We conducted a prospective, randomized clinical trial. Our hospital Ethics Committee (CEIm-PSMAR) approved this study (reference code: 2023/10996). Prior to patient enrollment we obtained a signed informed consent from the neonate's parents. This study was conducted according to the ethics code of the Barcelona Medical Association and the principles of the Helsinki-Fortaleza Declaration 2013, at the neonatal unit of a tertiary care hospital in Barcelona (Spain) within an area of influence of approximately 400,000 people, which experiences approximately 1,400 births per year. The target population for this study, and thus, inclusion criteria were healthy full-term neonates born at our center or less than 15 days old referred for a frenotomy, who had ankyloglossia according to the Hazelbaker tool [39] (Figure 1) between September 2023 and June 2024. We assess for the presence of ankyloglossia as part of the routine neonatal evaluation using the Hazelbaker tool to evaluate its impact on tongue movement and on breastfeeding [39]. According to the Hazelbaker tool (see Figure 1), ankyloglossia exists if appearance scores 8 points or less and/or function scores 11 points or less. We offer a frenotomy to all patients with ankyloglossia. During the study period, if we identified a patient with ankyloglossia, we offered the patient's parents the opportunity to participate in this study.

Patients were enrolled if their parents agreed to and signed a written informed consent. We only included patients who were breastfed, as after randomization we may need to get colostrum. Enrolled patients were randomized into case or control group by simple random sampling using sequentially numbered containers. During the frenotomy the neonate was taken to the neonatal unit and monitored with a pulse-oximeter (COVIDIEN Nellcor Portable SpO_2 Patient Monitoring System PM10N, Covidien Ireland Limited, IDA Business & Technology Park, Tullamore, Ireland) before, during and after the procedure. For both groups we swaddled, administered 1 mL of oral sucrose,

and let the newborn suck for 2 minutes prior to the procedure. The control group had a 7 x 7 cm gauze pad with 1 drop (43.75 mg) of 100% pure LEO (Pranarôm España S.L.) placed 2 cm under their nose for 2 minutes prior to starting the frenotomy and for the duration of the procedure, whereas the experimental group had a gauze with 2 drops of colostrum of the patient's mother placed in the same way. We used two drops of colostrum but only one of LEO because the odor of colostrum is more subtle than that of LEO, and in our previous studies [32-33] we had used one drop of LEO, and that is how we routinely perform frenotomies. Once the procedure was completed, we removed the gauze pad and recorded vital signs, whether the baby cried or not, the seconds crying lasted, and the NIPS score on a data collection sheet. If a neonate cried, calming techniques such as holding, swaddling, and sucking were employed. A blinded observer assessed pain by means of the Neonatal Infant Pain Scale (NIPS) score (Figure 2) [40], crying duration, and whether there was a change in heart rate (HR) and/or in oxygen saturation (satO2) before and after the procedure.

In a previously published study where we compared performing frenotomies using complementary analgesia or not with inhaled LEO, we observed a mean (SD) crying time of 14.8 vs 24.6 (10.8 vs 27.6) seconds in favor of LEO [32]. In order to detect a difference of 10 seconds in crying time, we calculated that we needed a sample size of 71 patients per group in order to draw conclusions with a CI 95% and a power of 80%. We chose to evaluate pain by means of the increase of HR rather than via the NIPS score because in previous research, we obtained NIPS scores of 1.88-2.92 and 2.02-2.38, and a NIPS score less than 3 indicates no pain. We used the NIPS score to assess whether neonates exhibited pain when using inhaled colostrum instead of LEO.

We recorded demographic (sex, gestational age, birth weight, age in hours at the time of frenotomy) and clinical variables (HR and satO2 before, during, and after the

procedure, whether the patient cried or not during the procedure, length of crying time in seconds, presence of side effects during the procedure (apnea, desaturation, others) and highest NIPS score within the first 5 minutes after the procedure). The attending staff were trained to assess the NIPS score before we started recruiting patients. Even though patients were swaddled, it was feasible to feel if they moved their legs or arms. The independent variable was the use of inhaled colostrum or inhaled LEO during frenotomy. The dependent variables were HR and satO2 pre and post procedure, presence of crying and duration, hours of life at the time of the frenotomy, and the NIPS score. The controlled variables were gestational age, sex, and birth weight.

Statistical analysis: Quantitative variables (gestational age, birth weight, age at frenotomy, heart rate pre and post-procedure, increase in heart rate post-procedure, oxygen saturation pre and post-procedure, decrease in oxygen saturation post-procedure, and duration of crying) are described using the mean, standard deviation and range; experimental vs control groups were compared with a Student's t-test. We assessed the equality of variances by using Levene's test and applied the result of the Student's t-test accordingly. Sex, the presence of crying, and adverse effects between the two groups are presented in percentages and compared using Fisher's exact test. Statistical significance was set for a $p < 0.05$. To perform statistical analyses we used STATA version 16.1 (StataCorp, College Station, TX, USA).