [22]. The effectiveness of humming on preterm infants’ physiological parameters is associated with a decrease in HR to adequate values and an increase in O₂ saturation [11–13].

According to literature [23], more benefits using live music therapy (singing or humming) when compared with recorded music were found relative to the physiological stabilization of preterm infants (at 30 minutes after music therapy, a lower level of heart rate and a deeper sleep). Also, live lullabies improve the sleep state of preterm infants more than recorded lullabies [24]. However, both conditions were associated with a decrease in the infants’ heart rate (HR) and no changes were observed in oxygen saturation level [24]. This way, there were no significant differences in preterm infants’ physiological parameters in response to recorded lullabies compared to mothers’ live lullabies [25]. Lullabies together with multimodal stimulation induce benefits in weight gain/day and diminishes the number of days of preterm infants’ hospitalization [26].

Potentially adverse effects of recorded maternal voice through infant directed speech (ID speech) and infant directed singing (ID singing) were found, expressed in decreased O₂ saturation [27]. However, another study did not observe significant differences in HR or O₂ saturation when comparing recorded maternal voice against the control condition [28]. A decrease of HR was found when the recorded maternal voice (speaking or reading) was delivered [29,30], while an increase of HR was found when live maternal voice (speaking or singing) was directed to preterm infants [31].

Inconsistent results were observed about the effects of maternal voice for physiological outcomes such as HR, heart rate variability (HRV), O₂ saturation [7,11,26,27,30,32,33]. These inconsistencies may be due to methodological issues, namely the different conditions in which maternal voice was delivered (recorded, live, speaking, reading, singing, humming, etc.). Most studies used recorded maternal voice during a speech condition (speaking or reading) and few

studies used live maternal voice either spoken or sung [8]. Few studies included singing comparatively with speech and very few studies used humming when compared with singing. Nevertheless, there is a gap about studies of music therapy in NICU regarding the musical characteristics of singing or of humming.

Maternal ID singing is a specific way for mothers to connect infants with a positive affect [34,35]. Despite dyads' individual variations, maternal ID singing presents universal characteristics that discriminate it from singing directed to adults. Most studies on ID singing were conducted with term dyads, and especially with infants older than three months [36]. Few studies regarding ID singing to preterm infants were reported in literature.

Maternal ID singing is characterized by a slower tempo with a regular beat, a higher and consistent pitch level, and a more expressive vocal tone, when compared with adult-directed singing [36–38]. Many of these acoustic features are useful to capture the infants' attention [34,35] and to improve their self-regulation [39]. Remarkably, parents or mothers naturally change the way they sing based on infants' needs and their behavioral states [40].

Lengthening of the final note in a musical phrase is a key feature of parental ID singing. This is a crucial temporal marker that helps infants perceiving units such as vocal phrases and phrase groupings [41–43]. It is likely that the lengthening of the final note in humming phrases is longer than in singing phrases, giving infants cues that help them anticipate the end of humming phrases. This way, in a previous study carried on with the present sample, preterm infants' overlapping vocalizations occurred predominantly during the lengthening of the final note in humming phrases [44]. This suggests that preterm infants can predict the end of humming phrases. Maternal breathing that follows the lengthening of the final note may be considered as a physiological marker of the offset pauses between humming vocalizations. We think that this marker may induce the infants' vocal response at the end of the final note.

About the melodic contours of ID singing, Falk [45] observed six types of melodic contours: linear, rising, falling, bell-shaped, U-shaped, and sinusoidal contours. A microanalytical study with the sample of the present one found a predominance of sinusoidal contours (29.92%) followed by bell-shaped (23.11%), rising (20.72%), falling (15.55%), U-shaped (6.70%), and linear contours (3.97%) in maternal humming directed to preterm infants [46]. In addition, a predominance of infants' overlapping vocalizations during sinusoidal and bell-shaped contours was observed [46]. These melodic contours are predominantly observed in world lullabies characterized by smoothly oscillating sinusoidal contours as well as by descending, bell-shaped, and flat contours with a lengthening of the final note where the pitch range tends to be smaller [47]. Infant-directed lullabies aim to calm, decreasing the arousal level, ensuring an intimate and warm environment, and inducing a sleep state [40].

It would be important to understand if maternal humming during kangaroo care is able to improve physiological synchrony in preterm dyads. In short, does the regularity of humming in a lullaby way helps to stabilize mothers' cardiorespiratory function and to maintain them in a relaxation state during kangaroo care? More than that, does maternal relaxation through humming improve the infants' self-regulation?

1.1. Purpose of the present study

As a vibroacoustic experience that improves vagal activity [19], maternal humming during skin-to-skin contact may be even more effective than speech enhancing parasympathetic responses associated with a maternal relaxation state. Compared with speech, humming as associated with improvements in maternal respiratory flow, and in cardiovascular function, with a lower vocal intensity and a smaller tonal variation, may improve preterm infants' self-regulation.

Our study focused on humming effects upon physiological parameters of preterm dyads during kangaroo care. Among several aspects, we think that humming musical features such as melodic contours and the lengthening of the final note contribute to its effectiveness. There is a gap in research about musical characteristics of humming produced during music therapy sessions. Therefore, it is essential to know how the musical features of humming can help to improve preterm infants'

physiological stability. This will be useful for preterm mothers' guidance while improving humming addressed to their infants during kangaroo care.

Infants' physiological stability is ensured when an adequate level of oxygen (O₂) saturation is achieved with less effort in heart rate (HR) and breathing [1]. Thus, a new physiological measure such as proportion of O₂ saturation relative to HR (Prop. O₂ saturation/HR) should be considered to evaluate the effects of stimulation on preterm infants' cardiorespiratory stability.

The aims of the present study are to evaluate during kangaroo care: 1) the effect of maternal humming vs. speech on preterm infants' physiological parameters (infants' HR and infants' Prop. O₂ saturation/HR); 2) the effect of maternal humming vs. speech on mothers' physiological parameters (HR and O₂ Saturation), and 3) the impact of humming melodic contours (linear, rising, falling, bell-shaped, U-shaped, and sinusoidal) and of the lengthening of the final note on preterm infants' physiological parameters.

2. Method

2.1. Design

The present study is a single group repeated measures design, included in a broader study about preterm infants' vocal responsiveness in humming vs. speech conditions during kangaroo care (Blinded for review, [44,46,48], approved by the Ethical Committee of the Hospital [48]. Due to cultural and occupational issues, most of the fathers were not present at NICU during the observations. So, only mothers were recruited for this study. All mothers gave written informed consent and were interviewed with a sociodemographic and clinical questionnaire.

According to the study protocol, each preterm dyad was observed only once in the NICU during kangaroo care in a sequence composed by silent, and vocal conditions (humming and speech). The skin-to-skin contact followed the "kangaroo supported diagonal flexion positioning" [4], which aims to improve visual interaction between mother and infant. For this, a large scarf was provided with an additional strap for the support of the infant's neck. Mothers were invited to address their infants speaking (as usually) and humming (an improvised melody in a spontaneous way, with the mouth closed) while in kangaroo care. Also, no guidance was given to mothers regarding the use of musical repertoire and there was no support or orientation from a music therapist. The protocol alternated between each vocal condition and silent condition during 5 periods of 3 minutes each: 1) silent baseline, 2) maternal voice (speech or humming), 3) silence, 4) maternal voice (humming or speech), and 5) final silence. In baseline condition mothers were silent, and other modalities of maternal contact were not discouraged (gaze, touch, rocking, etc.). The order effect was controlled; after the silent baseline, mothers assigned with odd numbers started with the humming condition and mothers assigned with even numbers started with the speaking condition.

All observations occurred at the same time of the day 10 minutes after the infant has been placed in kangaroo care and before being fed. If the infant presented a state of restlessness or cry it was waited for the infant to become in a state of quiet alertness or drowsiness [[49], pp. 49–51) in order to be available for interaction. All observations took place in the neonatal intermediate care unit; a room where 6 incubators were isolated from external noises by a closed sliding door. Only the mother with the infant, the researcher who recorded the observations on video, and the nursing team were present.

All dyads' observations were audio and video recorded. For this, two cameras (Panasonic 4K HC-VX870) were used. The first camera was directed to the faces of the mother and infant in order to capture both partners' faces simultaneously in real time. To measure the vocalizations of the dyad, an external microphone was connected to the first camera. Video (MP4) and audio (WAV) recordings were made for the mother's and infant's vocalizations during speech and humming. For the present study, to analyze the dyads' physiological parameters, a second camera was directed to the NICU's monitors to observe the values of each infant's physiological parameters (O₂ saturation level and HR), and at the same time to the mother's oximeter (O₂ saturation level and HR) during the several

conditions. Before the beginning of the video recording, the same oximeter was attached to the index finger of each mother.

2.2. Measures

A sociodemographic and clinical questionnaire was used to collect basic information about participating dyads and their obstetric and pediatric background. ELAN software (EUDICO Linguistic Annotator version 4.9.4) was used to code the data (maternal humming, maternal speech, and physiological parameters of both partners) for microanalytical analyses.

2.2.1. Recording, minute-by-minute, mothers', and infants' HR and O2 saturation

To observe the effect of humming compared to maternal speech on the dyads' physiological parameters, values of HR and O2 saturation (displayed on the NICU's monitors and on the mother's oximeter) were recorded minute by minute throughout the observation (at the first, the second and the third minutes during humming and speech periods), using ELAN software. We also estimated the Prop. O2 saturation/HR of the mother as well as of the infant in each condition (silent baseline, humming and speech) to assess the cardiac effort needed to reach an optimal level of O2 saturation. Means of the three values were estimated for HR, for O2 saturation and for Prop. O2 saturation/HR in each condition.

2.2.2. Maternal vocalizations during the protocol

In a broader study [48], all maternal vocalizations during humming and during speech were codified (frequency of vocalizations per minute and duration of each vocalization in milliseconds). According to the criteria of Gratier and team [50], each maternal vocalization was identified as the production, by the mother, of an audible vocal sound without interruptions exceeding 300 ms. The first author was trained at the Baby Lab of the Université Nanterre La Defense for the management of ELAN Software. In order to achieve reliability a second researcher, trained by the first author, participated in data coding.

2.2.3. Humming melodic contours and the lengthening of the final note

With the same sample, a previous study coded the melodic contours as well as the lengthening of the final note of maternal vocalizations (N = 1761) during humming [46]. The coding was based on the classification of the six tonal contours developed by Falk [45]: 1) linear contour, when the humming vocalization is flat (the vocalization presents only one musical note); 2) rising contour, if there is a tonal increase; 3) falling contour, characterized by a tonal decrease; 4) bell-shaped contour, an increase followed by a decrease; 5) U-shaped contour, a decrease followed by an increase; and 6) sinusoidal contour, a bell-shaped followed by a rising contour or a U-shaped contour followed by a falling contour. These melodic contours are displayed in Figure 1.

ELAN software was used to code the melodic contours (frequency and duration of each humming vocalization) as well as to code the lengthening of the final note of melodic contours (duration of the final note in each humming vocalization), in the audio and video recordings. For this, there were two researchers (the first author and a student of musicology), both with high academic musical training (8th degree of musical training). They listened (simultaneously visualizing the audio-spectrogram displayed by ELAN software) the humming of each mother to identify the type according to the Falk protocol, [45] and the duration of the melodic contour, for each maternal vocalization. Also, the duration of the lengthening of the final note in each humming vocalization was coded in ELAN software by the same researchers.

2.3. Sample selection process

This study was carried out with a sample of preterm dyads in the NICU of the Hospital [48]. While recruiting dyads, the following exclusion criteria were applied to mothers: a) maternal age under 19 years old, b) difficulty understanding and speaking Portuguese language, c) presence of a

maternal auditory deficit, d) absence of medical supervision during gestation, e) history of psychiatric pathology or existence of serious negative emotional states, and f) addictions. Also, dyads were excluded if infants: a) had a post-menstrual age of less than 32 weeks or more than 37 weeks, b) presented unstable vital parameters, c) needed the support of CIPAP (Continuous Positive Airway Pressure), d) had intraventricular hemorrhages, e) had congenital or neurological anomalies of the auditory cortex, f) had a nasogastric tube, and g) when their breathing needed to be supported. Dyads were also excluded if kangaroo care had not been practiced at least once. The selection process of the sample is displayed in Figure 2.

2.4. Participants

Table 1 displays the sociodemographic and clinical data of dyads regarding maternal age, education, and nationality, as well as infants’ gestational age at birth, chronological age at observation, weight at birth, and weight at observation. Most of the mothers are Portuguese nationals, and African and Brazilian mothers are living in Portugal for at least 7 years. None of the mothers had formal musical training. Most infants are males.

Table 1. Descriptive statistic of sociodemographic and clinical data of preterm dyads (N = 36).

Variables	n	%	M	SD	min. – max.
maternal age			34.20	5.63	21 - 48
maternal education			15.33	3.69	6 - 24
Portuguese nationality	26	72.22			
African and Brazilian nationality	10	27.78			
male infants	20	55.56			
female infants	16	44.44			
infants’ gestational age at birth*			212.78	17.11	178 - 241
infants’ chronological age at observation*			26.5	19.99	4 - 81
infants’ weight at birth (g)			1265.47	308.20	590 - 2017
infants’ weight at observation (g)			1538.05	237.72	1060 - 2185

*number of days.

2.5. Reliability

For the broader study, two independent researchers coded 30% of all mothers’ vocalizations [48]. Intraclass correlation coefficients (ICC) were used to assess differences between the researchers. A high agreement was found for the frequency of maternal humming vocalizations (ICC = .999, p = .000) as well as for the frequency of maternal speech vocalizations (ICC = .991, p = .000).

Regarding the melodic contours of humming, also two researchers with a musical background (the first author and a student of musical sciences with musical training and experience in music analysis) carried out a reliability analysis on the melodic contours of humming [46]. They studied seven dyads (19.44% of the participating dyads), and reached a high degree of agreement for all melodic contours: 1) linear (ICC = .944, p = .002), 2) rising (ICC = .999, p < .001), 3) falling (ICC = .996, p < .001), 4) bell-shaped (ICC = .995, p < .001), 5) U-shaped (ICC = .933, p = .002), and 6) sinusoidal (ICC = .992, p < .001).

2.6. Analytical plan

A descriptive statistical analysis was performed using SPSS 27 software for preterm dyads’ physiological variables (HR, O2 saturation and Prop. O2 saturation/HR) during the silent baseline, humming and speech conditions. Also, comparative statistical analyses (Student’s-t) for paired samples were performed to identify significant differences between the physiological parameters (HR, O2 saturation and the Prop. O2 saturation/HR) of each partner during silent baseline, humming and speech conditions.

Regarding the effects of the duration of melodic contours (linear, rising, falling, bell-shaped, U-shaped, and sinusoidal) and of the lengthening of the final note on infants’ physiological parameters during humming, several multiple hierarchical linear regressions were performed.

3. Results

Table 2 presents the descriptive statistics of mothers’ HR, O2 saturation and Prop. O2 saturation/HR relative to periods of 3 minutes in each condition. According to results, a higher level in mothers’ HR is observable for both humming and speech conditions; the highest value is observed during speech. Mothers’ O2 saturation changes slightly across protocol conditions. Table 3 presents the descriptive statistics of infants’ HR, O2 saturation and Prop. O2 saturation/HR for the several 3 minutes periods in each condition. According to results, HR mean is lower during the humming condition, reaching its highest point during speech condition. Infants’ O2 saturation seems to remain stable.

Table 2. Descriptive statistics of mothers’ physiological variables (HR*, O2 saturation**, Prop. O2 saturation/HR***) during silent baseline, humming and speech conditions.

Conditions	Physiological parameters	M	SD	min. – max.
Silent baseline	HR	79.02	10.17	47.67 – 97.67
	O2 saturation	97.90	1.14	94.00 – 99.00
	Prop. O2 saturation/HR	1.26	.19	.99 – 2.06
Humming	HR	81.55	10.75	49.00 – 101.00
	O2 saturation	97.92	1.10	94.33 – 99.00
	Prop. O2 saturation/HR	1.22	.19	.95 – 2.00
Speech	HR	83.47	10.39	50.67 – 101.00
	O2 saturation	97.75	1.14	94.33 – 99.00
	Prop. O2 saturation/HR	1.19	.18	.95 – 1.93

* heart rate, ** oxygen saturation, *** proportion of oxygen saturation relative to heart rate.

Table 3. Descriptive statistics of infants’ physiological variables (HR*, O2 saturation**, Prop. O2 saturation/HR ***) during silent baseline, humming and speech conditions.

Conditions	Physiological parameters	M	SD	min. – max.
Silent baseline	HR	156.24	11.37	137.00 – 181.00
	O2 saturation	97.68	3.33	86.36 – 100.00
	Prop. O2 saturation/HR	.63	.05	.52 – .73
Humming	HR	153.31	13.54	119.00 – 185.67
	O2 saturation	97.19	3.15	88.00 – 100.00
	Prop. O2 saturation/HR	.64	.06	.50 – .74
Speech	HR	157.32	12.08	130.00 – 188.00
	O2 saturation	97.11	3.02	88.00 – 100.00

Prop. O2 saturation/HR .62 .06 .51 – .77

* heart rate, ** oxygen saturation, *** proportion of oxygen saturation relative to heart rate.

3.1. Comparison of Outcomes Across Conditions

Table 4 displays the significant differences in the mothers’ physiological variables. According to results, we found a significantly higher mean of HR during humming ($t = -4.889$, $df = 33$, $p < .001$, Cohen’s $d = -.839$) as well as during speech ($t = -5.397$, $df = 33$, $p < .001$, Cohen’s $d = -.926$) when compared to silent baseline. Both significant differences present a very large effect size according to Cohen’s d statistics. However, only a slight difference was found between maternal HR in humming and in speech ($t = -2.037$, $df = 32$, $p = .050$, Cohen’s $d = -.355$) with a higher mean HR in speech than in humming. This difference presents a medium effect size for Cohen’s d statistics. For Prop. O2 saturation/HR, significant differences having Cohen’s d statistics ranging from medium to very large effect sizes were found between: 1) silent baseline and humming ($t = 5.060$, $df = 33$, $p < .001$, Cohen’s $d = .868$), with a higher Prop. O2 saturation/HR in silent baseline than in humming; 2) silent baseline and speech ($t = 5.511$, $df = 33$, $p < .001$, Cohen’s $d = .945$), with a higher Prop. O2 saturation/HR in silent baseline than in speech, and 3) humming and speech ($t = 2.289$, $df = 32$, $p = .029$, Cohen’s $d = .398$) with a higher Prop. O2 saturation/HR in humming than in speech. These significant differences must be taken into account once that their effects present values from medium to very large.

Table 4. Comparative statistics between mothers’ physiological variables (HR*, O2 saturation**, Prop. O2 saturation/HR ***) during silent baseline (BL), humming (Hum), and speech (Sp) conditions.

Physiological parameters	<i>t</i>	<i>df</i>	<i>p</i>	Cohen's <i>d</i>
HR				
BL vs. Hum	-4.89	33	< .001	-.839 (e)
BL vs. Sp	-5.39	33	< .001	-.926 (e)
Hum vs. Sp	-2.04	32	.050	-.355 (c)
O2 saturation				
BL vs. Hum	-.11	33	.917	-.018 (b)
BL vs. Sp	1.59	33	.121	.273 (b)
Hum vs. Sp	1.75	32	.089	-.355 (c)
Prop. O2 saturation/HR				
BL vs. Hum	5.06	33	< .001	.868 (e)
BL vs. Sp	5.51	33	< .001	.945 (e)
Hum vs. Sp	2.29	32	.029	.398 (c)

* heart rate; ** oxygen saturation; *** proportion of oxygen saturation as a function of heart rate.(a) very small (0.0 - < 0.1); (b) small (0.1 - < 0.30); (c) medium (0.30 - < 0.50); (d) large (0.50 - < 0.80); (e) very large (0.80 - < 1.20); (f) huge (1.20 – 2.0).

Optimal values in mothers’ O2 saturation ($M = 97$, $min. = 94$, $max. = 99$) were found for all conditions and no significant differences were observed.

Table 5 displays the significant differences in the infants’ physiological variables. According to results, the mean HR was lower during humming ($M = 153.315$) when compared to speech ($M = 157.324$) and this difference is statistically significant presenting a medium effect size according to

Cohen’s d statistics ($t = -2.293$, $df = 35$, $p = .028$, Cohen’s $s = -.382$). No significant differences were found for infants’ means of O2 saturation between speech and humming conditions. Also, O2 saturation presents optimal values ($M = 97$; $min. = 88$; $max. = 100$) in both conditions. However, the Prop. O2 saturation/HR is higher during humming than during speech and this difference is significant having a medium effect size in Cohen’s d statistics ($t = 2.309$, $df = 34$, $p = .027$, Cohen’s $s = .390$). Also this difference is to be considered since its effect size is medium in Cohen’s d terms.

Table 5. Comparative statistical analyses between infants’ physiological variables (HR*, O2 saturation**, Prop. O2 saturation/HR ***) during silent baseline (BL), humming (Hum), and speech (Sp) conditions.

Physiological Parameters	<i>t</i>	<i>df</i>	<i>p</i>	Cohen’s <i>d</i>
HR				
BL vs. Hum	1.62	35	.114	.270 (b)
BL vs. Sp	-.95	35	.349	-.158 (b)
Hum vs. Sp	-2.29	35	.028	-.382 (c)
O2 saturation				
BL vs. Hum	1.06	35	.297	.177 (b)
BL vs. Sp	1.32	34	.195	.224(b)
Hum vs. Sp	.00	34	1.000	.000 (a)
Prop. O2 saturation/HR				
BL vs. Hum	-1.26	35	.218	-.209 (b)
BL vs. Sp	1.37	34	.179	.232 (b)
Hum vs. Sp	2.31	34	.027	.390 (c)

* heart rate, ** oxygen saturation, *** proportion of oxygen saturation relative to heart rate. (a) very small (0.0 - < 0.1); (b) small (0.1 - < 0.30); (c) medium (0.30 - < 0.50); (d) large (0.50 - < 0.80); (e) very large (0.80 - < 1.20); (f) huge (1.20 – 2.0).

3.2. Effects of melodic contours and lengthening of the final note on preterm infants’ physiological parameters

Table 6 displays the single significant result obtained for preterm infants’ HR during humming as dependent variable and mean durations of sinusoidal contours and mean lengthening of the final notes as independents variables. Using non sinusoidal contours (linear, falling, rising, bell-shaped, and U-shaped) together with the lengthening of the final note as independent variables did not yield any significant results. As sociodemographic and clinical variables of preterm infants may influence their HR, in Model 1 infants’ gestational age at birth, infants’ chronological age at observation, and infants’ gender parameters were included as predictor variables. As infants’ physiological system may be deeply connected to their mothers’ physiological system during kangaroo care, maternal physiological parameters (mean mothers’ HR in humming and mean mothers’ O2 Saturation in humming) were included as predictor variables in Model 2. Finally, the mean durations of each melodic contour during humming (linear, rising, falling, bell-shaped, U-shaped and sinusoidal), as well as the mean lengthening of the final notes in humming, both as independents variables, were included in Model 3. According to the enter method, Model 2 included all independent variables of Model 1, and Model 3 included all independent variables present in Model 2. According to Table 6, independent variables included in Model 1 and in Model 2 do not seem to contribute for the

explanation of the variance of the dependent variable. However, relative to Model 3, a significant contribution is found. According to results, only the mean duration of humming sinusoidal contours together with the lengthening of the final notes impacts preterm infants’ HR during humming ($p = .025$).

Table 6. Multiple linear hierarchical regression for DV infants’ HR during humming and IV’s average duration of sinusoidal contours and of lengthening of the final notes.

Model	R	R ²	Adjusted R ²	St. error of estimate	R ² change	F change	df1	df2	Sig. of F change
1	.331	.109	.020	13.186	.109	1.228	3	30	.317
2	.336	.113	-.046	13.624	.003	.051	2	28	.951
3	.575	.331	.151	12.275	.218	4.246	2	26	.025

HR: heart rate; Model 1: infants’ gestational age at birth, infants’ chronological age and infants’ gender; Model 2: mothers’ HR in humming, mother’s O2 saturation in humming; Model 3: average lengthening of the final notes, average duration of humming sinusoidal contours; DV: dependent variable; IV’s: independent variables.

Table 7 shows results for preterm infants’ Prop. O2 saturation/HR during humming as dependent variable and mean duration of each melodic contours of humming as principal independent variables. As sociodemographic and clinical variables of preterm infants may influence preterm infants’ Prop. O2 saturation/HR, infants’ gestational age at birth, infants’ chronological age at observation, and infants’ gender parameters were included as predictor variables in Model 1. For the same reason presented in the previous analyses, maternal physiological parameters (mean mothers’ HR in humming and mean mothers’ O2 Saturation in humming) were included as predictor variables in Model 2. Finally, the mean durations of each melodic contour during humming (linear, rising, falling, bell-shaped, U-shaped and sinusoidal), as well as the mean lengthening of the final notes in humming, both as independent variables, were included in Model 3. In the same way of the previous regression analysis, the enter method was used, and therefore the independent variables of each Model were included in the next Model. According to results, variables of Model 1 and Model 2 do not contribute to explain the variance of the dependent variable. In contrast, the predictors variables of Model 3 offer a significant contribution for the explanation of the dependent variable ($p = .037$). The mean duration of sinusoidal contours together with the mean lengthening of the final notes in humming can be considered as good predictors of the infants’ Prop. O2 saturation/HR in humming.

Table 7. Multiple linear hierarchical regression for DV infants’ Prop. O2 saturation/HR* during humming and IV’s average duration of sinusoidal contours and of lengthening of the final notes.

Model	R	R ²	Adjusted R ²	St. error of the estimate	R ² change	F change	df1	df2	Sig. of F change
1	.369	.136	.050	.051	.136	1.574	3	30	.216
2	.395	.156	.005	.052	.020	.327	2	28	.724
3	.587	.345	.168	.048	.189	3.749	2	26	.037

* Proportion of O2 saturation relative to HR; Model 1: infants' gestational age at birth, infants' chronological age and infants' gender; Model 2: mothers' HR in humming, mother's O2 saturation in humming; Model 3: average lengthening of final note, average duration of humming sinusoidal contours; DV: dependent variable; IV's: independent variables.

4. Discussion

So far, according to our knowledge, this is probably a pioneering study as it aimed at understanding the effects of melodic contours and of the lengthening of the final note in maternal ID humming on preterm infants' autonomic system during kangaroo care. Regarding physiological measures used to assess preterm infants' ANS (HR mean, HR range, HRV and O2 saturation), as highlighted in the state of the art [7,11,17,26,28,30,32,33], our study added Prop. Infant O2/HR saturation as a new and promising physiological measure to this research field. This was based on the idea that autonomic stability can be improved if it is achieved while preserving infants' cardiac resources; this is relevant to ensure preterm infants' physiological stability [1].

Regarding the first aim of this study our results show a lower level (group mean) of preterm infants' HR during maternal humming than during maternal speech. Also, a higher level (group mean) of infants' Prop. O2 saturation/HR was found during humming when compared with speech. As a consequence, the Prop. O2 saturation/HR shows that the optimal value of O2 saturation can be achieved with a lesser cardiac effort. This suggests that humming is a favorable condition for decreasing preterm infants' HR while, at the same time, O2 saturation reaches an optimal level of O2 saturation.

About the second aim, a higher maternal Prop. O2 saturation/HR in humming than in speech was found. This suggests that mothers' humming during kangaroo care may improve maternal cardiorespiratory function, as highlighted in studies about the physiological benefits of vocal toning in adults [18–21].

Concerning the third aim of this study, it was observed that the mean duration of humming sinusoidal contours together with the lengthening of the final notes helps to decrease preterm infants' HR. Furthermore, the stability of O2 saturation is achieved with a lesser cardiac effort. Once that O2 saturation is crucial for human infants' viability, and that HR is indispensable to achieve its' regulation [1], it is important to relate both variables. Because preterm infants are particularly vulnerable to cardiac efforts, O2 saturation should not be regarded in the absence of HR.

Despite mothers were not asked to hum lullabies or in a lullaby style, our results show that maternal humming presents structural and melodic features of that style. Sinusoidal contours and the lengthening of the final notes are usually observed in lullaby style [47]. As highlighted by music therapy in NICU [11–15] our results suggest that maternal humming in a lullaby style can improve preterm infants' physiological stability.

Concerning infants' vocal responsiveness, in a previous study made with the same sample it was observed that more than a half of the infants' overlapping vocalizations took place during the final note [51]. Probably the lengthening of the final note can be perceived by the infants as a temporal marker helping to respond during the end of the humming phrase.

As mentioned in literature, humming has a strong effect on the vagal system [19] inducing a relaxation state, and wellbeing releasing endorphins, enhancing sleep, lowering heart rate, and blood pressure, decreasing the cortisol and increasing oxytocin [52]. Therefore, humming in a lullaby style attuned to preterm infants' behavior is commonly used on music therapy in NICU, [11–15].

Our study highlights the important role of the musical characteristics of humming that has been overlooked in studies of music therapy and other neonatal stimulation approaches directed at preterm infants in NICU. When mothers hum to their preterm infants' during kangaroo care, guidance about humming features should be offered to optimize infants' self-regulation. Maternal humming is likely to be an easy way to improve mother-infant relationship during kangaroo care. If maternal humming in kangaroo care is offered in a lullaby style and keeping the voice calm, slow, simple, predictable, and repetitive, it can optimize the cardiorespiratory functioning of both mother and preterm infant [12].

When the infant is agitated and presenting tachycardia, maternal humming should be repetitive, having continuous notes with short intervals, predominance of sinusoidal or descending melodic contours and with repetition of the final notes or its' lengthening. On the other hand, it is important that these guidelines do not induce an excessively relaxed state that could induce severe bradycardia. This reinforces the use of contingent singing including humming in music therapy applied to NICU contexts [12–15].

The joint effect of O2 saturation with HR assessed by the Proportion of O2 saturation/HR is a promising physiological variable that should be considered in future studies to clarify the benefits of maternal humming in preterm infants' cardiorespiratory stabilization.

4.1. Limitations

Our study has several limitations. One of these is that we didn't analyze the impact of the NICU's noise, which could have affected the physiological parameters of the dyads. Although infants' behavioral states and clinical status were controlled as baseline conditions, these aspects were not monitored during the protocol sequence. It is possible that these factors were affected by the protocol conditions and, this way, changing the infants' physiological parameters. In addition, mothers varied their tactile behavior during the interaction, touching and caressing the infant while they spoke or sang and during silent pauses. The possible effects of maternal touch and rocking directed at the infant during humming were not analyzed. Finally, we didn't analyze the maternal emotional state during the observation, which is another factor that could have affected mothers' vocal expressiveness as well as their physiological parameters.

Following studies using multimodal stimulation in music therapy programs with preterm infants [26,53] it is important to deepen the joint effect of humming with kangaroo care on infants' self-regulation. Among multimodal stimulations there are rocking, and touch coordinated with rhythmic features of maternal humming/singing during kangaroo care, i.e., rocking associated with a vestibular experience that can have an impact on the preterm infants' ANS.

5. Conclusion

Our study contributes to the current literature on maternal humming effects in NICU underlining the important contribute of musical features of humming such as sinusoidal contours and the lengthening of the final note on preterm infants' physiological parameters.

Maternal humming can improve maternal cardiorespiratory function and therefore should be recommended in NICU as an easy personal resource to foster maternal well-being and on preterm infants' self-regulation. The use of maternal contingent humming in a lullaby style during kangaroo care In NICU should be encouraged. More studies are needed to deepen our preliminary results.

Institutional Review Board Statement: This study follows the principles of the Declaration of Helsinki.

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