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Non-nutritive sucking, oral breast milk, and facilitated tucking relieve preterm infant pain during heel-stick procedures: A prospective, randomized controlled trial

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ABSTRACT

Background: Preterm infant pain can be relieved by combining non-nutritive sucking (sucking), oral sucrose, and facilitated tucking (tucking), but the pain-relief effects of oral expressed breast milk (breast milk) are ambiguous.
Aims: We compared the effects of combined sucking + breast milk, sucking + breast milk + tucking, and routine care on preterm infant pain during and after heel-stick procedures.
Design: A prospective, randomized controlled trial.
Settings: Level III neonatal intensive care unit and a neonatal unit at a medical center in Taipei.
Participants/subjects: Preterm infants (N = 109, gestational age 29–37 weeks, stable disease condition) needing procedural heel sticks were recruited by convenience sampling and randomly assigned to three treatment conditions: routine care, sucking + breast milk, and sucking + breast milk + tucking.
Methods: Pain was measured by watching video recordings of infants undergoing heel-stick procedures and scoring pain at 1-min intervals with the Premature Infant Pain Profile. Data were collected over eight phases: baseline (phase 1, 10 min without stimuli before heel stick), during heel stick (phases 2 and 3), and a 10-min recovery (phases 4–8).
Results: For infants receiving sucking + breast milk, pain-score changes from baseline across phases 2–8 were 2.634, 4.303, 2.812, 2.271, 1.465, 0.704, and 1.452 units lower than corresponding pain-score changes of infants receiving routine care (all p-values < 0.05 except for phases 6 and 7). Similarly, for infants receiving sucking + breast milk + tucking, pain-score changes from baseline were 2.652, 3.644, 1.686, 1.770, 1.409, 1.165, and 2.210 units lower than corresponding pain-score changes in infants receiving routine care across phases 2–8 (all p-values < 0.05 except for phase 4). After receiving sucking + breast milk + tucking and sucking + breast milk, infants' risk of mild pain (pain score ≥ 6) significantly decreased 67.0% and 70.1%, respectively, compared to infants receiving routine care. After receiving sucking + breast milk + tucking and sucking + breast milk, infants' risk of moderate-to-severe pain (pain score ≥ 12) decreased 87.4% and 95.7%, respectively, compared to infants receiving routine care.
Conclusion: The combined use of sucking + breast milk + tucking and sucking + breast milk effectively reduced preterm infants' mild pain and moderate-to-severe pain during heel-stick procedures. Adding facilitated tucking helped infants recover from pain across eight phases of heel-stick procedures. Our findings advance knowledge on the effects of combining expressed breast milk, sucking, and tucking on preterm infants' procedural pain.

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What is already known about the topic?

- The analgesic effects of supplemental breast milk on short-term pain during a single painful procedure (heel stick or venipuncture) in preterm infants are ambiguous.
- Facilitated tucking used in combination with oral sucrose has added value in lowering infant pain as measured by the Bernese Pain Scale for Neonates.
- Combined use of sucrose and non-nutritive sucking significantly decreases Premature Infant Pain Profile scores during heel-stick procedures.
- Combining non-nutritive sucking, oral sucrose, and facilitated tucking more effectively reduces the frequency of infants' stress-related or withdrawal behaviours than routine care across heel-stick procedures.

What this paper adds

- Preterm infants' pain and moderate-to-severe pain during heel-stick procedures were effectively reduced by two combinations of three nonpharmacological treatment modalities: 1) non-nutritive sucking, breast milk, and facilitated tucking, and 2) non-nutritive sucking and breast milk.
- Facilitated tucking facilitated preterm infants' recovery from pain and helped stabilize them.
- When facilitated tucking cannot be provided in a unit due to staffing issues, the combination of non-nutritive sucking and breast milk also effectively relieved preterm infants' pain.

1. Introduction

About 15 million infants worldwide are born preterm each year (before 37 weeks' gestation) (March of Dimes, 2017) or about 1 in 10 infants (World Health Organization, 2016). In Taiwan, 18,940 infants were born prematurely (20–37 weeks' gestation) in 2014, and the preterm birth rate has remained at 8.9–9.4% since 2014 (Health Promotion Administration, Ministry of Health and Welfare, 2015). Preterm birth is the leading killer of newborn infants worldwide (World Health Organization, 2016).

The survival of these immature infants depends on receiving specialized care in neonatal intensive care units, where they may experience numerous painful procedures. These immature infants are more vulnerable to procedural pain than older infants because they have a lower pain threshold and immature systems for modulating pain and maintaining homeostasis (Grunau, 2013). Repeated exposure to pain may change preterm infants' brain structure and organization as well as impair brain development through oxygen desaturation, leading to generation of free radicals that can damage fast-growing tissues (Bellieni et al., 2009; Brummelte et al., 2012). These effects can lead to complications such as retinopathy, chronic lung disease, cerebral palsy, and developmental delays that have been associated with preterm birth (Brummelte et al., 2012; Grunau, 2013) and may cause disability later in life. To improve preterm infants' neurodevelopmental outcomes, it is essential to adopt proper interventions to alleviate their pain during neonatal intensive care unit procedures.

Strategies successfully used to relieve short-term pain in preterm infants during procedures include non-nutritive sucking (sucking) (Liaw et al., 2012; Naughton, 2013), oral sucrose (sucrose) (Cignacco et al., 2012; Stevens et al., 2016), facilitated tucking (tucking) (Cignacco et al., 2012; Yin et al., 2015), and the combined use of sucrose and sucking (Thakkar et al., 2016; Yin et al., 2015). Sucking has been hypothesized to produce analgesia by stimulating orotactile and mechanoreceptors in the mouth to modulate nociception transmission or processing by the endogenous nonopioid system (Mohri et al., 2005). Oral sucrose has a sweet taste suggested to alleviate pain by stimulating orogustatory receptors that activate endogenous opioid pathways (Bach, 1997). Tucking involves holding a newborn with warm hands to offer tactile and thermal sensory stimulation to modulate pain during invasive procedures (Axelin et al., 2006, 2009).

Besides sucking and tucking, an agent shown to be as effective as sucrose in relieving preterm infants' pain is breastfeeding or breast milk (Shah et al., 2012). Breast milk, a natural, safe and highly beneficial food for preterm infants, has also been shown to protect very low birthweight infants from necrotizing enterocolitis (Johnson et al., 2015). Furthermore, in preterm infants undergoing heel-stick procedures, mean Premature Infant Pain Profile scores did not differ significantly when receiving breast milk (either breastfed or bottle-fed) or sucrose combined with sucking and Newborn Individualized Developmental Care and Assessment Program support, a combination known to relieve preterm infant pain (Simonse et al., 2012). Similarly, breast milk from a milk bank combined with tucking had the same pain-relieving effect as sucrose combined with tucking in preterm infants undergoing ophthalmoscopy to diagnose retinopathy of prematurity (Ribeiro et al., 2013). Furthermore, preterm infants' pain scores and cortisol levels were significantly lower during heel stick if they were exposed to the odor of breast milk than if they were exposed to the odor of formula milk (Badiee et al., 2013). Similarly, breast milk odor decreased the painful effects of venipuncture significantly more than the odor of vanilla by decreasing preterm infants' heart rate variability and blood oxygen saturation (Neshat et al., 2016).

Breast milk might relieve pain by two mechanisms: (1) its sweet taste from lactose, flavor (Blass, 1997), and odor (Badiee et al., 2013; Neshat et al., 2016), and (2) its high level of tryptophan (Heine, 1999). Tryptophan is a melatonin precursor that may increase secretion of the endogenous opioid, beta endorphin (Barrett et al., 2000).

Of note, expressed breast milk was not generally recommended for pain relief in neonates by a systematic Cochrane review of 20 studies (Shah et al., 2012). In that review, providing supplemental breastmilk during painful procedures (heel stick or venous puncture) resulted in inconsistent pain relief compared to that of infants receiving sucrose. For example, pain scores decreased only when measured by one validated pain scale (the Neonatal Facial Coding System), but not by two others (the Neonatal Infant Pain Scale and the Douleur Aigue Nouveaune), and most of the reviewed studies examined the pain-relieving effect of breastfeeding and supplemental breast milk in healthy full-term or stable late-term infants. Preterm infants' pain responses to breast milk were assessed in only one of 20 studies reviewed (Shah et al., 2012). In two other studies, a single dose of breast milk before heel stick was not as effective as oral sucrose in relieving pain among late preterm infants (gestation age = 34–36 weeks) (Bueno et al., 2012) and among full-term neonates (Ozdogan et al., 2010). On the other hand, breast milk (5 ml) given before venipuncture effectively reduced pain scores and pain indicators more than water in full-term neonates (Upadhyay et al., 2004).

Combining breast milk with other effective pain-relief strategies such as sucking and tucking may generate sufficient analgesic effects during intrusive procedures. However, little is known about the pain-relief effects of combining breast milk and sucking or tucking in preterm infants during heel-stick procedures. Furthermore, most Taiwanese neonatal intensive care units are still not supplied with 24% sucrose products (personal communication), despite 24% sucrose solution having been suggested as an effective pain-management strategy for preterm infants before heel stick (Bueno et al., 2013; Stevens et al., 2016; Witt et al., 2016). Based on our informal phone survey of 10 neonatal intensive care units in northwest Taiwan, neonatal clinicians in Taiwan do not use oral sucrose to relieve preterm infants' short-term pain during intrusive procedures.

Therefore, the purposes of this study were to examine the effects of combining sucking + breast milk or sucking + breast milk + tucking on preterm infant pain before, during, and after heel-stick procedures. Based on the above literature review and mechanisms, we hypothesized that: a) preterm infants receiving sucking + breast milk + tucking or

sucking + breast milk and after heel-stick during procedures would have lower pain scores and odds ratios for mild pain and moderate-to-severe pain than those receiving routine care, and b) the analgesic effects of sucking + breast milk + tucking would be greater than the analgesic effects of sucking + breast milk. Thus, we asked the research questions: "What are the differences in pain scores among preterm infants receiving sucking + breast milk + tucking, sucking + breast milk, and routine care before, during, and after heel-stick procedures? What are the odds ratios for mild pain and moderate-to-severe pain among preterm infants receiving sucking + oral breast milk + tucking, sucking + oral breast milk, and routine care before, during, and after heel-stick procedures?"

2. Methods

2.1. Design

A prospective randomized controlled trial with a repeated-measures design was used to compare the effects of combined use of sucking + breast milk, sucking + breast milk + tucking, and routine care on pain scores and pain risk before, during and after heel-stick procedures. The study outcomes were infants' pain scores as well as odds of mild pain and moderate-to-severe pain during heel sticks. Preterm infants needing heel sticks were randomly assigned by a blinded statistician using Clinstat block randomization (Altman and Bland, 1999) to three treatment conditions: (1) routine care, (2) sucking + breast milk, (3) sucking + breast milk + tucking. This report follows CONSORT guidelines (Schulz et al., 2010).

2.2. Sample and setting

A convenience sample of infants meeting the inclusion criteria was recruited from a level III neonatal intensive care unit and a neonatal special care unit in northern Taiwan from 2013 to 2014. Inclusion criteria were: (1) gestational age 27–37 weeks and postmenstrual age 27.4–38 weeks, (2) postbirth age 3–28 days, and (3) disease condition acceptable for observation (illness severity indicated by the Neonatal Therapeutic Intervention Scoring System score ≤20). Infants were excluded if they had a condition that might influence their physiological and responses to pain, e.g., congenital anomalies, surgery, or severe illnesses requiring treatment with sedatives, muscle relaxants, antiepileptics, or analgesic drugs.

After screening 179 infants, 153 met our criteria (Fig. 1). Of these infants, 34 did not receive heel sticks, and 10 were not included because their parents refused participation. The remaining 109 were randomly assigned to three conditions: routine care, sucking + breast milk, and sucking + breast milk + tucking. Participating infants did not differ significantly from those whose parents refused participation in terms of sex, gestational age, postmenstrual age, age (days since birth), birth weight, and prior painful experiences. Study power was estimated using G*Power version 3.1.9.2 (Faul et al., 2007) and calculated *a priori* based on repeated measures within factors. The results showed that the effect size on outcome variables was 0.63. Based on this effect size and a significance level of 0.05, the study power (two tailed) with 109 infants was 0.99.

2.3. Measures

2.3.1. Pain

Preterm infants' pain across heel-stick procedures was assessed using the Premature Infant Pain Profile (Stevens et al., 1996), which has been shown to be a reliable and valid instrument for pain assessment in preterm and full-term infants (28–42 weeks gestational age) (Balantyne et al., 1999; Stevens et al., 1996; Vederhus et al., 2006). The Premature Infant Pain Profile assesses pain by seven indicators: three behavioral (facial expressions: brow bulge, eye squeeze, nasolabial furrow), two physiologic (heart rate and oxygen saturation) and two contextual (gestational age and sleep/wake state) (Balantyne et al., 1999; Stevens et al., 1996; Vederhus et al., 2006). Each indicator is scored for pain on a 4-point scale (0–3); the total score can range from 0 to 21, depending on infant gestational age and sleep/wake state. Premature Infant Pain Profile scores ≥ 6 are suggested to indicate at least mild pain, and scores ≥ 12 are suggested to indicate moderate-to-severe pain (Balantyne et al., 1999). Pain was scored by a well-trained research assistant from videotapes of infants' faces 10 min before, 2 min during, and 10 min after heel-stick procedures (see Section 2.4.1). Time-triggered coding was used to score Premature Infant Pain Profile pain indicators in every minute of each videotaped heel stick. Pain indicators were scored in this order: sleep/wake state, brow bulge, eye squeeze, nasolabial furrow, gestational age, heart rate, and oxygen saturation. The inter-rater reliability of the Premature Infant Pain Profile ranged from 0.85-0.92 (0.89 for baseline [heel-stick phase 1], 0.86, 0.86, 0.86, 0.90, 0.91, 0.89 and 0.92 for heel-stick phases 2–8, respectively).

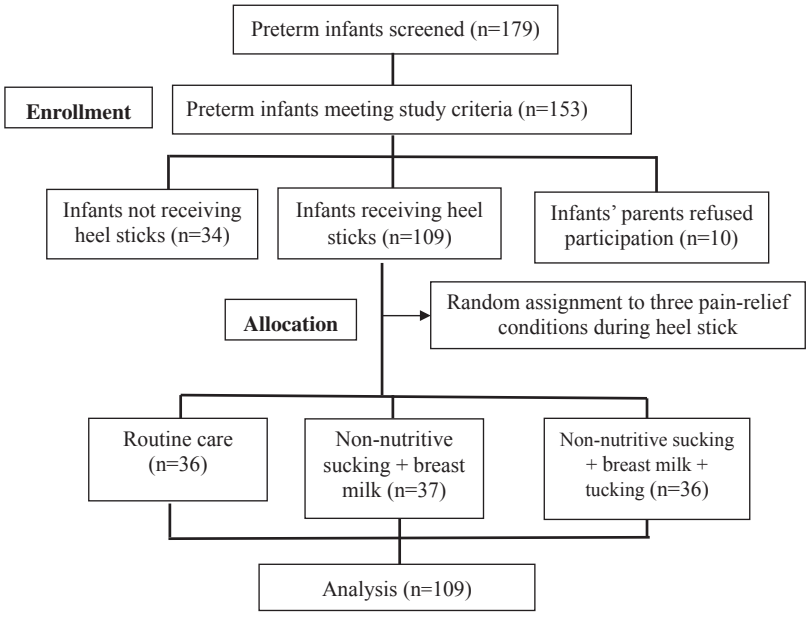

Fig. 1. Flowchart of participant recruitment.

2.3.2. Physiological parameters

Heart rate and oxygen saturation were measured using Philips electrocardiographic bedside monitors and continuously recorded by custom computer software. The electrocardiographic monitors were regularly calibrated by certified technicians, and computer function was checked before data collection. Heart rate leads were attached to each infant's front chest, and the oxygen saturation probe was attached to each infant's big toe. The two physiological parameters were digitally sampled at 1-min intervals by computer. Each infant's heart rate and oxygen saturation readings were recorded during the 10-min baseline phase before heel stick; the average baseline heart rate and oxygen saturation values were compared with the corresponding values for each phase. Changes in heart rate and oxygen saturation were calculated and scored based on Premature Infant Pain Profile item descriptions at each minute across the heel-stick procedure. For example, heart rates that increased 0–4, 5–14, 15–24 and > 25 beats were scored 0–3, respectively. Oxygen saturations that decreased 0–2.4%, 2.5–4.9%, 5.0–7.4%, and > 7.5% were scored 0–3, respectively.

2.3.3. Illness severity

Infants' illness severity was measured by a senior nurse using the Neonatal Therapeutic Intervention Scoring System, which examines the therapeutic intensity and complexity of neonatal intensive care unit therapies (Gray et al., 1992). This 63-item instrument measures illness severity in eight subcategories based on the care (treatments) received by infants. Items (treatments) are rated yes/no; total scores range from 0 to 47. The Neonatal Therapeutic Intervention Scoring System internal consistency was 0.84 (α coefficient), and its validity was estimated by correlation between its scores and mortality risk (Gray et al., 1992). In this study, the instrument interrater reliability between the principal investigator and senior nurse was 0.95.

2.4. Procedures

After this study was approved by the study site's institutional review board, parents of infants who met the study criteria received a pamphlet introducing the study and a reply sheet indicating parents' willingness for their infant to participate. Interested parents returned the response sheet to the first author, who explained the study in more detail and obtained parents' signed consent.

2.4.1. Heel sticks

Infants' blood was collected during heel sticks by a senior nurse with more than 10 years of clinical experience in the neonatal intensive care unit. She had been trained in the neonatal intensive care unit to follow its standard procedures for collecting blood from preterm infants. Time for heel-stick procedures was controlled at 2.00 min for infants in all three conditions. All heel sticks occurred over eight phases: phase 1 (10 min without stimuli [baseline]), phases 2 and 3 (the 2nd and 3rd minutes during heel-stick procedures), and phases 4–8 (recovery, starting when the senior nurse finished collecting blood and left the infant, through the 1st to 4th minutes after heel stick [phases 4–7] and the 10th minute [phase 8]) (Fig. 2). These phases were based on our previous study, in which preterm infants in the neonatal intensive care unit took almost 10 min to completely recover from heel-stick pain (Liaw et al., 2012). During all eight phases, infants' reactions were video recorded for data collection (see Section 2.4.3), with the camera lens focused on each infant's face.

2.4.2. Treatment conditions

One hour before heel stick, regardless of treatment condition, infants were placed in a supine position with support (rolled towels). Before, during, and after heel stick, infants in the control condition (routine care) received only gentle touch and verbal comfort if they were crying. For ethical reasons, infants in the routine care condition received two validated forms of analgesia: position support (Kostandy and Ludington-Hoe, 2017; Liaw et al., 2012) and gentle touch (Bahman Bijari et al., 2012; Herrington and Chiodo, 2014).

Intervention fidelity was established by the same intervener (the second author) being trained by the principal investigator (the last author) to consistently provide tucking, sucking, and breast milk. For the sucking condition, infants received a standard silicone newborn pacifier 2 min before their foot was touched to start heel-stick procedures. For the breast milk condition, infants were orally fed 0.5–2.0 ml of expressed breast milk through a syringe 2 min before heel stick. The volume of breast milk fed to the infant was based on gestational age (gestational age 27–28 weeks: 0.5 ml; gestational age 28.1–30 weeks: 1.0 ml; gestational age 30.1–32 weeks: 1.5 ml; gestational age 32.1–37 weeks: 2 ml). For the tucking condition, infants were gently held in warm hands before the heel stick, keeping the infant in a flexed, midline position with his/her four limbs close to his/her own body and minimum restraint of the infant's head and body (Axelin et al., 2006; Valizadeh et al., 2016). To maintain consistency in the three treatment conditions, the principal investigator regularly met with the intervener and checked procedures twice a month.

2.4.3. Data collection

Data on infant pain were collected by a research assistant from video recordings of infants' faces during the 8-phase heel-stick procedures and scored at 1-min intervals using the Premature Infant Pain Profile (see Section 2.3.1). Infants' background data, including number of previous painful procedures (invasive, tissue-damaging cutaneous procedures, i.e., heel stick, intravenous or arterial line insertion, chest tube insertion, lumbar puncture, endotracheal intubation and intramuscular injection) were collected from medical and nursing charts by the first author, who knew the research purpose and plans. This author also recruited infant participants.

2.4.4. Study fidelity

Study fidelity was established by weekly meetings between the investigators, research assistant, and senior nurse to review procedures and discuss any problems encountered. The first author worked regularly with the principal investigator to confirm that data were consistently collected from medical and nursing charts. As an additional fidelity check, videotapes were coded in random order. The research assistant, who was blinded to the study purpose and infants' clinical information, was trained by the principal investigator to score pain. All videotapes were scored in a quiet room to maintain consistency and accuracy.

2.5. Data analysis

The data were analyzed using IBM SPSS Statistics, version 21.0. Infants' background data were compared by chi-square and one-way analysis of variance (ANOVA). Data were described using means and standard deviations (SD) for continuous variables and frequencies for categorical data. To account for within-subject dependency due to

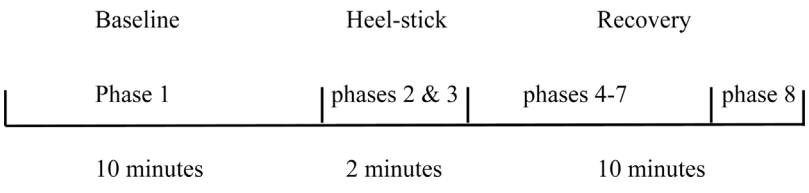

Fig. 2. Heel-stick phases.

repeated measurements, we used the generalized estimating equation (GEE) (Liang and Zeger, 1986) method's generalized linear models. To compare differences in pain at different heel-stick phases among infants in the three treatment conditions, the GEE method's generalized linear models included three conditions, eight phases, and their interaction terms after adjusting for the effects of baseline pain scores and post-menstrual age. To compare the risk of pain (in terms of odds ratio) among the three conditions at different phases, we defined two event variables: 1) mild pain was defined as Premature Infant Pain Profile scores ≥ 6 (event = 1 if Premature Infant Pain Profile > 6; event = 0 if Premature Infant Pain Profile < 6), moderate-to-severe pain was defined as Premature Infant Pain Profile scores ≥ 12 (event = 1 if Premature Infant Pain Profile > 12; event = 0 if Premature Infant Pain Profile < 12). The odds for infants in each condition to experience at least mild pain (Premature Infant Pain Profile score ≥ 6) and moderate-to-severe pain (Premature Infant Pain Profile score ≥ 12) were analyzed separately by the GEE method's multiple logistic regression models. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Infant characteristics

The sample included 109 preterm infants with a mean gestational age of 31.21 ± 2.87 weeks. The majority was male (50.9%) and born by caesarean delivery (76.5%). The infants' mean age was 13.10 ± 11.69 days, mean birth weight was 1563.17 ± 483.92 g, and they had 62.65 ± 65.04 prior painful experiences. At baseline, infants in the three treatment conditions did not differ significantly in gestational age, postmenstrual age, birth weight, age, Neonatal Therapeutic Intervention Scoring System score, Apgar score, and number of prior painful experiences (Table 1).

3.1.1. Pain

In Fig. 3, for simple comparisons, we used clustered error-bar plots to visualize time trends in Premature Infant Pain Profile scores for our three treatment conditions at each phase. Infants receiving sucking + breast milk + tucking or sucking + breast milk during heel-stick procedures had significantly lower mean (SD) pain scores than those receiving routine care after phase 2.

Phase $_1$, baseline (no stimulation); phase$_2$, the 1st minute of heel stick; phase$_3$, the 2nd minute of heel stick; phase$_4$, the 1st minute after heel stick; phase$_5$, the 2nd minute after heel stick; phase$_6$, the 3rd minute after heel stick; phase$_7$, the 4th minute after heel stick; phase$_8$, the 10th minute after heel stick.

For more detailed comparisons of pain differences among infants in the three treatment conditions at different heel-stick phases, after adjusting for the effects of baseline Premature Infant Pain Profile and postmenstrual age, we used the Premature Infant Pain Profile score as the dependent variable and included all three conditions, eight phases, and their interaction terms in the GEE method's multiple linear regression model. As shown in Table 2, the three conditions did not differ significantly at baseline (condition 2 *vs.* condition 1, $p = 0.830$, and condition 3 *vs.* condition 1, $p = 0.957$) and for infants receiving routine care (condition 1), Premature Infant Pain Profile scores at phases 2–5 were on average 5.626, 6.911, 3.483, and 2.626 units significantly higher than those at baseline, respectively, with all p-values < 0.001. For infants receiving sucking + breast milk during heel-stick procedures (condition 2), the changes in Premature Infant Pain Profile scores at phases 2–8 were on average 2.634, 4.303, 2.812, 2.271, 1.465, 0.704, and 1.452 units significantly lower than the changes in Premature Infant Pain Profile scores at the corresponding phases for infants receiving routine care, respectively (all p-values < 0.05 except for phases 6 and 7), after adjusting for the effects of baseline pain scores and infants' postmenstrual age (Table 2).

Similarly, for infants receiving sucking + breast milk + tucking (condition 3), the changes in Premature Infant Pain Profile scores were on average, 2.652, 3.644, 1.686, 1.770, 1.409, 1.165, and 2.210 units significantly lower than those changes for infants in the control condition across phases 2–8, respectively, with all p-values < 0.05 (except phase 4), after adjusting for the effects of baseline pain scores and infants' postmenstrual age (Table 2).

To compare the odds of infants having at least mild pain among three conditions at different phases, we used the GEE method's multiple

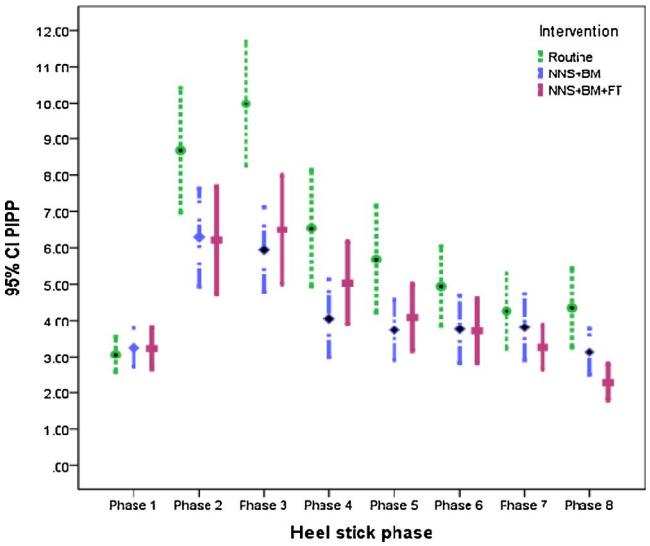

Fig. 3. Premature Infant Pain Profile (PIPP) scores of preterm infants in the three treatment conditions (routine care, non-nutritive sucking [NNS] + oral expressed breast milk [BM], and NNS + BM + facilitated tucking [FT]) over eight heel-stick phases.

Table 1
Characteristics of infants in the control, non-nutritive sucking + breast milk, and non-nutritive sucking + breast milk + facilitated tucking conditions ($N = 109$).

Characteristic	Control, $n = 36$ Mean ± SD	NNS + BM, $n = 37$ Mean ± SD	NNS + BM + FT, $n = 36$ Mean ± SD	F	p[a]
Gestational age (weeks)	31.16 ± 2.99	31.28 ± 2.84	31.31 ± 2.87	0.025	0.975
Postmenstrual age (weeks)	32.83 ± 2.32	33.18 ± 2.34	33.04 ± 2.38	0.210	0.811
Birth weight (g)	1556.78 ± 495.66	1572.95 ± 474.86	1559.5 ± 498.37	0.012	0.988
Body weight during heel-stick procedures (g)	1568.86 ± 462.67	1564.49 ± 437.71	1523.12 ± 451.48	0.110	0.896
Age (days)	12.8 ± 12.83	13.82 ± 11.99	12.68 ± 10.14	0.175	0.839
Time from previous feeding (minutes)	156.5 ± 106.89	173.18 ± 36.02	167.06 ± 35.66	0.571	0.567
Number of prior painful procedures	65.39 ± 71.93	60.67 ± 62.96	60.56 ± 60.35	0.064	0.938
NTISS score	10.89 ± 2.1	10.31 ± 2.94	10.32 ± 2.92	0.552	0.578
Apgar score: 1 min	5.19 ± 1.69	5.36 ± 1.72	5.24 ± 1.72	0.094	0.91
Apgar score: 5 min	7.28 ± 1.45	7.38 ± 1.43	7.32 ± 1.41	0.053	0.948

Note: SD, standard deviation; NNS, non-nutritive sucking; BM, oral breast milk; FT, facilitated tucking; NTISS, neonatal therapeutic intervention scoring system.
[a] One-way ANOVA.

Table 2
Changes in Premature Infant Pain Profile pain scores for the control, non-nutritive sucking + breast milk, and non-nutritive sucking + breast milk + facilitated tucking conditions predicted by the generalized estimating equation method's multiple linear regression (N = 109).

Variable	*B*	*SE*	Wald Chi-square	*p*	95% Confidence Interval	
					Lower	Upper
Condition effects						
$Condition_3$ vs. $Condition_1$	0.010	0.196	0.003	0.957	−0.394	0.373
$Condition_2$ vs. $Condition_1$	0.047	0.218	0.046	0.830	−0.381	0.475
Phase effects						
$Phase_8$ vs. $Phase_1$	1.283	0.508	6.365	0.012	0.286	2.279
$Phase_7$ vs. $Phase_1$	1.197	0.484	6.127	0.013	0.249	2.145
$Phase_6$ vs. $Phase_1$	1.883	0.551	11.692	0.001	0.804	2.962
$Phase_5$ vs. $Phase_1$	2.626	0.743	12.504	< 0.001	1.170	4.081
$Phase_4$ vs. $Phase_1$	3.483	0.790	19.456	< 0.001	1.935	5.033
$Phase_3$ vs. $Phase_1$	6.911	0.795	75.520	< 0.001	5.353	8.470
$Phase_2$ vs. $Phase_1$	5.626	0.758	55.023	< 0.001	4.139	7.112
Interaction effects						
$Condition_3$* $phase_8$	−2.21	0.565	15.433	< 0.001	−3.329	−1.113
$Condition_3$ * $phase_7$	−1.165	0.578	4.049	0.044	−2.299	−0.030
$Condition_3$ * $phase_6$	−1.409	0.708	3.964	0.046	−2.797	−0.022
$Condition_3$* $phase_5$	−1.770	0.869	4.146	0.042	−3.473	−0.066
$Condition_3$ * $phase_4$	−1.686	0.939	3.223	0.073	−3.526	0.155
$Condition_3$ * $phase_3$	−3.644	1.029	12.553	< 0.001	−5.660	−1.628
$Condition_3$ * $phase_2$	−2.652	0.986	7.230	0.007	−4.585	−0.719
$Condition_2$ * $phase_8$	−1.452	0.669	4.720	0.030	−2.763	−0.142
$Condition_2$ * $phase_7$	−0.704	0.747	0.888	0.346	−2.167	0.760
$Condition_2$ * $phase_6$	−1.465	0.800	3.351	0.067	−3.033	0.104
$Condition_2$ * $phase_5$	−2.271	0.907	6.266	0.012	−4.050	−0.493
$Condition_2$ * $phase_4$	−2.812	1.018	7.636	0.006	−4.806	−0.817
$Condition_2$ * $phase_3$	−4.303	0.995	18.710	< 0.001	−6.252	−2.353
$Condition_2$ * $phase_2$	−2.634	0.957	7.577	0.006	−4.510	−0.758
Infant characteristics						
Postmenstrual age	0.222	0.705	9.934	0.002	0.084	0.360
Baseline PIPP	0.710	0.112	40.061	< 0.001	0.490	0.930

Note: Control variables: $Condition_1$, routine care; $Condition_2$, non-nutritive sucking (NNS) + oral expressed breast milk (BM); $Condition_3$, NNS + BM + facilitated tucking (FT); $Phase_1$, baseline (no stimulation); $phase_2$, the 1st minute during heel stick; $phase_3$, the 2nd minute during heel stick; $phase_4$, the 1st minute after heel stick; $phase_5$, the 2nd minute after heel stick; $phase_6$, the 3rd minute after heel stick; $phase_7$, the 4th minute after heel stick; $phase_8$, the 10th minute after heel stick; T_1, $Condition_3$ * $phase_1$; P_1, $Condition_2$ * $phase_1$; SE, standard error.

Table 3
Odds ratios for preterm infants' mild pain (Premature Infant Pain Profile score ≥ 6) *vs*. no pain by the generalized estimating equation method's multiple logistic regression (N = 109).

Variable	*B*	*SE*	Wald chi-square	*p*	Odds ratio	95% Confidence Interval	
						Lower	Upper
Condition effects							
$Condition_3$ vs. $Condition_1$	−1.107	0.335	10.956	0.001	0.330	0.171	0.637
$Condition_2$ vs. $Condition_1$	−1.209	0.324	13.964	< 0.001	0.299	0.158	0.563
Phase effects							
$Phase_8$ vs. $Phase_1$	0.939	0.542	3.002	0.083	2.557	0.884	7.396
$Phase_7$ vs. $Phase_1$	1.114	0.539	4.275	0.039	3.047	1.060	8.759
$Phase_6$ vs. $Phase_1$	1.116	0.543	4.232	0.040	3.052	1.054	8.838
$Phase_5$ vs. $Phase_1$	1.486	0.532	7.805	0.005	4.418	1.558	12.528
$Phase_4$ vs. $Phase_1$	2.114	0.475	19.805	< 0.001	8.278	3.263	20.997
$Phase_3$ vs. $Phase_1$	3.324	0.471	49.835	< 0.001	27.783	11.039	69.926
$Phase_2$ vs. $Phase_1$	3.182	0.441	52.102	< 0.001	24.088	10.153	57.147
Postmenstrual age	0.238	0.065	13.412	< 0.001	1.269	1.117	1.441
Baseline PIPP score	0.381	0.082	20.894	< 0.001	1.463	1.243	1.722

Note: Control variables: $Condition_1$, routine care; $Condition_2$, non-nutritive sucking (NNS) + oral expressed breast milk (BM); $Condition_3$, NNS + BM + facilitated tucking (FT); $Phase_1$, baseline (no stimulation); $phase_2$, the 1st minute of heel stick; $phase_3$, the 2nd minute of heel stick; $phase_4$, the 1st minute after heel stick; $phase_5$, the 2nd minute after heel stick; $phase_6$, the 3rd minute after heel stick; $phase_7$, the 4th minute after heel stick; $phase_8$, the 10th minute after heel stick.
PIPP, Premature Infant Pain Profile; SE, standard error.

logistic regression with three conditions, eight phases, and their interaction terms. All the coefficients of both interaction terms (condition 2 × phases, and condition 3 × phases) were negative (range: −2.212 to −0.414, data not shown), indicating that the relative risks (odds ratios) of at least mild pain in both conditions were lower than that in the routine care condition (condition 1) for phases 2–8 (*vs*. phase 1). However, since the interaction effects were non-significant (*p*-value range: 0.056- 0.742, data not shown), we present the results of the logistic regression model with main effect terms in Table 3. After adjusting for the effects of baseline pain scores, infants' postmenstrual age, and phase, the odds ratios in favor of at least mild pain for infants receiving sucking + breast milk + tucking and sucking + breast milk *vs*. infants receiving routine care were 0.330 (p = 0.001) and 0.299 (p < 0.001), respectively. In other words, for infants who received sucking + breast milk + tucking and sucking + breast milk during heel-stick procedures, the odds of at least mild pain were 64.0% and 70.1% less than that for infants who received routine care, respectively. The odds of at least mild pain in preterm infants were significantly

Table 4
Odds ratios for moderate-to-severe pain (Premature Infant Pain Profile score ≥ 12) vs. no pain by the generalized estimating equation method's multiple logistic regression (N = 109).

Variable	*B*	*SE*	Wald Chi-square	*p*	Odds ratio	95% Confidence Interval	
						Lower	Upper
Condition effects							
$Condition_3$ vs. $Condition_1$	−2.073	0.627	10.922	0.001	0.126	0.037	0.430
$Condition_2$ vs. $Condition_1$	−3.136	0.676	21.548	< 0.001	0.043	0.012	0.163
Phase effects							
$Phase_8$ vs. $Phase_2$	−2.851	0.628	20.631	< 0.001	0.058	0.017	0.198
$Phase_7$ vs. $Phase_2$	−2.825	0.698	16.395	< 0.001	0.059	0.015	0.233
$Phase_6$ vs. $Phase_2$	−1.755	0.574	9.336	0.002	0.173	0.056	0.533
$Phase_5$ vs. $Phase_2$	−1.219	0.507	5.778	0.016	0.295	0.109	0.798
$Phase_4$ vs. $Phase_2$	−1.095	0.445	6.056	0.014	0.335	0.140	0.800
$Phase_3$ vs. $Phase_2$	0.591	0.278	4.525	0.033	1.806	1.048	3.112
Postmenstrual age	0.356	0.121	8.712	0.003	1.428	1.127	1.810
Baseline PIPP score	0.593	0.126	22.093	< 0.001	1.810	1.413	2.318

Note: Control variables: $Condition_1$, routine care; $Condition_2$, non-nutritive sucking (NNS) + oral expressed breast milk (BM); $Condition_3$, NNS + BM + facilitated tucking; $Phase_1$, baseline (no stimulation); $phase_2$, the 1st minute of heel stick; $phase_3$, the 2nd minute of heel stick; $phase_4$, the 1st minute after heel stick; $phase_5$, the 2nd minute after heel stick; $phase_6$, the 3rd minute after heel stick; $phase_7$, the 4th minute after heel stick; $phase_8$, the 10th minute after heel stick.
PIPP, Premature Infant Pain Profile; SE, standard error.

related to their postmenstrual age and baseline pain score (both p-values < 0.001), after adjusting for the effects of conditions and phases.

Similarly, the odds ratios in favor of moderate-to-severe pain for infants receiving sucking + breast milk + tucking and sucking + breast milk *vs.* infants receiving routine care were 0.126 (p = 0.001) and 0.043 (p < 0.001), respectively, after adjusting for the effects of phase, baseline pain scores, and infants' postmenstrual age (Table 4). In other words, for infants receiving sucking + breast milk + tucking and sucking + breast milk during heel-stick procedures, the odds of moderate-to-severe pain were 87.4% and 95.7% less than that for infants who received routine care, respectively. The odds of preterm infants' moderate-to-severe pain were significantly related to their postmenstrual age and baseline pain score (p-value = 0.003 and < 0.001), after adjusting for the effects of condition and phase.

4. Discussion

The current study advances knowledge on the effects of combined use of oral expressed breast milk, non-nutritive sucking, and facilitated tucking on preterm infants' pain (measured by Premature Infant Pain Profile pain scores and odds ratios for pain) across different phases of heel-stick procedures. Our findings suggest that using sucking + breast milk + tucking and sucking + breast milk more effectively reduced preterm infants' pain scores and their odds ratios for mild pain and moderate-to-severe pain than using routine care, thus supporting not only our study hypotheses, but also the combined use of syringe-fed breast milk with sucking and tucking 2 min before heel stick to relieve infant pain.

Our findings suggest that the combined use of syringe-fed breast milk and sucking with/without tucking has analgesic effects on preterm infants undergoing heel stick. We could not find any study that compared the analgesic effects of breast milk and sucking with/without tucking in preterm infants to the analgesic effects of routine care, but we note that breast milk has been shown to relieve preterm infants' procedural pain as effectively as sucrose in combination with other non-pharmacological interventions. For example, breast milk from a milk bank and tucking had the same effect as sucrose and tucking in relieving premature infants' pain associated with ophthalmoscopy to diagnose retinopathy of prematurity (Ribeiro et al., 2013). Similarly, breast milk (either breastfed or bottle-fed) had the same pain-relieving effect on preterm infants' pain during heel stick as the combined use of sucrose, sucking, and Newborn Individualized Developmental Care and Assessment Program support (Simonse et al., 2012). Furthermore, breast milk (5 ml) given to full-term neonates before venipuncture reduced pain and pain indicators more than distilled water (Upadhyay et al., 2004). The authors of the two studies on premature infants recommend using breast milk as a noninvasive, safe method for these infants' procedural pain in neonatal intensive care units (Ribeiro et al., 2013; Simonse et al., 2012).

The difference between our results and those of others showing that breast milk relieves pain in preterm infants (Ribeiro et al., 2013; Simonse et al., 2012), offers poor pain relief in late preterm infants (Bueno et al., 2012), and has inconsistent pain-relieving effects in healthy term infants (Ozdogan et al., 2010; Shah et al., 2012) cannot be explained by neonates' gestation age. Indeed, breast milk (5 ml) given to full-term neonates before venipuncture effectively reduced pain scores and pain indicators more than water (Upadhyay et al., 2004). Other factors that might have explained the difference between our results and those of other studies include the interventions, comparison groups, pain measurements, and types of procedural pain. For example, our interventions combined expressed breast milk + sucking with/without tucking, whereas one study on preterm infants combined breast milk from a milk bank with tucking (Ribeiro et al., 2013) and other studies on preterm infants did not combine breast milk with other non-pharmacological analgesic agents (Bueno et al., 2012; Simonse et al., 2012). In the single study that included 66 preterm infants (Skogsdal et al., 1997) in the 20-study review by Shah et al., 2012, breast milk alone had no analgesic effect during heel stick. Similarly, the analgesic effect of our breast milk interventions was compared with that of routine care, whereas other studies compared breast milk + tucking with sucrose + tucking (Ribeiro et al., 2013), breast milk (breastfed or bottle-fed) with the combined use of sucrose, sucking, and Newborn Individualized Developmental Care and Assessment Program support (Simonse et al., 2012), and breast milk with 25% sucrose (Bueno et al., 2012).

Pain measurements varied, including Premature Infant Pain Profile scores (the present study; Bueno et al., 2012; Simonse et al., 2012), crying time (Bueno et al., 2012; Ribeiro et al., 2013; Skogsdal et al., 1997), heart rate (Ribeiro et al., 2013; Skogsdal et al., 1997) and salivary cortisol (Ribeiro et al., 2013). Types of procedural pain also varied, including heel-stick (the present study; Bueno et al., 2012; Simonse et al., 2012; Skogsdal et al., 1997) and ophthalmoscopy (Ribeiro et al., 2013). All these different factors could have explained the different results, suggesting the need for further studies on preterm infants to clarify the analgesic effects of breast milk during painful procedures

Based on these results, we recommend using breast milk as an alternative to/in combination with other non-pharmacologic analgesic methods shown to effectively relieve pain in preterm infants (tucking, gentle touch, or non-nutritive sucking). Our findings suggest that both

sucking + breast milk + tucking and sucking + breast milk significantly reduced the odds of preterm infants' mild pain and moderate-to-severe pain. Clinicians can provide sucking + breast milk + tucking and sucking + breast milk to reduce preterm infants' short-term mild pain or moderate-to-severe procedural pain.

When comparing the pain-relief effects of sucking + breast milk (condition 2) and sucking + breast milk + tucking (condition 3) with condition 2 as reference, we found no significant interaction effects at different heel-stick phases (data not shown). Although we found that the mean pain score of infants receiving sucking + breast milk + tucking during heel stick was 0.20 points lower than the pain score of infants receiving sucking + breast milk, these results do not mean that sucking + breast milk + tucking more effectively relieved pain than sucking + breast milk. This finding only indicates that adding tucking generated a non-statistical increment in pain relief, not completely consistent with previous reports that facilitated tucking stabilizes infants and reduces their pain/stress behaviors (Cignacco et al., 2012; Liaw et al., 2012; Yin et al., 2015). On the other hand, our preterm infants who received sucking + breast milk + tucking during heel-stick procedures recovered sooner than infants receiving sucking + breast milk or routine care (Fig. 3), confirming that tucking helps stabilize preterm infants during stress/painful situations (Cignacco et al., 2012; Liaw et al., 2012; Yin et al., 2015). In other words, tucking might not strongly relieve preterm infants' pain due to heel stick, but it comforted and stabilized them, thus decreasing the time of pain perception.

Because we found considerable variation in preterm infants' postmenstrual age, number of previous painful experiences, and baseline Premature Infant Pain Profile scores, these factors were adjusted for in our GEE method's multiple linear and logistic regression models to account for within-subject dependency due to repeated measurements. We found that the pain of infants in the control condition increased during phases 2–3 and decreased during recovery (phases 4–8). Furthermore, preterm infants' pain was associated with their postmenstrual age and baseline Premature Infant Pain Profile pain scores, consistent with a previous report (Liaw et al., 2012). These factors (heel-stick phase-related effects, postmenstrual age, baseline pain scores) should be considered when providing supportive interventions to ensure their appropriate timing, dosage and duration. During heel-stick phases 2 and 3, when pain increases for infants, clinicians could relieve pain by providing sucking and tucking as well as increase the frequency of gentle touch. During heel-stick phases 4–6, clinicians could still provide sucking and tucking and reduce the frequency of gentle touch. For infants who are irritable before heel stick, clinicians may need to first comfort the infant with gentle touch before providing breast milk, sucking and tucking. For infants who are stable and calm, clinicians may need to provide sucking and breast milk only. For infants with a greater postmenstrual age, clinicians may need to provide more supportive interventions (sucking + breast milk + tucking + gentle touch). Clinicians can adjust the use of supportive interventions to each infant's characteristics.

The most beneficial outcome of this study is that oral expressed breast milk, when combined with sucking and tucking, effectively relieved preterm infants' pain during heel-stick procedures. This result suggests that feeding breast milk by syringe could overcome problems for preterm infants who cannot repeatedly receive oral sucrose for pain relief and cannot be held by their mothers for breastfeeding due to severe illness. Although oral sucrose or oral sucrose with sucking has been shown to relieve preterm infants' procedural pain (Cignacco et al., 2012; Thakkar et al., 2016) and crying/fussy state (Liaw et al., 2013), sucrose products are not available in most Taiwanese neonatal intensive care units (personal communication). Despite oral sucrose being encouraged in pain-management guidelines to alleviate preterm infants' pain (Bueno et al., 2013; Stevens et al., 2016; Witt et al., 2016), Taiwanese clinicians have difficulty following these guidelines due to lack of sucrose products. Our study findings provide an alternative to sucrose and multiple supportive interventions to relieve preterm infants' pain.

4.1. Clinical implications

Our findings confirm the effectiveness of providing sucking + breast milk + tucking and sucking + breast milk to preterm infants during painful procedures. For neonatal intensive care units where sucrose is not available or not preferred by clinicians, breast milk and sucking, with or without tucking, can be provided to relieve preterm infants' short-term pain. If tucking cannot be provided in a unit due to staffing issues, sucking + breast milk also effectively relieves pain. Clinicians in neonatal intensive care units and neonatal units should be educated about the analgesic mechanisms and effects of breast milk and how to combine it with sucking and tucking for preterm infants' pain relief. Our results suggest that sucking, breast milk, and tucking could be combined in two different ways during standard care to help alleviate pain in preterm infants during painful procedures.

4.2. Strengths and limitations

This study has several strengths. It was a randomized controlled trial. Study fidelity was maintained by several methods: (1) different persons provided the interventions and assessed Premature Infant Pain Profile pain scores to minimize measurement bias, (2) one senior nurse performed heel stick based on standard protocols and the same person (the second author) consistently offered the pacifier, oral expressed breast milk, and facilitated tucking to infants in the intervention conditions, (3) physiological data were measured every 30 s from Philips electrocardiographic bedside monitors and continuously recorded by custom computer software, and (4) heel sticks were controlled to last within 2 min, and infants' pain was scored every minute during heel-stick procedures.

Nonetheless, the study had some limitations. Although the sample size was sufficient, these infants were stable and from only one medical center in Taiwan. Future studies should recruit larger samples and increase the number of study settings. Another limitation is that we did not include a sucrose treatment. Future studies might consider including another condition to compare breast milk + sucking with sucrose + sucking. Although outcome assessors were different from interveners, it was not possible for them to be completely blind to all research processes. The control condition for this study was routine care, which might have led to unnecessary pain for infants assigned to this condition. However, this limitation was minimized by offering infants in this condition gentle touch and position support, two validated forms of analgesia. Furthermore, the effects of sucking + breast milk + tucking or sucking + breast milk were examined only during heel-stick procedures, which is only one of many invasive neonatal intensive care unit procedures. In addition, since heel stick is a short procedure, it is not known whether our combined interventions can effectively relieve pain due to longer procedures. Future studies should include more preterm infants with different gestational ages (< 26 weeks), block gestational age before randomization, and better control factors that may influence outcome variables. Future studies can adopt a crossover design to decrease the effects of variable infant characteristics. Researchers can evaluate the effects of breast milk plus other supportive non-pharmacological or pharmacological interventions, e.g., leg massage, gentle touch, swaddling, on relieving pain during different procedures. Finally, the only outcomes we measured were pain scores or odds of pain. Future studies might consider measuring other outcome variables such as biological outcomes (e.g., sleep, salivary cortisol levels), behavioral outcomes (e.g., facial expressions, body movements), or clinical outcomes (e.g., complications due to prematurity, oxygen demands).

5. Conclusions

The combined use of sucking, breast milk, and tucking effectively reduced preterm infants' pain and odds of mild pain and moderate-to-severe pain during heel-stick procedures. The risks of mild pain and moderate-to-severe pain were lower in infants receiving sucking + breast milk + tucking and sucking + breast milk during heel-stick procedures than in infants receiving routine care. Clinicians can incorporate the combined use of sucking, breast milk, and tucking into caregiving to relieve pain while preterm infants undergo invasive procedures. Tucking might not significantly reduce pain, but could facilitate recovery from pain and help stabilize infants. The results of this study add to a growing body of evidence supporting the combined use of non-nutritive sucking, expressed breast milk, and facilitated tucking for non-pharmacological pain relief in preterm infants undergoing painful procedures.

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