Article title: Comparison of the analgesic effect of inhaled lavender vs vanilla essential oil for neonatal frenotomy: a randomized clinical trial (NCT04867824)

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Abstract:

Background: It is necessary to treat neonatal pain because it may have short and long-term adverse effects. Frenotomy is a painful procedure where sucking, a common strategy to relieve pain, cannot be used because the technique is performed on the tongue. In a previous randomized clinical trial, we demonstrated that inhaled lavender essential oil (LEO) reduced the signs of pain during neonatal frenotomy. We aimed to find out whether inhaled vanilla essential oil (VEO) is more effective in reducing pain during frenotomy than LEO.

Methods: Randomized clinical trial with neonates who underwent a frenotomy for type 3 tongue-ties between May and October 2021. Pain was assessed using pre and post-procedure heart rate (HR) and oxygen saturation (SatO2), crying time and NIPS score. Neonates were randomized into “experimental” and “control” group. In both groups, we performed swaddling, administered oral sucrose, and let the newborn suck for two minutes. We placed a gauze pad with one drop of LEO (control group) or of VEO (experimental group) under the neonate’s nose for two minutes prior to and during the frenotomy.

Results: We enrolled 142 neonates (71 per group). Both groups showed similar NIPS scores (2.02 vs 2.38) and crying times (15.3 vs 18.7 s). We observed no differences in HR increase or in SatO2 decrease between both groups. We observed no side effects in either of the groups.

Conclusions: We observed no appreciable difference between LEO and VEO, therefore we cannot conclude which of them was more effective in treating pain in neonates who underwent a frenotomy.

Keywords: ankyloglossia; aromatherapy; frenotomy; lavender; neonate; neonatal pain; tongue-tie; vanilla

Abbreviations: HR: heart rate; LEO: Lavender Essential Oil; NIPS: Neonatal Infant Pain Scale; SatO2: oxygen saturation; VEO: Vanilla Essential Oil.

What is known:

- Pain management is one of the most important goals of neonatal care as it can have long-term neurodevelopmental effects.
- Lavender essential oil can help relieve pain due to its sedative, antispasmodic and anticolic properties.

What is new:

- Lavender and vanilla essential oils are safe, beneficial, easy to use and cheap in relieving pain in neonates who undergo a frenotomy for type 3 tongue-ties.

This clinical trial is registered with www.clinicaltrials.gov with NCT04867824.

Funding:

We declare that we have not received any funds or grants for this research. Pranarôm España S.L. (Barcelona, Spain) provided the samples of lavender and vanilla essential oils used in the present study free of charge. Pranarôm España S.L. had no role in study design, data collection, analysis, decision to publish, or preparation of the manuscript.

Acknowledgments:

We would like to thank Jennifer Bricker-Bolton for proofreading this manuscript and helping with the English language. We would like to thank Pranarôm España S.L. for providing the samples of lavender and vanilla essential oils used in the present study at no cost.

Introduction

Neonates routinely undergo painful procedures such as blood sampling for the early diagnosis of inborn errors of metabolism. According to the International Association for the Study of Pain, “pain” is as an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage [1]. Over the last years, neonatal units have shown an increasing interest in studying neonatal pain. Historically, newborns were thought to have no pain due to the immaturity of their nervous system [2]. Evidence however demonstrated that newborns feel pain and may even have increased sensitivity to it and to its long-term negative effects due to this immaturity. Repeated, unrelieved pain can cause adverse physiologic effects in all systems, including the brain, potentially affecting long-term development [3]. This fact has driven development-based care, a model which promotes individualized care based on the observation of the neonates' behaviors and on the knowledge of their physical and family environment. Developmental care focuses on avoiding painful procedures such as blood sampling as much as possible, grouping interventions in order to minimally manipulate the newborn, and managing pain, either by administering analgesia, physically restraining the patient, or combining both [4]. It is important to recognize pain and relieve it because it may lead to hemodynamic instability, decreased oxygen saturation, and increased intracranial pressure [5]. Non-pharmacological pain relief interventions are important in neonatology because there is evidence that they reduce pain (even though they do not eliminate it) and distress, but also because pharmacological treatments have potential adverse effects. They include: sensory stimulation (positioning or swaddling, vestibular action or rocking, aromatherapy, non-nutritive sucking, musical therapy), nutritive (oral sweet solutions) and maternal interventions (maternal odor and voice, breastfeeding, skin-to-skin contact) [4,6-11]. Olfaction is essential for neonatal behavioral adaptation in many mammals, including humans [12]. Olfactory signals help the newborn baby localize and attach to the nipple at the first sucking bout [13]. Aromatherapy uses the healing effects of volatile essential oils in different ways and has been widely used for centuries in traditional and modern medicine as complementary therapy [6,14,15]. Aroma stimulates the olfactory bulb, anatomically close to the limbic system which is responsible for the emotions. Effects of essential oils on the limbic system lead to encephalin, endorphin and serotonin release [16]. Lavender essential oil (LEO), of all the essential oils, has been studied most by healthcare professionals [17]. LEO may relieve pain [8,14,16] through inhibition of nociceptive stimuli by stimulating the olfactory system, inducing relaxation and stimulating endogenous opioids [7].

In our service we perform painful techniques following administration of oral sucrose, performing swaddling, and allowing neonates to breastfeed or suck, which helps prevent crying. However, these measures are

not enough during frenotomies because they are performed in the mouth and neonates cannot suck during the procedure. We have a high prevalence of neonatal ankyloglossia (32.5%) in our center, for which frenotomy is a common treatment [18]. In a former clinical trial (NCT04877392), we compared the use of common pain control strategies (swaddling, administration of oral sucrose, and suck for two minutes prior to the procedure) with the use of those plus inhaled LEO, and observed that signs of pain (duration of crying and NIPS score) were lower in the experimental group. From that moment on, we have routinely used inhaled LEO when performing a frenotomy [19]. Other authors have observed the benefits of using vanilla (*Vanilla fragrans*) essential oil (VEO) for pain control in neonates [20,21]. The aim of this study was to demonstrate if the use of inhaled VEO was more effective than inhaled LEO (*Lavandula angustifolia ssp angustifolia*) in reducing signs of pain during frenotomy in healthy, full-term neonates. Our hypothesis was that signs of pain (crying time and NIPS score) would be lower in the experimental group than in the control group. We chose LEO (control group) and VEO (experimental group) because these are the fragrances for which more studies have been performed in neonates and infants [6-8,14,19,21-29]. As far as we know, there are no previous studies which have analyzed the potential benefit of LEO vs VEO to relieve pain in neonatal frenotomy.

Patients and methods

We conducted a blinded randomized clinical trial (registered at https://clinicaltrials.gov with the identifier NCT04867824, under the title "The Use of Lavender vs Vanilla Essential Oil as Complementary Analgesia for Frenotomy in Healthy Newborns"). Our hospital Ethics Committee (CEIm-PSMAR) reviewed and approved this study on May 4, 2021 (approval number: 2021/9731/I). This study was conducted according to the ethics code of the Barcelona Medical Association and the principles of the Helsinki-Fortaleza Declaration 2013.

This study was conducted at the neonatal unit of a tertiary care hospital in Barcelona, Spain, which experiences approximately 1,400 births per year from a multiethnic population with Spanish, Pakistani and Bangladeshi being the most frequent nationalities of our patients [30]. We have high breastfeeding rates: around 85% at discharge from the maternity ward (86.8% in 2018) [18], 82% at the age of three months, and 54% at six months. We assess for the presence of ankyloglossia as part of the routine neonatal evaluation and classify it based on Coryllos's criteria [31] and the Hazelbaker tool [32]. A lingual frenulum is symptomatic if it scores eight points or less in appearance and/or 11 or less in function according to Hazelbaker. Advice and help with positioning and attachment for breastfeeding is provided to all the mothers by lactation support providers.

During the study period, if we identified a symptomatic neonate with a type 3 tongue-tie which affected breastfeeding, we offered the neonate's parents to participate. We considered a tongue-tie to be symptomatic if it scored 8 points or less in appearance and/or 11 points or less in function according to Hazelbaker and the mother experienced nipple pain or bruises, or if the neonate had problems latching onto the breast after a neonatal nurse assessed feeding and corrected other reasons for maternal pain such as retrognathia, micrognathia, incorrect positioning, insufficient mouth opening, and latching onto the nipple onlyWe chose type 3 tongue-ties because they are the most common in our population [18] and, due to their anatomical features (thick and submucosal), they seem to make breastfeeding more difficult. Neonates were enrolled if their parents agreed to and signed a written informed consent prior to the procedure, then they were allocated into the experimental or the control group by simple random sampling using the program OxMAR (Online Minimization and Randomization for Clinical Trials) [33]. Prior to recruitment, we generated a list of 142 numbers, where each number was randomized to either the “VEO” or “control (LEO)” group. Neonates were enrolled in numerical order and assigned into the pre-determined group. The group into which a neonate had been enrolled was not known by the attending personnel until the moment of performing the frenotomy.

To perform the frenotomy, the neonate was taken to the neonatal unit and monitored with a pulse-oximeter (COVIDIEN Nellcor Portable SpO2 Patient Monitoring System PM10N, Covidien Ireland Limited, IDA Business & Technology Park, Tullamore, Ireland) while preparing the neonate for the frenotomy, throughout the procedure and until five minutes after completing it. For both groups we swaddled, administered 1 mL of oral sucrose, and let the newborn suck for two minutes prior to the procedure. The control group had a 7 x 7 cm gauze pad with one drop of 100% pure LEO (Pranarôm España S.L.) placed 2 cm under their nose for two minutes prior to starting the frenotomy and for the duration of the procedure; whereas in the experimental group the drop on the gauze pad was of 100% pure VEO (Pranarôm España S.L.) instead. The bottles of both LEO and VEO have a dropper that always dispenses the same amount of oil per drop. We did not start the procedure until the neonates were calm and had a NIPS score of 0. Frenotomy was performed by one of the three staff neonatologists by placing a sterile groove director under the tongue holding the frenulum in place with visualization of tongue base and frenulum, then snipping the frenulum with a blunt tip scissor along the underside of the tongue to its base just proximal to the genioglossus muscle until a full release was achieved [31]. We assessed pain by means of crying time and the highest Neonatal Infant Pain Scale (NIPS) score in the five minutes post procedure, and whether there was an increase in heart rate (HR) and decrease in oxygen saturation (SatO2) (comparing pre- and post-procedure HR and SatO2). NIPS evaluates facial expression, crying, breathing pattern, arm and leg position, and state of arousal on

a scale from 0 to 7, where 0-2 means no pain to mild pain, 3-4 mild to moderate pain, and >4 severe pain [34]. A blinded neonatologist who did not perform the frenotomy evaluated vital signs through the screen of the pulse-oximeter, NIPS score and crying time from a neighboring room through a glass, for which he/she could not smell or see which oil was being used. Vital signs, whether the baby cried or not, the seconds crying lasted, and the post procedure NIPS score were recorded on a data collection sheet. A chronometer was started when the neonate started crying and was stopped once he/she completely stopped crying. If he/she restarted crying after having initially stopped, the chronometer was started again and all the crying time was added up. If a neonate cried, the attending staff who performed the frenotomy provided calming techniques such as holding, swaddling, and sucking regardless of which essential oil was being used. These persons were not blinded. Once the procedure was completed, we removed the gauze pad and returned the neonate to the mother for breastfeeding.

Calculation of sample size: In an exploratory preliminary study prior to the intervention, we observed a mean (SD) crying time of 19.80 (21.14) seconds. We used this data as our baseline. In order to detect a difference of 10 seconds in crying time, we calculated that we needed a sample size of 71 neonates per group to be able to draw conclusions with a confidence interval (CI) 95% and a power of 80%. We used crying time to calculate sample size because it is an objective way to measure pain, whereas NIPS could be more person-specific.

The observer (a blinded neonatologist) saw the procedure from a neighboring room through a glass, from which the screen of the pulse-oximeter and the neonate were perfectly visible. This person recorded demographic (sex, gestational age, birth weight, age in hours at the time of frenotomy) and clinical data (HR and SatO2 before, during, and after the procedure, whether the neonate cried or not during the procedure, length of crying time in seconds, presence of side effects during the procedure (apnea, desaturation, distress, vomiting, changes in skin color) and highest NIPS score within the first five minutes after the procedure) on a data collection sheet. The primary outcome was difference in crying time between the experimental and the control group, and secondary outcomes were: difference of NIPS score, HR and SatO2 pre and post-procedure between the experimental and the control group. Participants' confidentiality was maintained because neither the name nor the medical record number were recorded on the data collection sheet.

Statistical analysis: Quantitative variables are described using the mean, standard deviation, and 95% CI; the experimental and the control groups were compared with a Student's t test. Qualitative variables are presented in percentages and compared using Fisher's exact test. We compared NIPS scores between both groups using the Wilcoxon rank-sum (Mann-Whitney) test. Statistical significance was set for a $p < .05$. To perform statistical analyses we used STATA version 16.1 (StataCorp, College Station, TX, USA).

177

178 **Results**

179 We enrolled 142 neonates until we reached 71 neonates in each group from a total of 155 potential
180 candidates between May 10, 2021 and October 9, 2021. Thirteen were excluded for the following reasons: eight
181 parents refused to participate in the study, there was a language barrier with four parents, and one was isolated due
182 to maternal COVID-19 infection. There was no follow up period therefore we did not lose any participants to
183 follow up. All the neonates were analyzed for the primary and secondary outcomes. We included 77 male (54.2%)
184 and 65 female (45.8%) newborns. Globally, mean (SD) gestational age was $39^{6/7}$ ($1^{1/7}$) weeks, and mean (SD) birth
185 weight, 3328.58 (488.04) g. The mean (SD) age at the time of the procedure was 43.0 (32.9) hours. Table 1 shows
186 the demographic characteristics of both groups. There were no differences between the two groups in terms of sex,
187 birth weight, gestational age, or age at the moment of the frenotomy.

188 Mean (SD) HR pre-procedure was 125.4 (17.3) bpm, and post-procedure 155.3 (16.8) bpm; mean (SD)
189 HR increase was 29.9 (15.6) bpm. Mean (SD) SatO2 pre-procedure was 99.3 (1.2) %, and post-procedure, 96.4
190 (3.1) %; mean (SD) SatO2 decrease was 2.9 (3.0) %. 140 neonates cried (99.3%) with a mean (SD) crying time of
191 17.0 (19.5) seconds. Mean (SD) NIPS score was 2.20 (1.05). There were no differences between the two groups
192 in terms of baseline HR. There were statistically different baseline SatO2 that had no clinical significance. Table
193 2 presents the outcomes of the experimental group and the control group.

194 There were no differences between the experimental group and the control group in terms of crying time,
195 NIPS scores, HR increase, or SatO2 decrease. Almost all neonates cried in both groups. We observed no adverse
196 effects with the use of LEO or VEO.

197

198 **Discussion**

199 Goubet conducted the first study of aromatherapy with neonates in 2003 [35]. Aromatherapy has been
200 used to treat pain in infants, showing an objective improvement in neonatal pain scale scores, decreased heart rate,
201 shorter crying time, and prevention of decreased oxygen saturation [6-8,14,19,22]. The main aromas used in
202 neonatology are lavender, vanilla, amniotic fluid and human milk [6,36].

203 LEO may alter the perception of pain by inhibiting nociceptive stimuli by means of stimulating the
204 olfactory system and inducing relaxation, providing a pleasant environment, distracting the mind from the pain,
205 and stimulating endogenous opioids [7]. Its sedative, antidepressant, as well as antispasmodic and anticolic
206 properties make it capable of relieving the symptoms of pain [8,14]. Inhaled LEO has demonstrated benefits in

reducing pain during neonatal blood sampling, heel puncture [7,8,22] and vaccination at the age of two months [23].

Several studies concluded that the use of a familiar odor (mainly breast milk, but also vanillin, present in VEO) helps to reduce agitation, apneas, and stress and adverse effects of neonatal pain [29,37,38]. Vanillin seems to be hedonically pleasant for full-term and preterm neonates, seems to influence pain reaction, and may even be analgesic, especially if the infant has been previously exposed to its odor [28,29,35,39]. We did not sensitize neonates with the aroma of VEO because we performed the frenotomy when it was indicated, and thus there was no time to sensitize them prior to the procedure. Goubet observed that VEO has soothing effects on premature neonates during venipuncture but not during heel stick [35], whereas some authors have observed that it is effective in full-term neonates during heel stick [21,28,39], venipuncture [20] and arterial puncture [40]. Sadathosseini observed that crying lasted significantly less and that variation in SatO2 was lower when the odor of VEO was familiar [40]. Other studies have found no effects of VEO in soothing pain in full-term infants if the patients have not been previously exposed to this odor [26]. Neshat et al. found no differences on prematures' heart rate and SatO2 during venipuncture even though the neonates had been previously familiarized with VEO [25].

We are aware that the prevalence of ankyloglossia we found in our population is higher than reported [18]. This can be explained by the fact that we designed a study to prospectively evaluate all the neonates for the presence of a tongue-tie. Most studies have focused their attention to the so-called "anterior tongue-ties", whereas the true prevalence of "posterior tongue-ties" remains unknown [41]. Most clinicians recognize an anterior frenulum and recommend a frenotomy if it affects breastfeeding. Posterior ankyloglossia is often undiagnosed, as it does not have the usual appearance of the traditional, anterior frenulum, and it is a relatively newly recognized entity [42].

To the best of our knowledge, this is the first study to evaluate the effect of inhaled LEO vs VEO as pain relief during neonatal frenotomy. In a previous clinical trial, which we conducted, we observed a significant decrease in crying time and NIPS scores in the LEO exposed group when compared to the control group and traditional pain control measures [19]. When planning this clinical trial, we chose VEO because it is the second most used aroma (apart from breastmilk) in neonatology. In this study, we observed no difference in crying time or the NIPS scores between the LEO and VEO groups. Thus, we can assume that VEO is a suitable alternative for LEO in treating neonatal pain during frenotomy.

None of the prior aromatherapy studies performed in infants have described any side effects, for instance nausea, vomiting or chills [24,27,29,40]. In keeping in line with them, we also have observed no side effects from

its use. Therefore, we conclude that using inhaled LEO and VEO for frenotomy is safe. Our study is easily reproducible.

One positive note is that the use of inhaled essential oils is cheap. A 10-mL bottle of Pranarôm LEO or VEO costs $7.60 US, and contains approximately 200 drops. We used one drop per neonate, which represents approximately $0.04 US.

We acknowledge that the study has limitations. The team who performed the frenotomies was not blinded, because the smell of LEO and VEO is too obvious to ignore. However, the person who recorded the data was blinded, as described in the "Patients and methods" section. Some candidates (8.39%) were not eligible to participate primarily because eight parents did not consent and four parents had language barrier issues for which they were not offered to participate. Another limitation is that more than one person performed the frenotomies, for which the technique could have minimal variations, however, all three staff neonatologists have similar experience and training.

Conclusions

In conclusion, we observed no differences in the signs of pain between the experimental and the control group. For this reason, we cannot conclude that LEO or VEO are more effective in treating pain in the neonates who underwent a frenotomy for type 3 tongue-ties. We observed no side effects from its use.

Author Declarations:

-Ethics approval and consent to participate: Our Ethics Committee approved the study (reference number: 2021/9731/I), which was conducted in accordance with the Declaration of Helsinki. Consent to participate: Yes.

-Consent for publication: N/A.

-Availability of data and materials: Our database is accessible from the authors upon request.

-Competing interests: We declare that we have no conflicts of interest to disclose.

-Funding: We declare that we have not received any funds or grants for this research. Pranarôm España S.L. (Barcelona, Spain) provided the samples of lavender and vanilla essential oil used in the present study free of charge. Pranarôm España S.L. had no role in study design, data collection, analysis, decision to publish, or preparation of the manuscript.

-Authors' contributions:

Dr. SM designed the study, collected data, analyzed the results, and drafted the initial manuscript.

Dr. MF designed the study, analyzed the results, and drafted the initial manuscript.

Ms. RL reviewed the literature and helped draft the initial manuscript.

Drs. JC, JG and ML collected data, and helped draft the initial manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

-Acknowledgments: We would like to thank Jennifer Bricker-Bolton for proofreading this manuscript and helping with the English language. We would like to thank Pranarôm España S.L. for providing the samples of lavender and vanilla essential oil used in the present study at no cost.

Compliance with Ethical Statements

Conflict of Interest: The authors declare that they have no conflict of interest. This research was not sponsored.

Funding: There is no funding source. Pranarôm España S.L. (Barcelona, Spain) provided the samples of lavender and vanilla essential oil used in the present study free of charge. Pranarôm España S.L. had no role in study design, data collection, analysis, decision to publish, or preparation of the manuscript.

Ethical approval: Our hospital Ethics Committee approved this study (reference number: 2021/9731/I), which was conducted in accordance with the Declaration of Helsinki.

References

[1] International Association for the Study of Pain. IASP Terminology. (2021) https://www.iasp-pain.org/Education/Content.aspx?ItemNumber=1698#Pain

[2] Urso, AM. (2007). The reality of neonatal pain and the resulting effects. *Journal of Neonatal Nursing*. https://doi.org/10.1016/j.jnn.2007.09.006

[3] Walker S. M. (2019). Long-term effects of neonatal pain. Seminars in fetal & neonatal medicine, 24(4), 101005. https://doi.org/10.1016/j.siny.2019.04.005

[4] Program Guide. Newborn Individualized Developmental Care and Assessment Program (NIDCAP). An Education and Training Program for Health Care Professionals (2015). http://nidcap.org/wp-content/uploads/2014/09/Program-Guide-Rev-22Sep2014.pdf

[5] Banga, S., Datta, V., Rehan, H. S., & Bhakhri, B. K. (2016). Effect of Sucrose Analgesia, for Repeated Painful Procedures, on Short-term Neurobehavioral Outcome of Preterm Neonates: A Randomized Controlled Trial.

Journal of tropical pediatrics, 62(2), 101–106. https://doi.org/10.1093/tropej/fmv079

[6] Fitri, S., Wardhani, V., Rakhmawati, W., Pahria, T., & Hendrawati, S. (2020). Culturally Based Practice in Neonatal Procedural Pain Management: A Mini Review. *Frontiers in pediatrics*, 8, 540. https://doi.org/10.3389/fped.2020.005400540

[7] Razaghi, N., Aemmi, S. Z., Sadat Hoseini, A. S., Boskabadi, H., Mohebbi, T., & Ramezani, M. (2020). The effectiveness of familiar olfactory stimulation with lavender scent and glucose on the pain of blood sampling in term neonates: A randomized controlled clinical trial. *Complementary therapies in medicine*, 49, 102289. https://doi.org/10.1016/j.ctim.2019.102289

[8] Razaghi, N., Sadat Hoseini, A., Aemmi, S., Mohebbi, T., & Boskabadi, H. (2015). The Effects of Lavender scent on pain of blood sampling in term neonates. *International Journal of Pediatrics*, 3(2.2): 535-541. https://doi.org/10.22038/ijp.2015.3830

[9] Stevens, B., Yamada, J., Ohlsson, A., Haliburton, S., & Shorkey, A. (2016). Sucrose for analgesia in newborn infants undergoing painful procedures. *The Cochrane database of systematic reviews*, 7(7), CD001069. https://doi.org/10.1002/14651858.CD001069.pub5

[10] Harrison, D., Larocque, C., Bueno, M., Stokes, Y., Turner, L., Hutton, B., & Stevens, B. (2017). Sweet Solutions to Reduce Procedural Pain in Neonates: A Meta-analysis. *Pediatrics*, 139(1), e20160955. https://doi.org/10.1542/peds.2016-0955

[11] Leng, H. Y., Zheng, X. L., Zhang, X. H., He, H. Y., Tu, G. F., Fu, Q., Shi, S. N., & Yan, L. (2016). Combined non-pharmacological interventions for newborn pain relief in two degrees of pain procedures: A randomized clinical trial. *European journal of pain (London, England)*, 20(6), 989–997. https://doi.org/10.1002/ejp.824

[12] Winberg, J., Porter, R. (1998). Olfaction and human neonatal behaviour: clinical implications. Acta Paediatr, 87, 6–10. https://doi.org/10.1080/08035259850157787

[13] Varendi, H., Porter, R., Winberg, J. (1994) Does the newborn baby find the nipple by smell? Lancet, 344, 989–990. https://doi.org/10.1016/s0140-6736(94)91645-4

[14] Çetinkaya, B., & Başbakkal, Z. (2012). The effectiveness of aromatherapy massage using lavender oil as a treatment for infantile colic. *International journal of nursing practice*, 18(2), 164–169. https://doi.org/10.1111/j.1440-172X.2012.02015.x

[15] López, V., Nielsen, B., Solas, M., Ramírez, M. J., & Jäger, A. K. (2017). Exploring Pharmacological Mechanisms of Lavender (Lavandula angustifolia) Essential Oil on Central Nervous System Targets. *Frontiers in pharmacology*, 8, 280. https://doi.org/10.3389/fphar.2017.00280

[16] Habanananda T. (2004). Non-pharmacological pain relief in labour. *Journal of the Medical Association of Thailand = Chotmaihet thangphaet*, 87 Suppl 3, S194–S202.

[17] Jones, J. E., & Kassity, N. (2001). Varieties of alternative experience: complementary care in the neonatal intensive care unit. *Clinical obstetrics and gynecology*, 44(4), 750–768. https://doi.org/10.1097/00003081-200112000-00012

[18] Maya-Enero, S., Pérez-Pérez, M., Ruiz-Guzmán, L., Duran-Jordà, X., & López-Vílchez, M. Á. (2021). Prevalence of neonatal ankyloglossia in a tertiary care hospital in Spain: a transversal cross-sectional study. *European journal of pediatrics*, 180(3), 751–757. https://doi.org/10.1007/s00431-020-03781-7

[19] Maya-Enero, S., Fàbregas-Mitjans, M., Llufriu-Marquès, R.M., Candel-Pau, J., Garcia-Garcia, J., & López-Vílchez, M. Á. (2022). Analgesic effect of inhaled lavender essential oil for frenotomy in healthy neonates: a randomized clinical trial. World Journal of Pediatrics. http.://doi.org/10.1007/s12519-022-00531-7 Accepted for publication. Not published yet.

[20] Jebreili, M., Neshat, H., Seyyedrasouli, A., Ghojazade, M., Bagher, M., Hamishehkar, H. Comparison of Breastmilk Odor and Vanilla Odor on Mitigating Premature Infants' Response to Pain During and After Venipuncture. Online: 21 Aug 2015https://doi.org/10.1089/bfm.2015.0060

[21] Molina-De La Garza, J., Ochoa-Correa, E., Saucedo-Rodríguez, E., Rodríguez-Balderrama, I., Cárdenas-Del Castillo, B., Martínez-Cobos, M., De La O-Cavazos, M. Vanilla essence non-nutritive sucking, an effective alternative for pain relief during heel-stick procedure in healthy term neonates: A randomized clinical trial. Revista Medicina Universitaria. 23(1), 4-10. https://doi.org/10.24875/RMU.21000006

[22] Akcan, E., & Polat, S. (2016). Comparative Effect of the Smells of Amniotic Fluid, Breast Milk, and Lavender on Newborns' Pain During Heel Lance. *Breastfeeding medicine: the official journal of the Academy of Breastfeeding Medicine*, 11(6), 309–314. https://doi.org/10.1089/bfm.2015.0174

[23] Vaziri, F., Khosropoor, M., Hidari, M., Pourahmad, S., Morshed Behbahani, B., & Saki, F. (2019). The Effect of Aromatherapy by Lavender Oil on Infant Vaccination Pain: a Double Blind Randomized Controlled Trial. *Journal of caring sciences*, 8(1), 17–21. https://doi.org/10.15171/jcs.2019.003

[24] Field, T., Field, T., Cullen, C., Largie, S., Diego, M., Schanberg, S., & Kuhn, C. (2008). Lavender bath oil reduces stress and crying and enhances sleep in very young infants. *Early human development*, 84(6), 399–401. https://doi.org/10.1016/j.earlhumdev.2007.10.008

[25] Neshat H, Jebreili M, Seyyedrasouli A, Ghojazade M, Hosseini MB, Hamishehkar H. Effects of Breast Milk and Vanilla Odors on Premature Neonate's Heart Rate and Blood Oxygen Saturation During and After

Venipuncture. Pediatr Neonatol. 2016;57(3):225-231. doi:10.1016/j.pedneo.2015.09.004

[26] Romantsik O, Porter RH, Varendi H. The effects of olfactory stimulation and gender differences on pain responses in full-term infants. Acta Paediatr. 2014;103(11):1130-1135. doi:10.1111/apa.12759

[27] Marlier L, Gaugler C, Messer J. Olfactory stimulation prevents apnea in premature newborns. Pediatrics. 2005;115(1):83-88. doi:10.1542/peds.2004-0865

[28] Goubet, N., Strasbaugh, K., Chesney J. Familiarity Breeds Content? Soothing Effects of a Familiar Odor on Full-Term Newborns. Journal of Developmental & Behavioral Pediatrics: June 2007 - Volume 28 - Issue 3 - p 189-194 doi: 10.1097/dbp.0b013e31802d0b8d

[29] Thiel MT, Längler A, Ostermann T. Systematic review on phytotherapy in neonatology. Forsch Komplementmed. 2011;18(6):335-344. doi:10.1159/000334712

[30] Maya-Enero, S., Candel-Pau, J., Garcia-Garcia, J., Giménez-Arnau, A. M., & López-Vílchez, M. Á. (2020). Validation of a neonatal skin color scale. European journal of pediatrics, 179(9), 1403–1411. https://doi.org/10.1007/s00431-020-03623-6

[31] Coryllos, EW., Genna, CW., Salloum, AC. (2004). Congenital tongue-tie and its impact on breastfeeding. American Academy of Pediatrics. Section on breastfeeding. https://www.researchgate.net/publication/301346077_Congenital_tongue-tie_and_its_impact_on_breastfeeding

[32] Hazelbaker, AK. (1993). The Assessment Tool for Lingual Frenulum Function: Use in a Lactation Consultant Private Practice. Pasadena, CA: Pacific Oaks College.

[33] O'Callaghan C. A. (2014). OxMaR: open source free software for online minimization and randomization for clinical trials. *PloS one*, 9(10), e110761. https://doi.org/10.1371/journal.pone.0110761

[34] Lawrence, J., Alcock, D., McGrath, P., Kay, J., MacMurray, S. B., & Dulberg, C. (1993). The development of a tool to assess neonatal pain. *Neonatal network: NN*, 12(6), 59–66.

[35] Goubet, N., Rattaz, C., Pierrat, V., Bullinger, A., & Lequien, P. (2003). Olfactory experience mediates response to pain in preterm newborns. Developmental psychobiology, 42(2), 171–180. https://doi.org/10.1002/dev.10085

[36] Bartocci, M., Winberg, J., Ruggiero, C., Bergqvist, L., Serra, G., Lagercrantz, H. (2000). Activation of Olfatory Cortex in Newborn Infants After Odor Stimulation: A Functional Near-Infrared Spectroscopy Study. Pediatric Research, 48(1), 18-23.
https://doi.org/10.1203/00006450-200007000-00006

[37] Edraki, M., Pourpulad, H., Kargar, M., Pishva, N., Zare, N., Montaseri, H. (2013). Olfactory stimulation by

vanilla prevents apnea in premature newborn infants. Iran J Pediatr, 23:261–268. https://doi.org/10.1542/peds.2004-0865

[38] Kanbur BN, Balci S. Impact of the odors of vanilla extract and breast milk on the frequency of apnea in preterm neonates. Jpn J Nurs Sci. 2020;17(1):e12271. doi:10.1111/jjns.12271

[39] Rattaz, C., Goubet, N., Bullinger, A. The calming effect of a familiar odor on full-term newborns. *J Dev Behav Pediatr*. 2005 Apr;26(2):86-92

[40] Sadathosseini, A. S., Negarandeh, R., & Movahedi, Z. (2013). The effect of a familiar scent on the behavioral and physiological pain responses in neonates. *Pain management nursing: official journal of the American Society of Pain Management Nurses*, 14(4), e196–e203. https://doi.org/10.1016/j.pmn.2011.10.003

[41] Walsh, J., & McKenna Benoit, M. (2019). Ankyloglossia and Other Oral Ties. Otolaryngologic clinics of North America, 52(5), 795–811. https://doi.org/10.1016/j.otc.2019.06.008

[42] Pransky, S. M., Lago, D., & Hong, P. (2015). Breastfeeding difficulties and oral cavity anomalies: The influence of posterior ankyloglossia and upper-lip ties. International journal of pediatric otorhinolaryngology, 79(10), 1714–1717. https://doi.org/10.1016/j.ijporl.2015.07.033

Table 1. Demographic characteristics of the experimental group and the control group.

Variables	Experimental group (VEO) *n* = 71 (%)	Control group (LEO) *n* = 71 (%)	*P* value
Male newborn	38 (53.5)	39 (54.9)	>0.99[a]
Birth weight (grams) (mean, SD)	3277.97 (494.71)	3379.20 (479.41)	0.217[b]
Gestational age (weeks) (mean, SD)	$39^{6/7}$ ($1^{1/7}$)	$39^{6/7}$ ($1^{2/7}$)	0.909[b]
Age at frenotomy (hours) (mean, SD)	43.6 (31.1)	42.4 (34.8)	0.819[b]

[a]Fisher's exact test, [b]Student's t-test, [c]Wilcoxon rank-sum (Mann-Whitney). LEO: Lavender Essential Oil, VEO: Vanilla Essential Oil.

Table 2. Outcomes of the experimental group and the control group. Control group is the reference.

Variables	Experimental group *n* = 71 (%)	Control group *n* = 71 (%)	*P* value	95% CI[d]
Crying (yes, %)	71 (100%)	70 (98.6%)	>0.99[a]	-
Crying (seconds) (mean, SD)	15.3 (16.5)	18.7 (22.0)	0.297[b]	-9.88 to +3.04
NIPS score (mean, SD) (range)	2.02 (0.97) (1-4)	2.38 (1.11) (0-4)	0.114[c]	-0.63 to +0.07
Heart rate (bpm)	125.1 (13.1)	125.8 (17.3)	0.781[b]	-5.83 to +4.39

pre-procedure (mean, SD) post-procedure (mean, SD)	155.8 (16.5)	154.9 (17.2)	0.762[b]	-4.74 to +6.46
Increase in heart rate post-procedure (bpm) (mean, SD)	31.3 (16.1)	30.6 (15.5)	0.549[b]	-3.62 to +6.77
Oxygen saturation (%) pre-procedure (mean, SD) post-procedure (mean, SD)	 99.1 (1.5) 96.1 (3.3)	 99.6 (0.9) 96.7 (2.9)	 **0.024[b]** 0.277[b]	 -0.87 to -0.06 -1.62 to +0.47
Decrease in oxygen saturation post-procedure (%) (mean, SD)	2.3 (2.7)	2.4 (2.9)	0.826[b]	-1.13 to +0.90
Presence of adverse effects (yes, %)	0 (0.0%)	0 (0.0%)	-	-

[a]Fisher's exact test, [b]Student's t-test; [c]Wilcoxon rank-sum (Mann-Whitney) test; [d]95% CI: 95% confidence interval of the difference between the experimental and the control group.