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# Breastfeeding or breast milk for procedural pain in neonates (Review)

Shah PS, Torgalkar R, Shah VS

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[Intervention Review]

# Breastfeeding or breast milk for procedural pain in neonates

Prakeshkumar S Shah[1], Ranjit Torgalkar[2], Vibhuti S Shah[1]

[1]Department of Paediatrics and Institute of Health Policy, Management and Evaluation, University of Toronto and Mount Sinai Hospital, Toronto, Canada. [2]Department of Paediatrics, Division of Neonatology, Kentucky Children's Hospital, University of Kentucky, Lexington, USA

**Contact:** Prakeshkumar S Shah, prakeshkumar.shah@sinaihealth.ca.

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## ABSTRACT

### Background

Pain in the neonate is associated with acute behavioural and physiological changes. Cumulative pain is associated with morbidities, including adverse neurodevelopmental outcomes. Studies have shown a reduction in changes in physiological parameters and pain score measurements following pre-emptive analgesic administration in neonates experiencing pain or stress. Non-pharmacological measures (such as holding, swaddling and breastfeeding) and pharmacological measures (such as acetaminophen, sucrose and opioids) have been used for analgesia. This is an update of a review first published in 2006 and updated in 2012.

### Objectives

The primary objective was to evaluate the effectiveness of breastfeeding or supplemental breast milk in reducing procedural pain in neonates. The secondary objective was to conduct subgroup analyses based on the type of control intervention, gestational age and the amount of supplemental breast milk given.

### Search methods

We searched CENTRAL, MEDLINE, Embase, CINAHL and trial registries (ICTRP, ISRCTN and clinicaltrials.gov) in August 2022; searches were limited from 2011 forwards. We checked the reference lists of included studies and relevant systematic reviews.

### Selection criteria

We included randomised controlled trials (RCTs) or quasi-RCTs of breastfeeding or supplemental breast milk versus no treatment/other measures in neonates. We included both term (≥ 37 completed weeks postmenstrual age) and preterm infants (< 37 completed weeks' postmenstrual age) up to a maximum of 44 weeks' postmenstrual age. The study must have reported on either physiological markers of pain or validated pain scores.

### Data collection and analysis

We assessed the methodological quality of the trials using the information provided in the studies and by personal communication with the authors. We extracted data on relevant outcomes, estimated the effect size and reported this as a mean difference (MD). We used the GRADE approach to assess the certainty of evidence.

### Main results

Of the 66 included studies, 36 evaluated breastfeeding, 29 evaluated supplemental breast milk and one study compared them against each other. The procedures conducted in the studies were: heel lance (39), venipuncture (11), intramuscular vaccination (nine), eye examination for retinopathy of prematurity (four), suctioning (four) and adhesive tape removal as procedure (one). We noted marked heterogeneity in the control interventions and pain assessment measures amongst the studies. Since many studies included multiple arms

with breastfeeding/supplemental breast milk as the main comparator, we were not able to synthesise all interventions together. Individual interventions are compared to breastfeeding/supplemental breast milk and reported. The numbers of studies/participants presented with the findings are not taken from pooled analyses (as is usual in Cochrane Reviews), but are the overall totals in each comparison.

Overall, the included studies were at low risk of bias except for masking of intervention and outcome assessment, where nearly one-third of studies were at high risk of bias.

**Breastfeeding versus control**

Breastfeeding may reduce the increase in heart rate compared to holding by mother, skin-to-skin contact, bottle feeding mother's milk, moderate concentration of sucrose/glucose (20% to 33%) with skin-to-skin contact (low-certainty evidence, 8 studies, 784 participants).

Breastfeeding likely reduces the duration of crying compared to no intervention, lying on table, rocking, heel warming, holding by mother, skin-to-skin contact, bottle feeding mother's milk and moderate concentration of glucose (moderate-certainty evidence, 16 studies, 1866 participants).

Breastfeeding may reduce percentage time crying compared to holding by mother, skin-to-skin contact, bottle feeding mother's milk, moderate concentration sucrose and moderate concentration of sucrose with skin-to-skin contact (low-certainty evidence, 4 studies, 359 participants).

Breastfeeding likely reduces the Neonatal Infant Pain Scale (NIPS) score compared to no intervention, holding by mother, heel warming, music, EMLA cream, moderate glucose concentration, swaddling, swaddling and holding (moderate-certainty evidence, 12 studies, 1432 participants).

Breastfeeding may reduce the Neonatal Facial Coding System (NFCS) score compared to no intervention, holding, pacifier and moderate concentration of glucose (low-certainty evidence, 2 studies, 235 participants).

Breastfeeding may reduce the Douleur Aigue Nouveau-né (DAN) score compared to positioning, holding or placebo (low-certainty evidence, 4 studies, 709 participants).

In the majority of the other comparisons there was little or no difference between the breastfeeding and control group in any of the outcome measures.

**Supplemental breast milk versus control**

Supplemental breast milk may reduce the increase in heart rate compared to water or no intervention (low-certainty evidence, 5 studies, 336 participants).

Supplemental breast milk likely reduces the duration of crying compared to positioning, massage or placebo (moderate-certainty evidence, 11 studies, 1283 participants).

Supplemental breast milk results in little or no difference in percentage time crying compared to placebo or glycine (low-certainty evidence, 1 study, 70 participants).

Supplemental breast milk results in little or no difference in NIPS score compared to no intervention, pacifier, moderate concentration of sucrose, eye drops, gentle touch and verbal comfort, and breast milk odour and verbal comfort (low-certainty evidence, 3 studies, 291 participants).

Supplemental breast milk may reduce NFCS score compared to glycine (overall low-certainty evidence, 1 study, 40 participants). DAN scores were lower when compared to massage and water; no different when compared to no intervention, EMLA and moderate concentration of sucrose; and higher when compared to rocking or pacifier (low-certainty evidence, 2 studies, 224 participants).

Due to the high number of comparator interventions, other measures of pain were assessed in a very small number of studies in both comparisons, rendering the evidence of low certainty.

The majority of studies did not report on adverse events, considering the benign nature of the intervention. Those that reported on adverse events identified none in any participants.

Subgroup analyses were not conducted due to the small number of studies.

**Authors' conclusions**

Moderate-/low-certainty evidence suggests that breastfeeding or supplemental breast milk may reduce pain in neonates undergoing painful procedures compared to no intervention/positioning/holding or placebo or non-pharmacological interventions. Low-certainty evidence suggests that moderate concentration (20% to 33%) glucose/sucrose may lead to little or no difference in reducing pain compared

to breastfeeding. The effectiveness of breast milk for painful procedures should be studied in the preterm population, as there are currently a limited number of studies that have assessed its effectiveness in this population.

## PLAIN LANGUAGE SUMMARY

### Breastfeeding or breast milk for procedural pain in newborn babies

#### Review question

We investigated how well breastfeeding or supplemental breast milk (expressed breast milk given via feeding tube or by placing breast milk in baby's mouth) works as a pain reliever in newborn babies while they undergo painful procedures (e.g. vaccination, heel prick, blood sampling for tests or eye examinations). The babies' pain responses (e.g. changes in heart rate, oxygen level, blood pressure, percentages of crying time, duration of crying etc.) were assessed by health care professionals to measure the pain that babies are experiencing.

#### Background

Breastfeeding may provide pain relief for newborn babies undergoing painful procedures. Medication for pain relief is commonly given for major painful procedures, but may not be given for minor painful procedures such as blood sampling (by heel prick or taking a sample from a vein). There are different forms of non-drug strategies that can be used to reduce pain in babies, such as holding or swaddling them, sucking on a pacifier, or giving sweet solutions (such as sucrose or glucose). Different studies done in newborn babies have shown that breastfeeding may be a good way to reduce the pain they feel when subjected to minor painful procedures. These studies have been done in full-term babies, and they have shown that breastfeeding may be effective by demonstrating that it reduces babies' crying time and various pain scores that have been validated for babies. Breast milk given by syringe has not shown the same efficacy as breastfeeding itself. Very few studies have been done in preterm babies, and so new studies are needed to determine if the use of supplemental breast milk in these small babies is effective in reducing their pain.

#### Study characteristics

We searched the medical literature thoroughly up to 1 August 2022 for studies that investigated the pain-relieving effect of breastfeeding or supplemental breast milk for minor medical procedures in newborn full-term and preterm neonates We included randomised trials only, as they provide the most reliable medical evidence. We identified 66 studies that reported on a total of more than 6200 infants in this Cochrane Review. Thirty-six studies evaluated breastfeeding, 29 studies evaluated supplemental breast milk, while one study compared both against each other. In over half of the studies, pain relief was during a heel prick procedure. In others, it was during vaccination, drawing blood from a vein or other procedures. The studies used a variety of comparative groups, for example, placebo, no intervention, maternal holding, skin-to-skin contact, similar volume of water, a pacifier, routine care, various concentrations of sucrose or glucose, 'facilitated tucking' (holding the infant in a flexed position with arms close to the body and hands placed to promote sucking), swaddling, heel warmth, anaesthetic cream for the skin, or a combination of these. The studies used a wide variety of pain scales as well as changes in heart rate and blood pressure and cry duration to assess pain.

#### Study funding sources

The studies included in the review were not externally funded according to information given in the reports.

#### Key results

Newborn babies in the breastfeeding group experienced a lower heart rate, shorter duration of cry, lower percentage of cry time and lower scores on the Neonatal Infant Pain Scale than babies who received no intervention. Moderate concentrations of glucose/sucrose may have similar effectiveness to breastfeeding. Studies of supplemental breast milk showed variable results. Supplemental breast milk was found to have a lower increase in heart rate when compared to water, and a lower duration of crying when compared to placebo.

#### What are the limitations of the evidence

We are moderately confident that breastfeeding reduces pain as assessed by heart rate, cry duration or validated pain scale compared to no intervention. Supplemental breast milk for painful procedures may reduce pain when compared to no intervention or placebo.

Due to the high number of comparator groups, other measures of pain were assessed in a very small number of studies. The majority of the studies did not report on any unwanted or harmful effects of treatment. Those that did this identified no unwanted or harmful effects of treatment in any infants.

## Summary of findings 1. Summary of findings - Breastfeeding versus control

**Breastfeeding compared with control for procedural pain relief**

**Patient or population:** term newborns (≥ 37 completed weeks' postmenstrual age) and preterm infants (< 37 completed weeks' postmenstrual age) up to maximum of 44 weeks' postmenstrual age, undergoing heel lance, venipuncture, intramuscular injection, eye examination or any other diagnostic and/or therapeutic procedures

**Settings:** neonatal units

**Intervention:** breastfeeding

**Comparison:** placebo, no treatment, sucrose, glucose, pacifiers, positioning or any other comparison as reported by authors

**Time**: within minutes of intervention

| Outcomes | Illustrative comparative risks* (95% CI) | | Relative effect (95% CI) | No. of participants (studies) | Certainty of the evidence (GRADE) | Comments |
|---|---|---|---|---|---|---|
| | Assumed risk | Corresponding risk | | | | |
| | Control | Breastfeeding | | | | |
| **Heart rate** (beats per minute) | The mean heart rate ranged across control groups from 93.5 to 177. | The mean heart rate ranged across breastfeeding groups from 123.3 to 175. | The range of mean differences between breastfeeding and various comparator groups ranged from +30 to -21 beats per minute.<br><br>A precise minimal clinically important difference has not yet been identified. | 784 (8) | ⊕⊕⊖⊖ **low** | The heart rate was lower in the breastfeeding group compared to holding by mother, skin-to-skin, bottle feeding mother's milk, moderate concentration of sucrose and moderate concentration of sucrose with skin-to-skin. The heart rate was similar when compared to pacifier, positioning, no intervention, breast milk odour and maternal heart beats. Heart rate was higher when compared to formula feeding.<br><br>Downgraded for indirectness and imprecision. |
| **Duration of crying (seconds)** | The mean duration of crying ranged across control groups from 0 to 184 seconds. | The mean duration of crying in the intervention groups was lower and ranged from 0 to 75.8 seconds. | The range of mean differences between breastfeeding and various comparator groups ranged from +6 to -130 seconds. | 1866 (16) | ⊕⊕⊕⊖ **moderate** | Duration of crying was lower with breastfeeding compared to no intervention, lying on table, rocking, heel warming, holding by mother, skin-to-skin contact, bottle feeding mother's milk and moderate concentration of glucose. Duration of crying was similar when compared to positioning, pacifier, water, |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | A precise minimal clinically important difference has not yet been identified. | | | moderate concentration of sucrose, formula, odour of breast milk or listening to maternal heart beats.<br><br>Downgraded for imprecision. |
| **Percentage of time crying** | The mean percentage of time crying ranged across control groups from 4% to 65.6%. | The mean percentage of time crying ranged across intervention groups from 3% to 33%. | The range of mean differences between breastfeeding and various comparator groups ranged from -1 to -49.<br><br>A precise minimal clinically important difference has not yet been identified. | 359 (4) | ⊕⊕⊖⊖<br>**low** | Percentage of time crying was lower compared to positioning, skin-to-skin contact, pacifier use (neonate held by research assistant) and moderate concentration of sucrose. Percentage of time crying was similar to pacifier use (neonate held by mother) and moderate concentration of sucrose with skin-to-skin contact.<br><br>Downgraded for inconsistency and imprecision. |
| **Neonatal Infant Pain Scale (NIPS)** | The mean NIPS score ranged across control groups from 0.3 to 7.37<br><br>(Lower score indicates lower pain, the range is 0 to 7). | The mean NIPS score in the intervention groups was lower, with a mean of 0.9 to 5.52. | The range of mean differences between breastfeeding and various comparator groups ranged from -0.9 to -3.0.<br><br>A precise minimal clinically important difference has not yet been identified. | 1432 (12) | ⊕⊕⊕⊖<br>**moderate** | NIPS scores were lower when compared to no intervention, holding by mother, heel warming, music, EMLA cream, moderate glucose concentration, swaddling, swaddling and holding. NIPS scores were similar when compared to positioning, moderate concentration of sucrose and non-nutritive sucking.<br><br>Downgraded for imprecision. |
| **Neonatal Facial Coding System (NFCS)** | The mean NFCS score ranged across control groups from 2.3 to 19.36.<br><br>(Lower score indicates lower pain; maximum score for preterm neonates is 9 and full-term neonates is 10; see comments for Leite et al scoring in text). | The mean NFCS score in the intervention groups was lower, ranging from 2.9 to 18.44. | The range of mean differences between breastfeeding and various comparator groups ranged from +0.6 to -4.2.<br><br>A precise minimal clinically important difference has not yet been identified. | 235 (2) | ⊕⊕⊖⊖<br>**low** | NFCS scores were lower when compared to no intervention, holding, pacifier and moderate concentration of glucose. NFCS scores were no different when compared to skin-to-skin and formula feeding.<br><br>Downgraded for inconsistency and imprecision. |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Douleur Aigue Nouveau-né (DAN) scale** | The mean DAN score ranged across control groups from 3 to 9.02.<br><br>(This scale scores pain from 0 to 10, where 0 is no pain and 10 is maximal pain). | The mean DAN score in the intervention groups was lower, ranging from 2.25 to 4.54. | The range of mean differences between breastfeeding and various comparator groups ranged from +1.4 to -6.9.<br><br>A precise minimal clinically important difference has not yet been identified. | 709 (4) | ⊕⊕⊖⊖ **low** | DAN scores were lower when compared to positioning, holding or placebo. DAN scores were similar when compared to no intervention, rocking, pacifier held by research assistant, water or moderate concentration of glucose. DAN scores were higher when compared to moderate concentration of sucrose.<br><br>Downgraded for inconsistency and imprecision. |
| **Any adverse outcome** | None of the studies reported adverse outcomes. | | | | | |

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
**CI:** confidence interval

GRADE Working Group grades of evidence
**High certainty :** further research is very unlikely to change our confidence in the estimate of effect.
**Moderate certainty:** further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
**Low certainty:** further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
**Very low certainty:** we are very uncertain about the estimate.

Since each comparison had several different control groups that were compared to breastfeeding, we could not report a single relative effect measure in this table, or report details of the certainty of evidence for one intervention.
We downgraded the evidence by one level for heterogeneity of effect and one level for the small number of comparisons for each of the interventions. This was done in the individual comparison.

### Summary of findings 2. Summary of findings - Supplemental breast milk versus control

**Supplemental breast milk compared with control for procedural pain relief**

**Patient or population:** term newborns (≥ 37 completed weeks' postmenstrual age) and preterm infants (< 37 completed weeks' postmenstrual age) up to maximum of 44 weeks' postmenstrual age, undergoing heel lance, venipuncture, intramuscular injection, eye examination or any other diagnostic and/or therapeutic procedures

**Settings:** neonatal units

**Intervention:** supplemental breast milk

**Comparison:** placebo, no treatment, sucrose, glucose, pacifiers, positioning or any other comparison as reported by authors

**Time:** within minutes of intervention

| Outcomes | Illustrative comparative risks* (95% CI) | | Relative effect (95% CI) | No. of participants (studies) | Certainty of the evidence (GRADE) | Comments |
|---|---|---|---|---|---|---|
| | Assumed risk | Corresponding risk | | | | |
| | Control | Supplemental breast milk | | | | |
| **Heart rate** (beats per minute) | The mean heart rate ranged across control groups from 141 to 177. | The mean heart rate ranged across breastfeeding groups from 154 to 175. | The range of mean differences between breastfeeding and various comparator groups ranged from +2 to -20 beats per minute.<br><br>A precise minimal clinically important difference has not yet been identified. | 336 (5) | ⊕⊕⊖⊖ **low** | Heart rate was lower when compared to water or no intervention; and no different when compared to moderate concentration of glucose, moderate concentration of sucrose, eye drops, smell of breast milk or taste and smell of breast milk; and higher compared to positioning.<br><br>Downgraded for indirectness and imprecision. |
| **Duration of crying (seconds)** | The mean duration of crying ranged across control groups from 2 to 162 seconds. | The mean duration of crying in the intervention groups was similar to the control groups, ranging from 22.04 to 151.34 seconds. | The range of mean differences between breastfeeding and various comparator groups ranged from +52 to -55.<br><br>A precise minimal clinically important difference has not yet been identified. | 1283 (11) | ⊕⊕⊕⊖ **moderate** | Duration of crying was lower compared to positioning, massage and placebo; no different compared to artificial sweetener and low concentration of glucose; and higher when compared to no intervention, rocking, pacifier, glycine, moderate concentration of glucose, high concentration of glucose, low and moderate concentration of sucrose and 2 doses of low concentration of sucrose.<br><br>Downgraded for imprecision. |
| **Percentage time crying** | The mean percentage of time crying ranged across control groups from 76 to 90. | The mean percentage of time crying in the intervention group was 91. | The range of mean differences between breastfeeding and various comparator groups ranged from +1 to +9 suggesting an increase.<br><br>A precise minimal clinically important difference has not yet been identified. | 60 (1) | ⊕⊕⊖⊖ **low** | Percentage time crying was no different when compared to placebo or glycine, and was higher when compared to artificial sweetener.<br><br>Downgraded for inconsistency and imprecision. |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Neonatal Infant Pain Scale (NIPS)** | The mean NIPS score ranged across control groups from 0.46 to 6.51.<br><br>(Lower score indicates lower pain, the range is 0 to 7). | The mean NIPS score in the intervention groups ranged from 0.42 to 6.29. | The range of mean differences between breastfeeding and various comparator groups ranged from -0.3 to +1.2.<br><br>A precise minimal clinically important difference has not yet been identified. | 291 (3) | ⊕⊕⊖⊖ **low** | NIPS scores were no different compared to no intervention, pacifier, moderate concentration of sucrose, eye drops, gentle touch and verbal comfort, and breast milk odour and verbal comfort.<br><br>Downgraded for inconsistency and imprecision. |
| **Neonatal Facial Coding System (NFCS) score at 3 minutes** | The mean NFCS score at 3 minutes ranged across control groups from 2.6 to 3.54.<br><br>(Lower score indicates lower pain, maximum score for preterm neonates was 9 and for full term neonates was 10). | The mean NFCS score at 3 minutes in the intervention groups was similar, ranging from 0.6 to 3.08. | The range of mean differences between breastfeeding and various comparator groups ranged from -0.2 to -0.9.<br><br>A precise minimal clinically important difference has not yet been identified. | 40 (1) | ⊕⊕⊖⊖ **low** | NFCS score at 3 minutes was lower compared to glycine, and no different when compared to placebo or artificial sweetener.<br><br>Downgraded for inconsistency and imprecision. |
| **Douleur Aigue du Nouveau-né (DAN) scale** | The mean DAN score ranged across control groups from 1.1 to 6.63.<br><br>(This scale scores pain from 0 to 10, where 0 is no pain and 10 is maximal pain). | The mean DAN score in the intervention groups ranged from 2.2 to 5.63. | The range of mean differences between breastfeeding and various comparator groups ranged from -1.1 to +1.1.<br><br>A precise minimal clinically important difference has not yet been identified. | 224 (2) | ⊕⊕⊖⊖ **low** | DAN scores were lower when compared to massage and water; no different when compared to no intervention, EMLA and moderate concentration of sucrose; and higher when compared to rocking or pacifier groups.<br><br>Downgraded for inconsistency and imprecision. |
| **Any adverse outcome** | None of the studies reported any adverse outcomes. | | | | | |

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
**CI:** confidence interval

GRADE Working Group grades of evidence
**High certainty:** further research is very unlikely to change our confidence in the estimate of effect.
**Moderate certainty:** further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

**Low certainty:** further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
**Very low certainty:** we are very uncertain about the estimate.

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Since each comparison had several different control groups that were compared to supplemental breast milk, we could not report a single relative effect measure.
We downgraded the evidence by one level for heterogeneity of effect and one level for the small number of comparisons for each of the interventions. This was done in the individual comparison.

# BACKGROUND

## Description of the condition

Pain is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage" (AAP 2000). Evaluation of pain in neonates is difficult due to the subjective nature of pain and the inability of neonates to verbally express pain (Roue 2023). Surrogate measures used to describe pain in neonates include motor responses (Craig 1993; Marshall 1980), facial expressions (Grunau 1987; Stevens 1993), cry (Grunau 1987; Johnston 1993) and changes in physiologic parameters like heart rate, blood pressure, oxygen saturation and respiratory rate. Various measures have been developed to assess pain in neonates (Abu-Saad 1998). Validated scores for the assessment of pain include the Neonatal Facial Coding System (NFCS) (Craig 1994), Neonatal Infant Pain Scale (NIPS) (Lawrence 1993) or Premature Infant Pain Profile (PIPP) (Stevens 1996). These reactions to pain may contribute to the development of hypoxia, hypercarbia, acidosis, ventilator asynchrony, pneumothoraces, reperfusion injury and venous congestion, and subsequent late intraventricular haemorrhage or late extension of early intraventricular haemorrhage and periventricular leukomalacia (Abdel-Rahman 1994; Anand 1998). These behavioural changes may also disrupt postnatal adaptation, parent-infant bonding and feeding schedules.

## Description of the intervention

Clinical studies have shown beneficial effects of pre-emptive analgesic administration in reducing neonatal pain and stress (Anand 1989). Pharmacological interventions include acetaminophen, sucrose and opioid analgesics. Non-pharmacological interventions include reduction of noxious stimuli (Anand 2001; Schechter 1997), implementation of neurobehaviourally supportive relationship-based care (Corff 1995; Gunnar 1984), and breastfeeding during the actual procedure.

## How the intervention might work

There are several potential mechanisms by which breast milk or breastfeeding might provide an analgesic effect. Components of breastfeeding that may be analgesic include presence of a comforting person (mother) (Blass 1995), physical sensation (skin-to-skin contact with comforting person) (Blass 1995), diversion of attention (Gunnar 1984) and sweetness of breast milk (presence of lactose or other ingredients present in the breast milk) (Blass 1997). Compared to artificial formula, breast milk contains a higher concentration of tryptophan (Heine 1999), a precursor of melatonin. Melatonin is shown to increase the concentration of beta-endorphins (Barrett 2000), and could possibly be one of the mechanisms for the nociceptive effects of breast milk. Preterm neonates incapable of direct breastfeeding from the mother may benefit from placement of breast milk on the tongue or the administration of breast milk via the naso/orogastric route (supplemental breast milk) through some mechanisms listed above. Amongst the analgesics studied for neonatal pain, breastfeeding/breast milk is natural, easily available, easy to use and potentially risk-free (Schollin 2004), and can be easily adopted from the perspectives of health care providers and parents. No adverse effects of breastfeeding have been reported, apart from rare transmission of micro-organisms.

In a systematic review, 24% sucrose was found to be effective in alleviating procedural pain in neonates (Stevens 2010). Both opioid and non-opioid mechanisms were suggested for its effectiveness. Breast milk contains only 7% lactose and may not be as effective as sucrose. On the other hand, interventions like pacifiers or positioning may result in an effect similar to breastfeeding or supplemental breast milk without interruption of the regular breastfeeding schedule.

## Why it is important to do this review

To our knowledge, the topic of breastfeeding or breast milk for procedural pain in neonates was not systematically evaluated prior to the first version of this review (Shah 2006). Breastfeeding or breast milk is a naturally available, non-toxic and humane way of providing pain relief. If found effective, it could change practice worldwide, which makes it very important to constantly update this review with new evidence.

# OBJECTIVES

## Primary

The primary objective was to evaluate the effectiveness of breastfeeding or supplemental breast milk in reducing procedural pain in neonates.

## Secondary

The secondary objective was to conduct subgroup analyses based on the type of control intervention, gestational age and the amount of supplemental breast milk given.

# METHODS

## Criteria for considering studies for this review

### Types of studies

We included randomised controlled trials (RCTs) or quasi-RCTs* of breastfeeding/supplemental breast milk (given via naso/orogastric tube or orally) to alleviate procedural pain in neonates. Cross-over studies and cluster-randomised controlled trials were not included.

(*In a quasi-RCT participants are allocated to different arms of the study using a method that is not truly random).

### Types of participants

We included both term (≥ 37 completed weeks' postmenstrual age) and preterm infants (< 37 completed weeks' postmenstrual age) up to a maximum of 44 weeks' postmenstrual age, undergoing heel lance, venipuncture, intramuscular injection, eye examination or any other diagnostic and/or therapeutic procedures. When the age of participants included in the study was not clear, we contacted the study authors to clarify. We only included studies with an eligible population.

### Types of interventions

We included breastfeeding or supplemental breast milk (breast milk placed on the tongue or in mouth) prior to or during the painful procedure versus placebo, no treatment, sucrose, glucose, pacifiers, positioning or any other comparison as reported by the study authors.

Co-interventions are described in the Characteristics of included studies table. We only included studies if co-interventions were applied in both groups (intervention and control group).

### Types of outcome measures

We considered primary outcomes of pain indicators and secondary outcomes reported by authors for this review. The study must have reported on either physiological markers of pain or validated pain scores.

#### *Primary outcomes*

Pain, as assessed by at least one of the following.

1. Physiological parameters
   a. Heart rate change
   b. Heart rate
   c. Respiratory rate change
   d. Oxygen saturation change
   e. Oxygen saturation
   f. Blood pressure change
   g. Systolic blood pressure change
   h. Diastolic blood pressure change
2. Cry variables
   a. Latency of first cry
   b. Duration of first cry
   c. Duration of crying
   d. Percentage time crying
   e. Time of first calming
3. Validated pain measures
   a. Neonatal Infant Pain Scale (NIPS) (Lawrence 1993)
   b. Premature Infant Pain Profile (PIPP) (Stevens 1996)
   c. Neonatal Facial Coding System (NFCS) (Craig 1994)
   d. Other pain scores as reported by the authors. (We identified during this review that authors had reported on other non-validated scores, such as the Douleur Aigue Nouveau-né score (DAN) (Carbajal 2003 (Venipuncture)), composite score (Shendurnikar 2005 (Heel lance)), Body Pain Score (Bucher 2000 (Heel lance)), visual analogue scale (VAS) (Gradin 2004 (Venipuncture)), COMFORTneo score (Simonse 2012 (Heel lance)), and Neonatal Pain, Agitation and Sedation Scale (N-PASS) (Ou-Yang 2013 (Heel lance)), and we have included them in this review.)

#### *Secondary outcomes*

1. Any clinically important outcome reported by authors (not prespecified).
2. Any harmful effects reported by any author.

## Search methods for identification of studies

Search methods are described below.

### Electronic searches

The Cochrane Neonatal Information Specialist, M Fiander, wrote new search strategies for this update to increase the sensitivity of the search terms. Searches were conducted in August 2022. We did not apply language or publication type limits, but did limit results from 2011 (date of the previous version of this review) forward. The following databases were used.

- Cochrane Central Register of Controlled Trials (CENTRAL 2022, Issue 8), 2 August 2022 (via Wiley)
- Ovid MEDLINE® and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations, Daily and Versions 1946 to 1 August 2022
- Embase 1974 to 1 August 2022 (via OVID)
- CINAHL Complete 1986 to 2 August 2022 (via EbscoHost)

We searched the following trial registries.

- The World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) (who.int/ictrp/search/en/)
- US National Library of Medicine's ClinicalTrials.gov (clinicaltrials.gov)
- ISRCTN Registry (http://www.isrctn.com/)

Search strategies are provided in: Appendix 1; Appendix 2; Appendix 3; Appendix 4; Appendix 5.

This is the third update of this review. Previous search details are listed in Appendix 6.

### Searching other resources

We searched the reference lists of studies selected for inclusion in this review for studies not identified by the electronic searches.

## Data collection and analysis

We followed the recommendations of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2020), and those of Cochrane Neonatal. We collected data from each trial using a data collection form developed for the review. We extracted the data from each of the included studies necessary to complete the study characteristics, risk of bias and data for analyses. For the excluded studies, we recorded the most important reason for exclusion.

### Selection of studies

RT and PS independently assessed all published articles identified as potentially relevant by the literature search for inclusion in the review. Disagreements were resolved by consensus and involving a third author (VS). RT and PS obtained data from the authors where published data provided inadequate information for the review or where relevant data could not be extracted. We managed the search results using a text file. We recorded the selection process in sufficient detail to complete PRISMA flow diagram.

### Data extraction and management

Data extraction was done by RT and PS (with VS rechecking for any discrepancy).

### Assessment of risk of bias in included studies

Two review authors (RT and VS) independently assessed the risk of bias (low, high or unclear) of all included trials using the Cochrane risk of bias tool (RoB 1) (Higgins 2011), for the following domains.

- Sequence generation (selection bias)
- Allocation concealment (selection bias)
- Blinding of participants and personnel (performance bias)

- Blinding of outcome assessment (detection bias)
- Incomplete outcome data (attrition bias)
- Selective reporting (reporting bias)
- Any other bias

We resolved any disagreements by discussion or by a third assessor (PS). See Appendix 7 for a more detailed description of risk of bias for each domain.

### Measures of treatment effect

We used Review Manager 5.4 for statistical analysis (Review Manager 2020).

#### *Dichotomous data*

For dichotomous data we planned to present results using risk ratios (RR) and risk differences (RD) with 95% confidence intervals (CIs). We aimed to calculate the number needed to treat for an additional beneficial outcome (NNTB), or number needed to treat for an additional harmful outcome (NNTH) with 95% CIs if there was a statistically significant reduction (or increase) in RD.

#### *Continuous data*

For continuous data we used the mean difference (MD) when outcomes were measured in the same way between trials. We used the standardised mean difference (SMD) to combine trials that measured the same outcome but used different methods. Where trials reported continuous data as median and interquartile range (IQR) and data passed the test of skewness, we converted mean to median and estimated the standard deviation as IQR/1.35. If data were not reported in an RCT in a format that we could enter directly into a meta-analysis, then we converted them to the required format using the information in Chapter 6 of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2020).

When meta-analyses was not possible or not appropriate, we described the studies reporting on a particular outcome.

### Unit of analysis issues

We performed the primary analysis per individual randomised. We did not include cluster-randomised trials. When trials had multiple arms that were compared against the same control condition and included in the same meta-analysis, we included all arms as individual comparisons and did not combine data specifically for that outcome as an overall meta-analytic estimate. We carried out analysis on an intention-to-treat basis for all included outcomes. We analysed all participants in the treatment group to which they were randomised, regardless of the actual treatment received.

### Dealing with missing data

Where data were missing, and could not be derived as described, we contacted the original study investigators to request the missing data. We did not impute any missing data.

### Assessment of heterogeneity

We described the clinical diversity and methodological variability of the evidence in the review text and with tables describing study characteristics including design features, population characteristics and intervention details. To assess statistical heterogeneity, we visually inspected forest plots and described the direction and magnitude of effects and the degree of overlap between confidence intervals. We also considered the statistics generated in forest plots that measure statistical heterogeneity. We used the $I^2$ statistic to quantify inconsistency amongst the trials in each analysis. We also considered the P value from the $Chi^2$ test to assess if this heterogeneity was significant ($P < 0.1$). When we identified substantial heterogeneity we reported the finding and planned to explore possible explanatory factors using prespecified subgroup analysis.

We graded the degree of heterogeneity as: 0% to 40% might not represent important heterogeneity; 30% to 60% may represent moderate heterogeneity; 50% to 90% may represent substantial heterogeneity; and more than 75% may represent considerable heterogeneity. We used this guideline to interpret the $I^2$ value rather than a simple threshold, and our interpretation took into account an understanding that measures of heterogeneity ($I^2$ and $Tau^2$) were estimated with high uncertainty when the number of studies was small.

### Assessment of reporting biases

We assessed reporting bias by comparing the stated primary outcomes and secondary outcomes and reported outcomes. Where study protocols were available, we compared these to the full publications to determine the likelihood of reporting bias. We planned to use the funnel plots to screen for publication bias where there were a sufficient number of studies (> 10) reporting the same outcome. If publication bias was suggested by a significant asymmetry of the funnel plot on visual assessment, we planned to incorporate this in our assessment of certainty of evidence. Most of the analyses in our review included few studies eligible for meta-analysis, thus we cannot rule out possible publication bias or smalls study effects.

### Data synthesis

We used Review Manager 5.4 for statistical analysis (Review Manager 2020). Statistical parameters included RR, RD, NNTB, NNTH, MD and SMD when appropriate. We reported 95% CIs for estimates of treatment effects. We used a random-effects model for meta-analyses since clinical heterogeneity was identified between studies.

### Subgroup analysis and investigation of heterogeneity

We compared the data for the outcomes outlined in the previous section as follows (planned primary and subgroup analyses). There were two main comparisons:

- Comparison 1: Breastfeeding versus control (the infant must be actually feeding from the breast at the time of intervention)
- Comparison 2: Supplemental breast milk versus control (the infant may be receiving breast milk via oral or nasogastric tube in the intervention group)

Within each comparison, we planned three subgroup analyses:

- Category 1: type of control intervention. We grouped results according to type of control intervention and only meta-analysed within subgroups. We have not compared between subgroups. Considering the marked heterogeneity in the control interventions, we did not conduct an overall meta-analysis.
- Category 2: type of procedure. The type of procedure for which authors used the intervention varied between studies

but mainly included heel lance, venipuncture, intramuscular injection and eye examination. Since the number of studies reporting a particular outcome in relation to a particular procedure was small, we indicated the type of procedure within the study ID so that when the reader reviews the results it becomes apparent in forest plot which procedure results are from each study. This allowed us to create a visual impression without conducting formal subgroup analyses in this version of the review, as the individual analyses were too small to create subgroups.

- Category 3: gestational age (subgroup 1: preterm (< 37 weeks' gestational age); subgroup 2: term (≥ 37 weeks' gestational age)). We did not have an adequate number of studies to conduct the planned subgroup analyses for category 3.

### Sensitivity analysis

No sensitivity analyses were planned.

### Summary of findings and assessment of the certainty of the evidence

We used the GRADE approach, as outlined in the GRADE Handbook (Schünemann 2013), to assess the certainty of evidence of the following (clinically relevant) outcomes: change in heart rate, duration of crying, percentage of time crying, NIPS score, NFCS, DAN score and any adverse outcomes.

Two review authors (RT and PS) independently assessed the certainty of the evidence for each of the outcomes above. We considered evidence from RCTs as high certainty but downgraded the evidence one level for serious (or two levels for very serious) limitations based upon the following: design (risk of bias), consistency across studies, directness of the evidence, precision of estimates and presence of publication bias. We used the GRADEpro GDT Guideline Development Tool to create summary of findings tables to report the certainty of the evidence. The GRADE approach results in an assessment of the certainty of a body of evidence as one of four grades.

- High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
- Moderate certainty: we are moderately confident in the effect estimate. The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
- Low certainty: our confidence in the effect estimate is limited. The true effect may be substantially different from the estimate of the effect.
- Very low certainty: we have very little confidence in the effect estimate. The true effect is likely to be substantially different from the estimate of effect.

We created two summary of findings tables - one for each comparison - reporting seven outcomes that assessed the effectiveness of the intervention (Summary of findings 1; Summary of findings 2).

The tables contain details for each outcome and an overall summary of reporting between different comparators. In the abstract results section, we have adopted a similar format but due to the word count restriction only the main comparative analyses are reported.

## RESULTS

### Description of studies

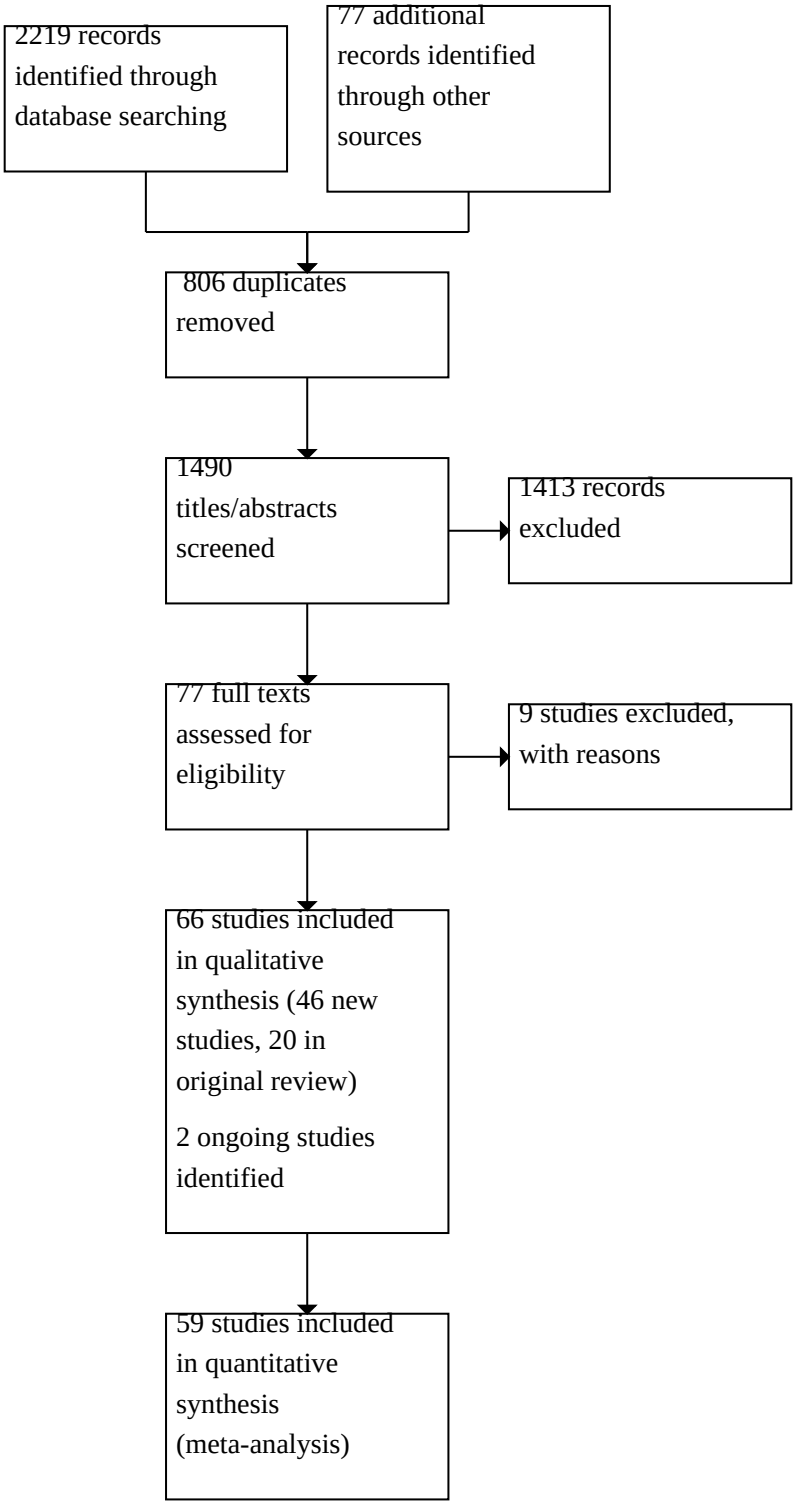
We identified studies assessing different control interventions. We have incorporated the type of painful procedure into the identifier (name) for each study, because subgroup analyses by type of procedure would have reduced the number of studies in each comparison (which was low to begin with).

### Results of the search

This is an update of our previous review (Shah 2006; Shah 2012).

The 2022 search (for the period 2011 to 2022) identified 2296 references. After removing 806 duplicates, 1490 references were available for title and abstract screening. We excluded 1413 based on title/abstract, and reviewed 77 full texts. We excluded nine studies, identified two ongoing studies (NCT00908401; NCT01355640), and included 66 studies (46 new studies; 20 in original review). See Figure 1.

**Figure 1. Study flow diagram.**



**Study funding sources:** studies included in the review were not externally funded according to information given in the papers.

Thirty-six studies evaluated breastfeeding, 29 studies evaluated supplemental breast milk and one study compared them against each other. The procedures conducted in the studies were: heel lance (39), venipuncture (11), intramuscular vaccination (nine), eye examination for retinopathy of prematurity (four), suctioning (two) and adhesive tape removal as procedure (one). Fifty-nine studies were included for meta-analysis.

Seven studies were not included in the quantitative synthesis: two studies provided results in a graphical manner, and it was difficult to obtain the information needed for use in meta-analyses (Blass 2001 (Heel lance); Peng 2018 (Heel lance)); two studies did not provide a measure of dispersion (Hashemi 2016 (Intramuscular injection); Zargham-Boroujeni 2017 (Venipuncture)); two studies did not provide actual scores (Lima 2013 (Venipuncture); Rioualen 2018 (Venipuncture)); and one study did not provide the actual number of participants in each group (Sabety 2013 (Venipuncture)).

Clinical details regarding the participants, interventions and outcomes are given in the Characteristics of included studies table.

Details of the excluded studies are provided in the Characteristics of excluded studies table, and details of the ongoing studies are in the Characteristics of ongoing studies table.

**Included studies**

Avcin 2021 (Heel lance) evaluated the effects of breastfeeding, kangaroo care and facilitated tucking during heel lance on pain in healthy term neonates. This was a quasi-randomised controlled trial of 140 neonates. Participants included were term neonates (38 to 42 weeks of gestation) who underwent heel lance for Guthrie (PKU) test. Neonates were randomised into four groups.

- Group 1: Breastfeeding (n = 35)
- Group 2: Kangaroo care group (n = 35)
- Group 3: Facilitated tucking group (n=35)
- Group 4: Control (n = 35)

In the breastfeeding group (n = 35, mean gestational age (GA) in weeks 38.5, mean birthweight (BW) in grams 3184), breastfeeding was started five minutes before blood collection. The nurse performed the heel stick procedure at the end of the five minutes when the participant was in the breastfeeding position on its mother's lap. In the kangaroo care group (n = 35, mean GA in weeks 38.6 mean BW in grams 3293), each participant received kangaroo care during blood collection. The baby was undressed, and the mother unbuttoned her shirt and placed her baby on her chest according to the kangaroo care guideline specified by WHO (WHO 2023). The room temperature was set at 24 °C to 26 °C. The mother was placed in the semi-Fowlers position, with her back supported by a pillow. The baby was facing the mother to ensure the maternal bond. The baby was wrapped in a blanket, with constant contact with its mother. The mother and the baby were in this position for 15 minutes, after which a blood sample was collected while the baby was still in the kangaroo care position. In the Facilitated Tucking Position Group (n = 35, mean GA in weeks 38.4, mean BW in grams 3261), each participant received facilitated tucking one minute before blood collection with its lower and upper extremities held in lateral flexion and close to the midline. The facilitated tucking position was maintained during and for one minute after blood collection. In the control group (n = 35, mean GA in weeks 38.3, mean BW in grams 3361), each participant underwent a routine heel stick procedure. No intervention was applied to the control group in this study. Outcomes measured were the Neonatal Infant Pain Scale during the procedure, total crying time in seconds and vital signs (oxygen saturations, body temperature, heart rate and respiratory rate).

Aydin 2019 (Heel lance) evaluated the effects of breastfeeding and heel warming during heel lance procedures on pain in healthy term neonates. This was a randomised controlled trial (RCT) of 150 neonates. Term neonates (38 to 42 weeks of gestation) who underwent heel lance for routine metabolic screening and were aged two to four days were included. Neonates were randomised into three groups:

- Group 1: Breastfeeding (n = 50)
- Group 2: Heel warming (n = 50)
- Group 3: Control (n = 50)

In the breastfeeding group (n = 50, mean GA in weeks 39.3, mean BW in grams 3394), breastfeeding was started just before the procedure (one minute before) and continued for a minimum of two minutes during and after the procedure. In the heel warming (n = 50, mean GA in weeks 39.1, mean BW in grams 3344) group, a thermal bag was used to warm the heel area before the heel lance procedure. Water was put at 40 °C in the thermal bag and applied against the puncture point for three to five minutes before the heel stick procedure. In the control group (n = 50, mean GA in weeks 38.98, mean BW in grams 3241), the heel lance procedure was conducted using the standard method, and the neonates received no intervention during the procedure. Outcomes measured were the Neonatal Infant Pain Scale during the procedure and total crying time in seconds.

Aziznejad 2013 (Venipuncture) compared the effects of supplemental breast milk to sucrose, EMLA cream and control. This was an RCT of 120 full-term neonates. Inclusion criteria for this study were neonates with a diagnosis of jaundice; no cardiovascular, respiratory, infectious or neural diseases; no congenital malformations or chromosomal syndromes confirmed by an attending physician; who required daily venipuncture to monitor bilirubin; had an Apgar score below seven at five minutes, and were stable and conscious before venipuncture.

Neonates were randomly divided into four groups of 30 each.

- Group 1: Control group (no intervention) (n = 30)
- Group 2: Sucrose group (n = 30)
- Group 3: Breast milk group (n = 30)
- Group 4: EMLA cream group (n = 30)

Venipuncture was carried out in the first group (control) without any specific treatment. In the second and third groups, venipuncture was performed two minutes after feeding neonates (dropping the substances on the neonate's tongue using a syringe) with respectively 2 mL of 25% sucrose and 2 mL of breast milk. In the fourth group, a layer of 2.5% EMLA cream (1 g) was first topically applied in the antecubital area. Outcomes measured were pain score via DAN scale, duration of crying in seconds, change in heart rate, arterial oxygen saturation and respiratory rate.

Bavarsad 2018 (Intramuscular injection) compared the effects of breast milk and powdered milk on pain severity after a muscular injection in one day-old neonates. Included participants for this study were full-term neonates, weighing 2500 g or more, born via vaginal delivery, having an Apgar score > 7, lacking any disease or congenital disorder, breastfed at least once, at least 2 hours after birth, and having a consent form completed. It was an RCT and 100 infants were randomly divided into four groups of 25 each.

- Group 1: Breastfeeding (n = 25)
- Group 2: Bottle feeding mother's milk (n = 25)
- Group 3: Bottle feeding powdered formula (n = 25)
- Group 4: Control (mother holding but no feeding) (n = 25)

The hepatitis B vaccine was injected in neonates in the control group routinely with no feeding. The vaccine was injected in the other three groups during oral feeding in different conditions: feeding from the mother's breast, bottle feeding of mother's milk and feeding of powdered formula. A similar method was considered for three feeding groups, i.e. neonates in a calm environment started feeding for two minutes and were still feeding during the injection for at least two minutes. Outcomes for this study were change in heart rate, arterial oxygen saturation, crying duration in seconds, facial grimace, limb movements and vocal response.

Bembich 2013 (Heel lance) evaluated both cortical and behavioural responses of healthy term newborns to a painful procedure during two non-pharmacologic analgesic interventions, i.e. glucose solution and breastfeeding. This was an RCT of 30 healthy full-term newborns (GA range: 38 to 41 weeks; 16 males and 14 females) who underwent a heel lance procedure on their third day of life for metabolic screening and had not previously experienced any painful procedure.

- Group 1: Oral glucose group (n = 15)
- Group 2: Breastfeeding group (n = 15)

In the oral glucose group (n = 15), the neonate was first placed on his/her back on a baby changing-table and the fibres were positioned on the infant scalp. Two minutes before starting the blood sampling procedure, a bolus of 2 mL of 20% oral glucose solution was administered. In the breastfeeding group (n = 15), the neonate was tested in his/her mother's lap. After placing fibres on the neonate's scalp and waiting for the newborn to get used to the equipment, breastfeeding was started two minutes before the blood sampling procedure. Mothers were required not to talk to their newborns. Breastfeeding lasted until the blood sampling procedure was completed. Outcomes measured for this study were increase of cortical oxy-haemoglobin (HbO2) during the heel prick procedure as an estimate of cortical activation by use of multichannel near infra-red spectroscopy (NIRS) device and Neonatal Infant Pain Scale (NIPS) scores.

Bembich 2018 (Heel lance) studied neonatal cortical brain response to four types of non-pharmacological analgesia (oral glucose, expressed breast milk, maternal holding plus oral glucose, breastfeeding) and assessed the differential effect of oral solutions (glucose, breast milk) given alone or combined with the maternal-infant relationship (holding, breastfeeding). In this RCT, 80 healthy term newborns (GA: 37 to 42 weeks) who were undergoing a heel stick for metabolic screening on their third day of life (and who had started breastfeeding) were included. Neonates were randomised into four groups.

- Group 1: 20% Oral glucose solution (n = 20)
- Group 2: Expressed breast milk (n = 20)
- Group 3 Maternal holding + oral glucose solution (n = 20)
- Group 4: Breastfeeding (n = 20)

Neonates who were allocated to group 1 and group 2 were placed on a changing table, and fibres were positioned on the scalp. A waiting period was allowed for the infant to get used to the equipment. Two minutes before starting the heel stick procedure, a 2 mL bolus of 20% oral glucose solution (group 1) or a 2 mL bolus of the mother's breast milk (group 2) was administered directly into the neonates mouth with a syringe. Neonates in group 3 and group 4 were tested while in their mothers' arms (mother-infant relationship). Optical fibres were placed on the scalp, and a waiting period was allowed for the newborn to adapt. Two minutes before the heel stick procedure, a 2 mL bolus of 20% oral glucose solution was given directly into the neonate's mouth with a syringe (group 3) or breastfeeding was started (group 4). Breastfeeding lasted at least until the heel stick procedure was completed. Outcomes for this trial were pain measurement by NIPS score and cortical activity using multichannel NIRS.

Blass 2001 (Heel lance) compared the effects of supplemental breast milk (colostrum) to water and sucrose. This was a quasi-RCT of 60 full-term neonates. The infants were randomly assigned to one of the following groups (10 neonates in each group).

- Group 1: Water via syringe (n = 10)
- Group 2: Colostrum via syringe (n = 10)
- Group 3: Sucrose via syringe (n = 10)
- Group 4: Water on a pacifier (n = 10)
- Group 5: Colostrum on a pacifier (n = 10)
- Group 6: Sucrose on a pacifier (n = 10)

The neonates were between 30 and 55 hours of age at the time of blood collection for routine neonatal screening using the heel lance procedure. 2 mL of the allocated solution was given either by slow administration via syringe over a span of two minutes or by allowing the neonates to suck a pacifier dipped in the solution every 30 seconds for two minutes. Prior to the procedure, baseline data were obtained for 60 seconds and continuous monitoring was done throughout and after the procedure during the recovery time. The blood collection was done by an experienced phlebotomist for 49 of the 60 neonates. The outcomes measured were reduction in the percentage crying and grimacing time during the procedure, the mean crying time following the procedure, and the mean heart rate change during and following the procedure. Despite repeated requests, we were unable to obtain data regarding individual groups from the authors.

Bozlak 2017 (Eye examination) compared the effects of neonate swaddling with oral administration of sucrose, swaddling with oral administration of breast milk, and swaddling with oral administration of distilled water (control) on pain perception in preterm neonates during a screening examination for retinopathy of prematurity (ROP). This was an RCT that included neonates with gestational age less than 32 weeks and body weight less than 1500 g, parent approval on the consent form, no requirement for

invasive or noninvasive mechanical ventilator support during the procedure, no intake of analgesic or sedative drug in the past 24 hours, no contraindications to oral feeding and no congenital abnormalities. The 87 neonates were randomly divided into three groups.

- Group 1: Swaddling with sucrose administration (n = 29)
- Group 2: Swaddling with breast milk administration (n = 29)
- Group 3: Swaddling with distilled water administration (n = 29)

The oral sucrose was prepared as a 24% sucrose solution in the hospital pharmacy; breast milk was obtained from the mothers and stored under appropriate conditions. Ready-to-use sterile ampoules were used for the distilled water. The research nurse first swaddled the neonate, and, according to the particular study group, then put 0.2 mL of the solution, breast milk or distilled water into the injector. This study used the PIPP scale to measure outcomes.

Bucher 2000 (Heel lance) compared the effects of commercially available artificial sweetener (containing 10 parts cyclamate and one part saccharin) to glycine (sweet amino acid), expressed breast milk and sterile water. This was an RCT of 80 full-term neonates.

- Group 1: 2 mL of artificial sweetener via syringe (n = 20)
- Group 2: 2 mL of glycine via syringe (n = 20)
- Group 3: 2 mL of breast milk via syringe (n = 20)
- Group 4: 2 mL of sterile water via syringe (n = 20)

The neonates were studied on postnatal day four at the time of blood collection for routine neonatal screening using the heel lance procedure. 2 mL of the allocated solution was given via syringe on the anterior part of the tongue by a nurse not involved in the study. Prior to the procedure, baseline data were obtained, and continuous monitoring was done throughout and after the procedure during the recovery time. The blood collection was performed two minutes after administration of solution by a research nurse. The procedure was videotaped and evaluated by two independent observers unaware of allocation. The outcomes measured were heart rate change, percentage time crying, body pain score, facial pain score (five components of NFCS) and body pain score during and after blood collection (torso movements 1 = one side, 2 = both sides; head movements = 1; arm movements 1 = one arm, 2 = both arms; hand movements 1 = one hand, 2 = both hands; bringing hands to face (mouth) = 1 point; maximum score was 8 points, minimum score was 0 points). The data were presented in graphical format. We obtained numerical data by contacting the author.

Bueno 2012 (Heel lance) compared the efficacy of supplemental breast milk versus 25% glucose on pain responses of late preterm neonates during heel lancing. Eligible neonates were between 34 and 36 completed weeks of GA at birth; were between 24 and 72 hours old; had 5-minute Apgar scores of > 7; were fed at least 1 hour before data collection; had no syndromes, congenital anomalies or previous surgery; were not born to mothers with hepatitis C or HIV infection; were born to mothers not known to be users of illicit drugs; and had clinical indication for blood sampling. Infants were randomly divided into two groups.

- Group 1: Expressed breast milk (EBM) (n = 56)
- Group 2: Glucose (n = 57)

Interventions investigated were 2 mL of EBM and 2 mL of 25% glucose, applied via a needleless syringe to the anterior portion of the tongue two minutes before the lancing procedure. Outcomes measured for this trial were pain intensity assessed with the PIPP score, crying incidence and incidence of adverse events (e.g. nausea, regurgitation, vomiting, choking, desaturation, tachycardia and bradycardia).

Carbajal 2003 (Venipuncture) compared the effects of breastfeeding to positioning, sterile water and 30% glucose. This was an RCT of 180 term neonates. The infants were randomised to one of the following four groups.

- Group 1: Breastfeeding (n = 44)
- Group 2: Held in mother's arms without breastfeeding (n = 45)
- Group 3: Sterile water without pacifier (n = 45)
- Group 4: 30% glucose followed by a pacifier (n = 45)

In Groups 1 and 2, the interventions were started two minutes before the procedure and continued throughout the procedure. In groups 3 and 4, the intervention was commenced two minutes prior to the procedure. Venipuncture was performed when neonates were at least 24 hours of age and had not been fed for the previous 30 minutes. The primary outcome measure was the DAN scale (Carbajal 1997), a behavioural scale developed to rate acute pain in term and preterm neonates. The score is comprised of three items, namely facial expressions, limb movements and vocal expression, with values in each ranging from zero (no pain) to 10 (maximum pain). The secondary outcome measure was the PIPP score. Mothers were interviewed 48 to 72 hours after the study by standardised questionnaires to assess any change in the sucking behaviour. One neonate was excluded from the analysis as the outcome measure could not be assessed properly due to the mother's head partially covering her neonate's face. Data from all four groups were used in their respective appropriate comparisons.

Chiabi 2016 (Heel lance) compared the analgesic effect of breastfeeding and 30% glucose on pain induced in term newborns during a single painful procedure. It was an RCT of 100 neonates. Included participants were healthy term neonates of at least 24 hours of life. They first had a complete physical examination, and then a heel prick on the latero-external or the posteromedial surface using a 23 gauge needle. Breastfeeding or 30% glucose solution was then given. The newborns were divided into the two groups by drawing of lots.

- Group 1: Breastfeeding group (n = 50)
- Group 2: 30% Glucose solution group (n = 50)

In the breastfeeding group, mothers were placed in a calm room and the neonates placed on the breast after a period of about 30 minutes without feeding. Neonates were held in the arms of their mothers, and the heel prick was done two minutes later. In the 30% glucose solution group, neonates received two doses of 30% glucose solution in two minutes interval on the anterior surface of the tongue. The two doses were administered two minutes before doing the heel prick. Newborns weighing between 2.5 kg and 3 kg received 2 mL of the solution and those weighing more than 3 kg received 4 mL. Heel pricks were performed to determine the blood sugar. The outcome measured was NIPS to evaluate pain - at the baseline state, at the impact of the needle and two minutes after the capillary prick.

Codipietro 2008 (Heel lance) compared the efficacy of breastfeeding versus orally administered 25% sucrose solution.

- Group 1: Breastfeeding (n = 51)
- Group 2: Received 1 mL of 25% sucrose (n = 50)

Neonates underwent heel lance for routine newborn screening. Neonates in group 1 were held by mother and breastfed until there was a continuous active suction prior to heel lance. Group 2 neonates were laid on a changing table and a bolus of 1 mL of 25% sucrose solution was administered through a syringe in the mouth two minutes before the heel lance. The outcomes measured were the PIPP scale, changes in heart rate and saturation 30 seconds after the procedure, duration of first cry and percentage of crying in the first two minutes after the procedure. The procedure was taped (audio) and the tape recording was evaluated by two assistants (who were blinded to the groups) to assess crying behaviour.

Cordero 2014 (Heel lance) evaluated the effectiveness of a 24% oral glucose solution and breastfeeding during heel lance. This was an RCT in which a random sample of 93 newborns were taken from all the babies in neonatal care unit who were required to undergo the heel lance procedure. They were divided randomly into 3 groups.

- Group 1: Breast milk group (n = 31)
- Group 2: Oral 24% glucose group (n = 31)
- Group 3: Control group (received nothing) (n = 31)

The first group of newborns received breast milk; the second group was given a 24% oral glucose solution; and the third group, which was the control group, received nothing at all. Outcomes measured were change in heart rate before and after heel lance procedure and change in oxygen saturation.

Dar 2019 (Intradermal) compared effects of breastfeeding to routine care. In this RCT, neonates were randomly divided into two groups. Included infants were healthy full-term neonates who were partially or exclusively breastfed and who came for Bacillus Calmette–Guérin (BCG) vaccination to outpatient department.

- Group 1: Breastfeeding group (partial or exclusive) (n = 30)
- Group 2: Control group (only routine care; no breastfeeding) (n = 30)

The outcome measured for this study was duration of crying time in seconds.

Desai 2017 (Suctioning) assessed the pain associated with suctioning in preterm neonates on assisted ventilation and compared the use of expressed breast milk (EBM), sucrose and swaddling to alleviate pain. Included participants were preterm neonates on assisted ventilation requiring suction. Neonates were randomised into 3 groups.

- Group 1: Breast milk group (n = 36)
- Group 2: Swaddling group (n = 36)
- Group 3: Oral sucrose group (n = 36)

In the breast milk group, 2 mL of EBM was administered to the neonate two minutes before suctioning. In the swaddling group, neonates were swaddled for 10 to 15 minutes before suctioning, and in the oral sucrose group, 2 mL sucrose was administered to the neonate for two minutes before suctioning. The outcome measured was the Premature Infant Pain Profile Score.

Efe 2007 (Venipuncture) compared breastfeeding and 25% sucrose solution to reduce pain due to venipuncture in term neonates. They included 102 term neonates in a quasi-randomised trial.

- Group 1: Breastfeeding (n = 34)
- Group 2: 25% Sucrose solution (n = 34)
- Group 3: Control, no intervention (n = 34)

Neonates underwent venipuncture for routine screening of phenylketonuria and hyperbilirubinaemia. Neonates in group 1 were held in skin-to-skin contact with their mothers during the entire procedure. Three minutes after the first jaw movements were observed, the venous blood sample was taken. Neonates continued to breastfeed during and after the venipuncture. Group 2 neonates received 2 mL of 25% sucrose solution dipped into pacifiers. The neonates started to suck the pacifier with sucrose three minutes before the venipuncture and continued to suck during and after sampling. The control group neonates were wrapped in a blanket with only the hand that would be used for sampling outside the blanket. The mother stayed next to the infant trying to soothe him verbally. After the sample was collected, the neonate was cuddled by the mother and could be given a pacifier. The outcomes measured were NIPS, heart rate, oxygen saturation levels and duration of crying time. Crying time was assessed by audio tapes.

Erdogan 2022 (Venipuncture) evaluated the effects of breast milk taste and smell during blood draw on pain in healthy term neonates. This was a randomised controlled trial of 120 neonates. Participants included were term neonates (37 to 40 weeks of gestation) who underwent venipuncture. Neonates were randomised into four groups.

- Group 1: Breast milk taste (n = 30)
- Group 2: Breast milk smell group (n = 30)
- Group 3: Breast milk taste + smell (n=30)
- Group 4: Control (n = 30)

The interventions were started three or five minutes before blood collection. In the breast milk taste group (n = 30, mean GA in weeks 38.6, mean BW in grams 3185), 5 mL of milk was dripped into the infant's mouth to give the infant a taste of the breast milk. In the breast milk smell group (n = 30, mean GA in weeks 38.4 mean BW in grams 3221), 5 mL of milk-soaked cotton was placed at a 5 cm to 10 cm distance to the infant's nose for the infant to smell it. In the breast milk taste + smell group (n = 30, mean GA in weeks 38.4, mean BW in grams 3105), the procedures in both experimental group 1 and experimental group 2 were performed. In the control group (n = 30, mean GA in weeks 38.8, mean BW in grams 3044), no intervention was used. Outcomes measured were the Neonatal Pain, Agitation and Sedation Scale (N-PASS), oxygen saturations and heart rate.

Fallah 2017 (Intradermal) compared the analgesic effect of kangaroo mother care (KMC), breastfeeding and swaddling in healthy term neonates who received routine BCG vaccination in the first day after birth. Eligible participants included term neonates (GA of 37 to 42 weeks) who were born via normal vaginal delivery, awake and alert before vaccination, without systemic illness, in a

healthy medical condition and with birthweight of 2500 g to 4000 g. The neonates were randomly distributed into three groups.

- Group 1: Breastfeeding (n = 40)
- Group 2: KMC (n = 40)
- Group 3: Swaddling (n = 40)

In group 1, neonates were breastfed two minutes before, during and one minute after BCG vaccination. In group 2, neonates received KMC 10 minutes before, during and one minute after vaccination and in group 3, they were swaddled 10 minutes before, during and one minute after vaccination. Outcomes included NIPS score and duration of crying during BCG vaccination.

Gabriel 2013 (Heel lance) investigated the analgesic effects of breastfeeding (BF) in addition to skin-to-skin contact (SSC) versus other methods of non-pharmacological analgesia during blood sampling through heel lance in term neonates. Included infants were healthy term neonates (37 to 41 weeks of gestation) confirmed through a routine physical examination during the first 24 hours of life, with a wish to breastfeed and the absence of feeding during the previous 60 minutes. It was an RCT and neonates were randomly assigned to four groups:.

- Group 1: Breastfeeding and skin-to-skin contact (BF + SSC) (n = 35)
- Group 2: Sucrose + SSC (n = 35)
- Group 3: SSC (n = 33)
- Group 4: Sucrose (n = 33)

Mothers were allowed to speak to or touch their neonates in all the groups. In group 1, neonates dressed in a diaper were held in prone, in skin-to-skin contact with the mother; breastfeeding was started at least five minutes before heel lance and maintained during sampling. In group 2, neonates were held in prone between the mothers' breast at least five minutes before sampling and 2 mL 24% sucrose was given with a sterile syringe in the mouth two minutes before heel lance. In group 3, neonates were held between the mother's breast as in group 2, but no sucrose was given and in group 4, 2 mL 24% sucrose was administered through a sterile syringe in the mouth two minutes before heel lance to neonates laid supine on a cot; the procedure was done in the presence of the mother. Outcomes measured were pain score by NIPS, duration of crying, percentage of time crying and change in heart rate.

Gradin 2004 (Venipuncture) compared the effects of breastfeeding to sterile water and 30% glucose. This was an RCT where infants were randomised to four groups.

- Group 1: Breastfeeding and 1 mL of sterile water (n = 27)
- Group 2: Breastfeeding and 1 mL of 30% glucose (n = 29)
- Group 3: Fasting and 1 mL of sterile water (n = 26)
- Group 4: Fasting and 1 mL of 30% glucose (n = 29)

Neonates underwent a routine neonatal screening procedure using venipuncture at three to five days of age. The data from group 2 were not used for this review. For the breastfed group, the neonates were allowed breastfeeding ad libitum 45 minutes prior to blood sampling, while neonates in the fasting group had blood sampling performed at least two hours after the last feeding. 1 mL of either sterile water or 30% glucose was administered through a syringe into the neonates mouth, and one minute later the blood sampling was performed. After sampling, the neonates were left undisturbed for three minutes during the recovery phase. The outcomes measured were the PIPP score and mean crying time. Parents were asked to assess pain using a visual analogue scale (VAS). The agreement between the parental assessment of pain and the PIPP score and crying time was determined. The primary author provided missing data. Nine neonates were excluded from the study by the authors, mostly due to technical problems with the video recordings (n = 6) and maternal choice to withdraw their infants from the study (n = 3). Data from groups 1, 3 and 4 were used for this review as the combination of breastfeeding and glucose was not planned to be compared a priori.

Gray 2002 (Heel lance) compared the effects of breastfeeding to positioning. This was an RCT where full-term neonates were randomised to two groups (15 neonates in each group).

- Group 1: breastfed and cuddled with full body skin-to-skin contact (n = 15)
- Group 2: swaddled and placed on their side in the crib (n = 15)

All neonates underwent heel lance for routine neonatal screening procedure. Mean postnatal age at procedure was 46 hours in group 1 and 40 hours in group 2. The outcomes measured were differences in crying, grimacing and heart rate between the two groups before, during and after blood collection. The primary author provided additional information.

Hashemi 2016 (Intramuscular injection) investigated the effect of swaddling and breastfeeding, and their combined effect on the pain induced by BCG vaccination in healthy term neonates. This was an RCT in healthy term neonates. Included participants were full-term (37 to 42 weeks) neonates with postnatal age less than three days, no evidence of obvious abnormality or illness by the physician exam, Apgar score 7 to 10 at five minutes, no history of transfusions or invasive procedures except vitamin K injection, and breastfed at least once. Neonates were divided into four groups.

- Group 1: Breastfed (n = 33)
- Group 2: Swaddled (n = 34)
- Group 3: Combined group: swaddling and breastfeeding (n = 31)
- Group 4: Control group (n = 33)

In the breastfed group, neonates were breastfed within 45 minutes prior to vaccination and were not swaddled. In the swaddled group, neonates were swaddled a few minutes before vaccination and a few minutes later, while more than 45 minutes had passed from being breastfed. In the combined group, neonates were swaddled a few minutes before vaccination and a few minutes later, and breastfed within 45 minutes prior to vaccination. In the control group, neonates were vaccinated according to the hospital routine without any intervention. Outcomes reported in this study were pain score using NFCS, changes in heart rate and changes in $O_2$ saturation.

Holsti 2011 (Heel lance) compared the analgesic effects of breastfeeding with non-nutritive sucking. Included participants were neonates born between 30 and 36 completed weeks of gestation, normally breastfeeding infants, with blood collection required for clinical management on or after day 3 of life; mother/infant pairs were medically stable. It was an RCT and 57 neonates were randomly divided into two groups.

- Group 1: Non-nutritive sucking (n = 29)
- Group 2: Breastfeeding (n = 28)

In the non-nutritive sucking group, two minutes before the first contact by the laboratory technician, each neonate remained in their cot/isolette and was given a soother to suck on throughout the blood collection. The soother was held in the neonate's mouth by the research nurse to ensure that contact was maintained. In the breastfeeding group, five minutes before blood collection, neonates were given to their mothers. Two minutes before the heel lance, the mothers began breastfeeding their neonate and continued until the last contact by the laboratory technician. Outcomes reported were Behavioural Indicators of Infant Pain (BIIP), change in heart rate and Preterm Infant Breast-feeding Behaviour Scale (PIBBS).

Iqbal 2014 (Intradermal) assessed the efficacy of breastfeeding for pain relief during BCG vaccination. Participants were full-term neonates of gestational age between 38 and 42 weeks; had Apgar score of 7 and higher at five minutes after birth; delivered by spontaneous and vaginal delivery; were exclusively breastfed; postnatal age not more than 48 hours. It was an RCT and participants were randomly assigned to two groups.

- Group 1: Breastfeeding (n = 75)
- Group 2: Control (n = 75)

In the breastfeeding group, the mothers cradled their neonates in a breastfeeding position that maintained full-body, skin-to-skin contact during the entire procedure. A large amount of areola was placed into the neonate's mouth and two minutes after the first jaw movements were observed the BCG vaccine was given. Neonates were breastfeeding during and after the vaccination. The mothers were asked to continue breastfeeding their neonates even if they started to cry during and after the procedure. In the control group, the neonate was placed on the treatment table. The mother stayed next to the neonate and helped in holding the neonate. She tried to soothe the neonate verbally during and after the vaccination. The neonate was cuddled by the mother just after the injection. The outcome reported was pain score by DAN scale.

Jatana 2003 (Heel lance) compared the effects of breast milk versus different solutions of glucose. This was an RCT of 125 term infants, who were randomised to five groups.

- Group 1: Control, received 1 mL of sterile water (n = 25)
- Group 2: 1 mL of glucose 10% solution (n = 25)
- Group 3: 1 mL of glucose 25% solution (n = 25)
- Group 4: 1 mL of glucose 50% solution (n = 25)
- Group 5: 1 mL of expressed breast milk (EBM) (n = 25)

All neonates underwent heel lance for blood sampling. The solution tested was administered slowly over a period of 30 seconds by means of a syringe placed in the mouth. Two minutes after giving the oral solution, the heel lancing was performed. The outcomes assessed were duration of crying (first cry and total duration), change in heart rate, change in oxygen saturation and facial action score.

Kumar 2020 (Intramuscular injection) aimed to compare the effectiveness of breastfeeding with various non-pharmacological pain management methods in newborns. This study included term newborns (0 to 28 days old) receiving hepatitis B vaccination, with parents consenting to take part in the study. This was an RCT and 150 neonates were randomised into six groups.

- Group 1: Breastfeeding (n = 50). Breastfeeding started two minutes before the vaccination and continued until 120 seconds.
- Group 2: 25% sucrose (n = 50). 2 mL of 25% dextrose solution was given through mouth with sterile dropper two minutes prior to vaccination.
- Group 3: Distilled water (n = 50). 2 mL of distilled water was given through mouth with sterile dropper two minutes prior to vaccination.
- Group 4: Non-nutritive sucking (n = 50). A sterile silicon pacifier was held gently to stimulate sucking. Vaccination was given two minutes after the newborn started sucking, and it was continued until 120 seconds.
- Group 5: Rocking (n = 50). Newborns were given gentle rocking movement by lifting the head on the palm of the hand. Rocking started two minutes before vaccination and continued till 120 seconds.
- Group 6: None (n = 50). No intervention was used.

Outcomes reported were total cry duration and DAN score at 30, 60 and 120 seconds.

Lan 2021 (Heel lance) evaluated the efficacy of multisensory interventions that include breast milk odour, breast milk taste, gentle touch and verbal comfort on relieving newborn pain during blood draw in healthy term neonates. This was an RCT of 120 neonates. Participants included were term neonates (38 to 40 weeks of gestation) who underwent heel lance. Neonates were randomised into three groups.

- Group 1: Gentle touch and verbal comfort (n = 40)
- Group 2: Breast milk odour + gentle touch + verbal comfort (n = 40)
- Group 3: Breast milk odour + breast milk taste + gentle touch + verbal comfort (n = 40)

Duration of heel stick procedures was controlled at five minutes for each newborn of the three groups by adjusting the pressure on the heel to regulate blood flow. The heel stick procedures were divided into 11 phases: phase 1 (five minutes before heel stick without stimuli (baseline, mean per minute)), phases 2 to 6 (the first, second, third, fourth and fifth minutes during heel stick procedure), and phases 7 to 11 (recovery, from the time when the senior nurse completed blood sample collection to the end of the fifth minute after heel stick). Infants' behavioural and physiological responses to pain were recorded with the camera lens focused on the newborn's face, body and legs to collect data. Thirty minutes before heel stick, newborns in all three conditions were positioned supinely and supported with rolled towels.

In the gentle touch and verbal comfort group (routine care) (n = 40, mean GA in weeks 39.42, mean BW in grams 3121), a female nurse gently touched the head and spoke softly to comfort the newborn during and after the heel sticks for newborn screening.

In the breast milk odour + gentle touch + verbal comfort group (n= 40, mean GA in weeks 39.07, mean BW in grams 3109), breast milk was expressed manually immediately when the mother woke in the

morning (to reduce the influences of diet on the breast milk flavour) and was then refrigerated and stored in the nursery. Before the newborn screening, the breast milk was warmed up. Three minutes prior to the heel stick until the fifth minute of recovery, a cotton ball with the breast milk was put near the newborn's nostrils for breast milk odour. Gentle touch and verbal comfort were provided as described in group 1.

In the breast milk odour + breast milk taste + gentle touch + verbal comfort group (n= 40, mean GA in weeks 38.91, mean BW in grams 3070), for breast milk-taste, 3 ml of mother's breast milk were fed slowly through syringe dripping to the newborn's mouth two minutes before and during the heel stick. Gentle touch and verbal comfort were provided as described for group 1 and breast milk odour was provided as described in group 3. The outcome measured was the Neonatal Infant Pain Scale.

Leite 2009 (Heel lance) compared the effects of breastfeeding versus maternal holding in an RCT including 60 healthy term newborns.

- Group 1: Breastfeeding (n = 31)
- Group 2: Held by mother (n = 29)

Neonates underwent heel lance for routine newborn screening. Neonates in group 1 were held by the mother and were breastfeeding with effective sucking movements five minutes prior to the procedure. Group 2 neonates were held by the mother for the same length of time. The outcomes measured were NFCS and change in heart rate.

Leite 2015 (Intramuscular injection) compared the combination of skin-to-skin contact with breastfeeding to skin-to-skin contact during hepatitis B vaccination in newborns. Full-term newborns of up to 12 hours of life admitted to collective rooms, Apgar ≥ 7 at five minutes, exclusive breastfeeding on demand, who suckled the breast at least once after birth, and whose mothers had physical conditions to breastfeed were included in the study. This was an RCT and 55 neonates were randomised to two groups.

- Group 1: Breastfeeding with skin-to-skin contact (n = 27)
- Group 2: Skin-to-skin contact (n = 28)

In the breastfeeding with skin-to-skin contact group, newborns were kept in a common crib for five minutes (baseline period), and then put in skin-to-skin contact for five minutes, then combined with breastfeeding for 10 more minutes, and kept in this condition during periods of antisepsis/injection, compression and recovery (five minutes after the end of compression). In the skin-to-skin contact group, newborns were maintained for five minutes in a common crib (baseline period), and soon after, positioned in maternal skin-to skin contact for 15 minutes (treatment period), followed by the period of antisepsis/injection and compression with cotton soaked in 70% alcohol, and in skin-to-skin contact until five minutes after the procedure (recovery period). Outcomes measured were the NFCS score and change in heart rate. NFCS coding for this study was different. NFCS scores were displayed in terms of the maximum number of activities possible in each period. The denominator for the blood collection phase was reported as 24 (8 intervals of 2s x 3 phases of possible face action); for the other phases, the denominator was 30 (10 intervals of 2s x 3 phases of possible face actions x 10). The usual maximum for NFCS is 9 for preterm neonate and 10 for full term neonates but in this study it was reported differently.

Lima 2013 (Venipuncture) compared breastfeeding and non-nutritive stimuli efficacy in the newborn's response to pain during venipuncture. A random sample of rooming-in newborns with medical requests for venipuncture was chosen for the study. All newborns suffering from neurological damage, head and neck malformations, and heart diseases or with absence of sucking reflex or motion were excluded. The newborns were randomly divided into three groups.

- Group 1: Breastfeeding (n = 20)
- Group 2: Non-nutritive sucking (n = 21)
- Group 3: Control group (n= 23)

In the breastfeeding group, breastfeeding was started two minutes before the venipuncture, was continued through the puncture, and was maintained until one minute after the painful procedure completion. In the non-nutritive sucking group, newborns received non-nutritive sucking stimulation before and during the venipuncture. The non-nutritive sucking stimulus was performed through the introduction of the researcher little finger in the newborn's oral cavity. The finger was protected by non-surgical examination gloves. In the control group, participants did not receive any sucking stimulation. The outcome for this study was prevalence of pain by NIPS score.

Mathai 2006 (Heel lance) compared the effects of breast milk with 20% sucrose solution, distilled water, non-nutritive sucking, massaging and rocking. It was an RCT of 104 term neonates. The infants were randomised to one of the following groups.

- Group 1: Expressed breast milk (n = 18)
- Group 2: 20% sucrose solution (n = 17)
- Group 3: Distilled water (n = 15)
- Group 4: Non-nutritive sucking (n = 20)
- Group 5: Massaging (n = 17)
- Group 6: Rocking (n = 17)

Neonates underwent heel prick at more than 24 hours of age for collection of blood for bilirubin estimation. 2 mL of expressed breast milk, 20% sucrose or distilled water were administered in the neonate's mouth with a dropper. In the non-nutritive sucking group, a sterile pacifier was held gently in the neonate's mouth and the palate was tickled to stimulate sucking. This was continued during and for two minutes after the heel prick. In the massaging group, neonates were subjected to firm, gentle stroking with bare fingers in a rhythmical manner starting from the forehead and going down to the chest, arms and legs, during and for two minutes after the heel prick. In the rocking group, newborns were rocked by lifting the baby's head off the cot on the palm of the hand (without lifting the body off the cot) and making rocking movements in a gentle rhythmic manner for two minutes after the heel prick. The outcomes measured were duration of first cry, total crying time and DAN score at 30 seconds, one minute, two minutes and four minutes after the prick. For the purpose of this review, we analysed the DAN score at two minutes. Other outcome variables were heart rate increase and saturation reduction; however, results were not shown, but they commented that there were no significant differences.

Modarres 2013 (Intramuscular injection) examined the effect of breastfeeding on pain relief in full-term neonates during injection of hepatitis B vaccine. Included infants were full-term neonates, with an Apgar score of 7 or more at five minutes after birth, delivered by spontaneous and vaginal delivery, exclusively breastfed and of postnatal age not more than 24 hours. It was an RCT and 130 neonates were randomly divided into two groups.

- Group 1: Breastfeeding (n = 65)
- Group 2: Control (n = 65)

In the breastfeeding group, neonates were breastfed for two minutes before, during and after hepatitis B vaccination. At the end of the second minute of breastfeeding, while the neonates were still sucking, an experienced nurse performed the immunisation injections. In the control group, they were held in mothers' arms but not fed. Outcomes reported was DAN pain scale.

Nanavati 2013 (Adhesive tape removal) compared the pain relief effect of kangaroo mother care and expressed breast milk (EBM) on the pain associated with adhesive tape removal in very low birth weight (VLBW) neonates. VLBW infants requiring removal of adhesive tape (Micropore Medical Tape, 3M) during removal of intravenous cannula were included. Neonates with neurological abnormalities and major congenital defects and those receiving sedatives or analgesics were excluded from the study. It was an RCT and infants were randomly divided into two groups.

- Group 1: Kangaroo mother care (n = 25)
- Group 2: Expressed breast milk (n = 25)

In the kangaroo mother care group, the neonate was kept in kangaroo mother care for 15 minutes before the removal of the adhesive tape. In the EBM group, a swab soaked in EBM was kept in the neonate's mouth for two minutes before the removal of the adhesive tape and continued during the intervention. Outcomes reported were the PIPP score and its components.

Napiorkowska-Orkisz 2022 (Heel lance) evaluated the effects of breastfeeding, 20% glucose and non-nutritive sucking during heel lance on pain in healthy term neonates. This was a randomised controlled trial of 90 neonates. Participants included were term neonates (38 to 42 weeks of gestation) who underwent heel lance for newborn screening test. Neonates were randomised into three groups.

- Group 1: Breastfeeding (n = 30)
- Group 2: Oral 20% glucose (n = 30)
- Group 3: Non-nutritive sucking (n=30)

Group I (breastfeeding group) consisted of newborns who were attached to the mother's breast during the painful procedure. Group II (20% oral glucose) included those given 2 mL to 3 mL of 20% glucose orally. Group III (non-nutritive sucking) sucked a pacifier during the study. Outcomes measured were the Neonatal Infant Pain Scale during the procedure and heart rate variability.

Obeidat 2015 (Heel lance) studied breastfeeding with maternal holding as compared with maternal holding without breastfeeding in relieving painful responses during heel lance blood drawing in neonates. The study included full-term neonates (38 to 42 weeks of gestation) who underwent heel lance blood drawing for routine hypothyroidism screening, were aged four to six days, with no feeding occurring in the previous 30 minutes, and whose Apgar score ranged from "7 to 10" at "1 and 5" minutes. It was an RCT and eligible neonates were randomly divided into two groups.

- Group 1: Breastfeeding with maternal holding (n = 64)
- Group 2: Only maternal holding (n = 64)

In the breastfeeding with maternal holding group, neonates who underwent heel lance blood sampling were breastfed and held in their mothers' lap while their mothers were seated reclining on a comfortable chair. The mothers were instructed to continue breastfeeding and cuddling if the neonates started to cry during or after the heel lance blood drawing. In the only maternal holding group, neonates underwent sampling under the same conditions except for breastfeeding. The mothers were instructed to continue cuddling if the infants started to cry during and after the heel lance blood drawing. The outcome measured in this study was PIPP score.

Okan 2010 (Heel lance) compared skin-to-skin contact and breastfeeding with only skin-to-skin contact and no intervention (lying on the table). It was an RCT of 107 healthy, full-term neonates between 24 and 48 hours of age. Infants were randomised into three groups.

- Group 1: Breastfeeding with skin-to-skin contact (n = 35)
- Group 2: Held by mother with skin-to-skin contact (n = 36)
- Group 3: Lying on the table (n = 36)

Heel lancing was done for the purpose of metabolic newborn screening. Mothers and infants from groups 1 and 2 were left alone for 15 minutes to allow them to rest comfortably in skin-to-skin contact position. Mothers in group 1 were asked to begin to breastfeed their neonates during this time. In the no-contact group, tests were performed with the neonates lying on an examination table in a silent nursery. Neonates were wrapped in blankets and placed supine on the examination table. The outcomes measured were heart rate and saturation changes, total time of crying and NFCS in Groups 2 and 3 (calculated at the moment of heel lance, and after one, two, three, four and five minutes).

Ors 1999 (Heel lance) compared the effects of supplemental breast milk to water and 25% sucrose. This was an RCT of 102 healthy term neonates. The infants were randomised to three groups.

- Group 1: 25% sucrose (n = 35)
- Group 2: Breast milk (n = 33)
- Group 3: Sterile water (n = 34)

All infants underwent heel lance blood sampling by a single performer. The allocated solution was given by syringe into the neonates' mouth over one minute. The heel prick was performed two minutes after administration of the solution. Crying duration and heart rate at three minutes were recorded from the time of the heel prick. The outcomes measured were crying time, percentage change in heart rate and recovery time for the heart rate. The primary author provided additional information. Data from all three groups were used for this review in their respective appropriate comparisons.

Ou-Yang 2013 (Heel lance) compared expressed breast milk with distilled water and 25% glucose water for relief of procedural pain associated with heel lancing in preterm neonates. Included

neonates were preterm neonates (< 37 weeks GA) and age < 7 days at the time of study. Participants were randomly divided into three groups. All treatments were given by a single independent investigator as a 5 mL volume via a syringe tube inserted into the participant's oral cavity.

- Group 1: Distilled water (n = 44)
- Group 2: Expressed breast milk (n = 40)
- Group 3: 25% Glucose water (n = 39)

Outcomes measured in this study were duration of the first cry after heel lancing, pain scores (N-PASS: neonatal pain, agitation and sedation scale) at baseline and one, two and three minutes after heel lancing; and physiological parameters at baseline and three minutes after heel lance, heart rate, respiratory rate, oxygen saturations, systolic blood pressure and diastolic blood pressure.

Ozdogan 2010 (Heel lance) compared the effects of breast milk to sterile water and 12.5% sucrose solution. It was an RCT that included 142 healthy newborns. The infants were randomised to one of the six groups.

- Group 1: Single-dose of breast milk (n = 18)
- Group 3: Sterile water (n = 27)
- Group 3: Single-dose of 12.5% sucrose solution (n = 25)
- Group 4: Two doses of breast milk (n = 23)
- Group 5: Two doses of sterile water (n = 26)
- Group 6: Two doses of 12.5% sucrose solution (n = 23)

Infants underwent routine neonatal screening through heel lance. In all the groups, neonates received 2 mL of the test solutions through syringe onto the anterior part of the tongue, and they were not allowed to suck the syringe tip. In the single-dose groups, the test solution was given two minutes before the heel prick and in the repeated-dose groups the dose was repeated just prior to heel prick. The outcomes measured were total crying time and NFCS at zero, one, two and three minutes. For the purpose of this review, we analysed the NFCS values at two minutes.

Peng 2018 (Heel lance) compared the effects of combined non-nutritive sucking breast milk, non-nutritive sucking + breast milk + tucking, and routine care on preterm infant pain during and after heel stick procedures. Included participants were neonates born at GA 27 to 37 weeks and postmenstrual age 27.4 to 38 weeks, post birth age 3 to 28 days, and disease condition acceptable for observation (illness severity indicated by the Neonatal Therapeutic Intervention Scoring System score ≤ 20). It was an RCT and eligible neonates were randomly assigned to three conditions.

- Group 1. Routine care (n = 36)
- Group 2. Non-nutritive sucking + breast milk (n = 37)
- Group 3. Non-nutritive sucking + breast milk + tucking (n = 36)

The outcome measured in this study was the PIPP score.

Phillips 2005 (Heel lance) compared the effects of breastfeeding in three groups in an RCT of 96 healthy term neonates.

- Group 1: Breastfeeding (n = 32)
- Group 2: Neonates held by mother holding pacifier in infant's mouth (n = 39)
- Group 3: Neonates held by research assistant holding pacifier in infant's mouth (n = 25)

All neonates underwent heel lance blood sampling by a single performer. Mothers held neonates in their bed while giving pacifier (group 2) while research assistant held neonates in bedside chairs (group 3). The outcomes measured were crying duration, percentage of infants crying, changes in the heart rate, blood pressure and oxygen saturation. The primary author provided additional information. The purpose of studying three groups was to assess the differences in outcome measures caused by one of the components of the act of breastfeeding (maternal contact).

Rawal 2018 (Heel lance) compared the effect of 25% dextrose and EBM in pain relief during heel lance in late preterm neonates. Included neonates were haemodynamically stable newborns, who had not received any painful stimulus in the last 30 minutes, and had received feeds at least one hour before data collection. The 63 eligible neonates were randomised into three groups. Sterile water (group 1) was the control group and 25% dextrose (group 2) and EBM (group 3) were the intervention groups.

- Group 1: Sterile water group (n = 21)
- Group 2: 25% Dextrose group (n = 21)
- Group 3: EBM group (n = 21)

The enroled neonates were administered either 2 mL of test solution (25% dextrose or EBM) or sterile water orally. Outcomes measured in this study were PIPP score and heart rate and oxygen saturation change during the test.

Rioualen 2018 (Venipuncture) compared the efficacy of sucrose administration versus breastfeeding to decrease cortical responses to pain during venipuncture. Included infants were healthy, three day-old breastfed full term newborn infants (> 37 weeks' gestation). These 113 neonates were randomly assigned to either a breastfed group or a sucrose-administered group, which involved holding and non-nutritive sucking.

- Group 1: Breastfeeding group (n = 57)
- Group 2: Sucrose group (n = 56)

In the breastfeeding group, neonates were placed on their mother's chest and breastfed two minutes before and throughout the procedure. In the sucrose group, neonates were placed on their mother's arms and were given 2 mL of 24% sucrose two minutes before blood sampling. Neonates in this group were allowed to suckle their mother's finger throughout the procedure to avoid the confounding soothing effect of sucking in the breastfed group. Outcomes measured in this study were cortical responses to pain by recorded by near infrared spectroscopy (NIRS), pain score by NFCS, salivary cortisol level (mcg/dL) and skin conductance (mean peak/second).

Rodrigues 2017 (Suctioning) compared expressed breast milk and 25% dextrose for their analgesic efficacy during nasopharyngeal suctioning in preterm neonates on CPAP. Preterm neonates (< 37 weeks of gestation) who required CPAP were included in this study. Neonates with perinatal asphyxia (Apgar score at five minutes < 5/10), neuromuscular disorders, receiving analgesics, sedatives or anticonvulsants and those who were nil per os were excluded from the study. This was an RCT and 40 eligible neonates were randomised to one of two groups.

- Group 1: 25% Dextrose (n = 20)
- Group 2: Expressed breast milk (n = 20)

Neonates in group 1 received 25% dextrose orally during the first suctioning while neonates in group 2 received EBM orally. No intervention was given during the second suctioning. There was a gap of at least 24 hours between the first and second suctioning. For the second suction, the same procedure was followed, except that the neonates received standard care (no milk or 25% dextrose). In the intervention arm responses associated with first suctioning were included. We did not include groups during second suctioning.

Thus, during second suctioning, neonates served as their own controls. The outcome measured in this study was pain score by PIPP.

Rosali 2015 (Eye examination) assessed the effectiveness of EBM vs standard practices on neonatal pain during screening for retinopathy of prematurity (ROP). Included infants were babies with a GA of less than 35 weeks (as assessed by New Ballard Score), BW less than 2000 g, requiring an ROP screening and who were on at least partial oral feeds. It was an RCT and neonates were randomly divided into two groups.

- Group 1: EBM + standard practice (n = 20)
- Group 2: Control (only standard practice) (n = 20)

In the intervention group, neonates were given 2 mL of EBM orally by paladai (a small cup used to feed neonates) two minutes prior to the procedure along with standard practice. Neonates in the control group received only standard practice. Standard practice included that infants were nested, swaddled and received topical proparacaine. The outcome measured in this study was the PIPP scale.

Sabety 2013 (Venipuncture) compared the effects of oral glucose (50%), topical application of lidocaine, EBM and nothing per oral for reducing pain before venipuncture. Included neonates were term neonates (38 to 42 weeks GA), without evidence of poor feeding, in stable condition, receiving per oral feeding, with five minute APGAR > 7 and no history of narcotic usage. It was an RCT of 121 term neonates who were randomly divided into four groups.

- Group 1: Glucose
- Group 2: Lignocaine
- Group 3: Breast milk
- Group 4: Control

In group 1 participants, 2 mL glucose 50% was administered orally, two minutes before procedure. In group 2 (lignocaine group), 1 g of lidocaine gel (2%) was applied topically 13 minutes before venipuncture. In the breast milk group, 2 mL breast milk was administered orally via syringe at two minutes before venipuncture, and in group 4, routine venipuncture was performed without adding anything. Outcomes reported were crying time in seconds, DAN score, changes in respiratory rate and heart rate.

Sahoo 2013 (Venipuncture) compared the effect of EBM, 25% dextrose (25 D) and sterile water (SW) on procedural pain in neonates. Included infants were neonates born at ≥ 34 weeks gestation that required venipuncture for blood sampling and who were on oral feeds. It was an RCT and 160 eligible neonates were randomised into three groups.

- Group 1: Expressed breast milk (n = 62)
- Group 2: 25% Dextrose (n= 50)
- Group 1: Sterile water (n = 48)

The neonates requiring venipuncture were taken to a quiet room. The time interval between the procedure and previous breast milk intake was maintained for at least one hour. 2 mL of test solution was administered to the neonate through a sterile paladai (a traditional cup with a spout) by mouth by one staff nurse. The excess amount of test solution and the paladai were cleared. Outcome measures in this study were change in heart rate, change is oxygen saturation, crying time in seconds and pain score by PIPP.

Shendurnikar 2005 (Heel lance) compared the effects of breastfeeding to positioning (swaddling). The authors provided details about the study as it was published as a letter to the editor. This was an RCT of 100 full-term neonates. The infants were randomised to two groups (50 neonates in each group).

- Group 1: Breastfeeding (n = 50)
- Group 2: Swaddled and placed in a cradle (n = 50)

Infants in group 1 were breastfed for 15 minutes prior to the heel prick. All neonates underwent a heel lance procedure for clinical indication such as measurement of packed cell volume or bilirubin. The outcomes measured were behavioural (state of arousal, cry, facial expression, body movements); physiological (breathing pattern, heart rate) and composite score (non-validated) between the two groups before, during and after blood collection. The primary author provided additional information. The composite score was calculated using the following criteria.

- Heart rate (0 ≤ 120/minute; 1 = 120 to 160/minute and 2 ≥ 160/minute)
- Breathing (0 = relaxed; 1 = changed)
- Facial expression (0 = relaxed; 1 = grimaced)
- Body movements (0 = relaxed; 1 = no gross movement; 2 = gross body movement)
- State of arousal (0 = sleepy; 1 = awake; 2 = fussy)
- Cry (0 = no; 1 = whimper; 2 = vigorous) and combining the score

The minimum score was 0 and maximum score was 10. This study was published as a letter to the editor and authors provided additional data.

Simonse 2012 (Heel lance) investigated whether breast milk (either breastfed or bottle-fed) has a better analgesic effect than sucrose in newborns. Included infants were those born at a gestational age between 32 + 0 and 36 + 6 weeks, being nourished with breastfeeding, and had to have a clinical blood sample taken. It was an RCT and 71 neonates were randomly divided into three groups.

- Group 1: Breastfeeding (n = 23)
- Group 2: Bottle-fed (n = 23)
- Group 1: Sucrose solution (n = 25)

In the breastfeeding group, neonates were held in their mother's arms. In the bottle-fed group, neonates were held in the arms of an experienced nurse and were given supplemental breast milk by a sterile syringe. In the sucrose solution group, neonates lay in their cots and received 1 mL to 2 mL of 24% sucrose solution two minutes before the heel lance, combined with non-

nutritive sucking. Outcomes reported were pain assessed by the COMFORTneo scale and Pain score by PIPP.

Singh 2017 (Heel lance) investigated the analgesic effects of breastfeeding during blood sampling through heel lance in healthy term neonates. Included neonates were healthy, full-term, non-asphyxiated neonates up to seven days of life who were scheduled to receive heel sticks to collect blood for obligatory newborn screening, who were haemodynamically stable and were not receiving oxygen or any analgesia. It was an RCT and neonates were randomised into two groups (30 neonates each).

- Group 1: Breastfeeding (n = 30)
- Group 2: Control (n = 30)

The neonates in the breastfeeding group received breastfeeding during the heel prick procedure. Breastfeeding was initiated two minutes before the procedure and continued throughout. The neonates in the control group were not breastfed during the heel prick procedure. In the control group they were held in their mother's arms without breastfeeding; this was done two minutes before the heel prick. Reported outcomes in this study were total duration of cry over a 10-minute period after heel prick (seconds), change in heart rate, mean fall in transcutaneous oxygen saturation and mean change in blood pressure (both systolic and diastolic blood pressure).

Skogsdal 1997 (Heel lance) compared the effects of no intervention to 30% oral glucose, 10% oral glucose and breast milk. This was an RCT of 120 neonates (66 preterm neonates between 30 and 37 weeks and 54 term neonates). The neonates were randomly assigned to one of the following groups (30 neonates in each group).

- Group 1: no intervention (n = 30)
- Group 2: 1 mLof 30% glucose via syringe (n = 30)
- Group 3: 1 mL of 10% glucose via syringe (n = 30)
- Group 4: 1 mL of breast milk via syringe (n =30)

The neonates were studied on postnatal day five at the time of blood collection for their routine care using the heel lance procedure. 1 mL of the allocated solution was given via syringe by a nurse not aware of allocation. Prior to the procedure, baseline data were obtained, and continuous monitoring was done throughout and after the procedure during the recovery time. The blood collection was performed two minutes after administration of solution. The outcomes measured were heart rate change and duration of crying. The data were presented in graphical format, however the contact author provided the data necessary for the review.

Soltani 2018 (Heel lance) compared four methods of relieving infants' pain, i.e. breastfeeding, oral 25% dextrose, kangaroo mother care and EMLA cream based on a pain score level following heel prick sampling in term newborns. Infants included were with GA of 37 to 42 weeks, Apgar score of ≥ 9 at birth, birth weight of 2500 g to 4000 g, and ages between three and five days who were candidates for heel prick sampling. This was an RCT and infants were allocated randomly to four groups of interventions.

- Group 1: Breastfeeding (n = 42)
- Group 2: 25% Dextrose (n = 40)
- Group 3: Skin contact (kangaroo mother care method - KMCM) (n = 38)
- Group 4: EMLA ointment (n = 41)

All interventions were applied 15 minutes before heel prick procedure. Outcome reported were NIPS and total pain score.

Sujatha 2018 (Intradermal) aimed to identify the effect of facilitated tucking and oral administration of expressed breast milk with control group for pain management amongst neonates of study groups who received BCG vaccine. Included newborns were of 37 weeks of gestation and 145 infants were randomly divided into two groups.

- Group1: Breast milk (n = 45)
- Group 2: Control (n = 100)

In the breast milk group, 1 mL of breast milk was administered to the neonates of through a sterile disposable syringe from one corner of the mouth. In the control group of facilitated tucking, infants received routine care. Outcomes reported were change in oxygen saturation, change in heart rate, change in respiratory rate, pain score by NIPS and duration of cry in seconds.

Taplak 2017 (Eye examination) aimed to determine the effects of breast milk and sucrose in reducing pain in preterm neonates during retinopathy of prematurity (ROP) examination. Included neonates were preterm with a birth weight < 1500 g and gestational age of < 32 weeks. Preterm neonates diagnosed with a congenital anomaly, hydrocephalus, necrotising enterocolitis, indirect hyperbilirubinaemia and receiving ventilatory support or analgesic drug treatment were excluded. This was an RCT and neonates randomly divided into three groups.

- Group1: Breast milk (n = 20)
- Group 2: Sucrose (n = 20)
- Group 3: Control (n = 20)

Group 1 was provided with 1 mL breast milk, group 2 was provided with 1 mL 33% sucrose and Group 3 was provided with 1 mL distilled water via nipples + injector. Outcomes measured in this study were changes in oxygen saturation, in heart rate and pain score by PIPP.

Tavlar 2021 (Heel lance) evaluated the effects of breastfeeding, breast milk and maternal heart beats during heel lance on pain in healthy term neonates. This was a randomised controlled trial of 90 neonates. Participants included were term neonates (38 to 41 weeks of gestation) who underwent heel lance for blood draw. Neonates were randomised into three groups.

- Group 1: Breastfeeding (n = 30)
- Group 2: Breast milk odour (n = 30)
- Group 3: Mother's heart beats (n=30)

In the breastfeeding group (mean GA 39.3 in weeks, mean BW in grams 3191), newborns were breastfed starting from three minutes before to three minutes after the procedure. The right breast was preferred so that the newborn would not be affected by the mother's heartbeat. In the breast milk odour group (mean GA in weeks 39.3, mean BW in grams 3219), a total of 5 ml of breast milk sample was extracted from each baby's own mother before the procedure and poured onto a sterile odourless cloth. Then, the cloth was placed 3 cm from the newborn's nose and remained

there starting from three minutes before to three minutes after the procedure. In the mother's heartbeat sounds group (mean GA in weeks 39.3, mean BW in grams 3259), before the procedure, the maternal heartbeat sounds of each newborn were recorded from their mothers by means of a foetal hand Doppler. The mother's heartbeat sound was listened to the newborn starting from three minutes before to three minutes after the procedure (maximum sound level: 60 dB). Outcomes measured were ALPS-Neo score, heart rate, oxygen saturations and total crying time in seconds.

Turan 2021 (Eye examination) investigated the effect of breast milk and sucrose on pain scores and perfusion index (PI) during retinopathy of prematurity (ROP) examination. Participants were hospitalised neonates in the neonatal intensive care unit, whose GA was < 32 weeks and BW was < 1500 g. The preterm neonates (n = 51) who would undergo ROP examination were allocated to three groups according to simple randomisation method as follows.

- Group 1: Proparacaine HCl ophthalmic solution 0.5% only (n = 17)
- Group 2: Proparacaine HCl ophthalmic solution 0.5% plus breast milk (n = 17)
- Group 3: Proparacaine HCl ophthalmic solution 0.5% plus sucrose 24% (n = 17)

The pupils of the neonates were dilated using 2.5% phenylephrine and 0.5% tropicamide three times in five-minute intervals. Preterm neonates in all groups were administered one drop of proparacaine HCl 30 seconds prior to an eye examination. Preterm neonates in group 2 were given 2 mL of breast milk two minutes before the eye examination and the neonates in group 3 were administered 0.3 mL of sucrose 24% onto the anterior part of the tongue two minutes before the eye examination. Outcomes measured in this study were changes in oxygen saturation, heart rate, PI and NIPS.

Upadhyay 2004 (Venipuncture) compared the effects of supplemental breast milk to sterile water. This was an RCT of 87 full-term neonates. The neonates were randomised to two groups.

- Group 1: Expressed breast milk (n = 40)
- Group 2: Distilled water (n = 41)

Venipuncture was performed based on clinical indications. Three neonates from each group were excluded from the study by the authors due to venipuncture failure and failure to attain state 3 or 4 of wakefulness. Data from 81 neonates were analysed. The primary outcome was the duration of the cry after the venipuncture. The secondary outcomes included changes in physiological parameters, namely heart rate and oxygen saturation from baseline to one and three minutes after venipuncture and the modified NFCS. Only five easily recordable parameters of the NFCS (out of 10) were assessed by the investigators. Data on heart rate and oxygen saturation were provided as mean and SD at baseline and three minutes. We contacted authors to provide data on mean changes in these parameters, but no response was obtained. We calculated the MD and SD of the difference assuming 50% correlation between baseline and subsequent findings.

Uyan 2005 (Heel lance) compared the effects of supplemental breast milk (two groups fore-milk and hind-milk) to water. This was a quasi-RCT of 62 healthy term neonates. The infants were randomised to three groups.

- Group 1: Fore milk (n = 20)
- Group 2: Hind milk (n = 21)
- Group 3: Sterile water (n = 21)

All neonates underwent heel lance blood sampling by a single performer. The allocated solution was given by syringe into the baby's mouth. The heel prick was performed two minutes after administration of the solution. Crying duration and heart rate changes at one, two and three minutes were recorded from the time of the heel prick. The outcomes measured were crying time, percentage change in heart rate and NFCS at one, two and three minutes. The data from groups 1 and 2 were combined for the analyses. Authors provided data on combined groups.

Velumula 2022 (Heel lance) evaluated the effects of breast milk and 24% sucrose during heel lance on pain in preterm neonates. This was a randomised controlled trial of 88 neonates. Participants included were pre-term neonates (born between 30 weeks and 1 day to 36 weeks and 6 days of gestation) who underwent heel lance for blood draw. Neonates were randomised into two groups.

- Group 1: Breast milk (n = 44)
- Group 2: 24% Sucrose (n = 44)

In the breast milk group (mean GA in weeks 33.1, mean BW in grams 1869), neonates received 2 mL expressed breast milk via syringe, combined with swaddling, two minutes prior to scheduled heel lance. In the 24% oral sucrose group (mean GA in weeks 32.7 mean BW in grams 1948), 0.5 mL of 24% sucrose via syringe was administered orally, combined with swaddling, two minutes prior to scheduled heel lance. Outcomes measured were PIPP score and heart rate.

Weissman 2009 (Heel lance) compared breastfeeding, formula feeding, a 30% glucose solution, holding by mother and non-nutritive sucking with a control group in a total of 180 term neonates in a quasi-RCT.

- Group 1: Breastfeeding (n = 31)
- Group 2: Formula feeding (n = 30)
- Group 3: 2 mL of 30% glucose solution (n = 31)
- Group 4: Infants were held by their mothers (n = 29)
- Group 5: Non-nutritive sucking with pacifier (n = 30)
- Group 6: Control (no intervention) (n = 29)

All neonates underwent heel lance for routine neonatal screening. They were assigned to the six groups according to the mothers' preference. For neonates in group 3, the solution was given orally two minutes before the procedure, infants in group 2 were fed formula while in their cribs. The outcomes assessed were NFCS, through video recording, duration of cry and heart rate increase. The intervention was not blinded.

Wu 2021 (Heel lance) evaluated the effects of embraced breastfeeding heel lance on pain in neonates. This was a randomised controlled trial of neonates. Participants included were preterm neonates who underwent heel lance for newborn screening test. Neonates were randomised into two groups.

- Group 1: Embracing breast milk sucking (EBMS) (n = 48)
- Group 2: Control (n = 48)

In the embracing breast milk sucking group (n = 48, mean BW in grams 3290), neonates were given EBMS as follows. First, the room temperature and humidity were controlled before blood collection. After the neonates were bathed, they were placed on the mother's chests for skin-to-skin contact. Medical staff guided the mothers to simulate the process of communicating with the newborn during pregnancy, and comforted the newborn. In the control group (n = 48, mean BW in grams 3215), routine interventions were provided as follows: prior to heel blood sampling, the medical staff controlled the indoor temperature to about 23 °C and about 60% humidity, dedicated medical staff bathed the newborns and disinfected and collected blood from the heels of the newborns after the bath; and the newborn was wrapped in a quilt during blood collection to keep them warm. Outcomes measured were Neonatal Infant Pain Scale, total crying time in seconds, oxygen saturations and heart rate.

Yilmaz 2011 (Heel lance) compared the effects of supplemental breast milk to 20% sucrose, pacifier and a control group with no intervention. It was an RCT of 120 healthy term newborns. The neonates were randomised to four groups.

- Group 1: Breast milk (n = 30)
- Group 2: 20% Sucrose (n = 30)
- Group 3: Pacifier (n = 30)
- Group 4: No intervention (n = 30)

All neonates underwent heel lance blood sampling. The allocated solution was given by syringe into the neonate's mouth two minutes prior to the heel prick, avoiding contact of the syringe with the mouth and lips. Heart rate, respiratory rate, body temperature and saturation changes were measured. The neonates were videotaped to assess the behavioural responses through the NIPS.

Yilmaz 2020 (Heel lance) aimed to experimentally determine effects of three different methods (swaddling; swaddling and holding; swaddling, holding and breastfeeding) used during heel lancing on pain levels in healthy term neonates. Included neonates in this study were two to four days old (38 to 42 weeks gestation) who needed a routine heel lance. This was an RCT that randomised 160 newborns into four groups.

- Group 1: Swaddling (n = 40)
- Group 2: Swaddling and holding (n = 40)
- Group 3: Swaddling, holding and breastfeeding (n = 40)
- Group 4: Control (n = 40)

In the swaddling group, swaddling was carried out one minute before the heel stick procedure and continued two minutes after the procedure. In the swaddling and holding group, the mothers sat down on a comfortable chair and following the safe swaddling of the newborns, the neonate was placed in the mothers arms and the heel lance process was applied. In the swaddling, holding and breastfeeding group, the neonate was first swaddled, then held by mother and breastfeeding began immediately before the heel lance procedure (about one minute before) and continued for a minimum of two minutes during and after the procedure. In the control group, no intervention was performed for the newborns during the procedure. Outcomes measured in this study were NIPS total score, total crying time in seconds and time to first calming.

Zargham-Boroujeni 2017 (Venipuncture) compared the effects of massage and breastfeeding on the pain of the neonates. Included neonates were conscious neonates, neonates aged > 34 weeks, term and near-term neonates, with no limitation for breastfeeding, being on mother's milk feeding, having experience of being fed by mothers' breasts, with no paralysis in limbs, or major congenital abnormalities such as Down syndrome and asphyxiation, being relaxed, no cry before venipuncture and a need for venipuncture. This was an RCT and 75 neonates were randomly divided into 3 groups.

- Group 1: Breastfeeding (n = 25)
- Group 3: Massage (n = 25)
- Group 4: Control (n = 25)

In the breastfeeding group, the mother started breastfeeding until the researcher observed active sucking. This was continued for three minutes and then venipuncture was administrated. In the massage group, effleurage massage technique (stroking) was administrated on venipuncture site for three minutes and then venipuncture was administrated. In the control group, no intervention was administrated to manage pain and then venipuncture was done. The outcome reported in this study was mean NIPS pain score.

Zhu 2015 (Heel lance) tested the effectiveness of breastfeeding, music therapy, and combined breastfeeding and music therapy on pain relief in healthy term neonates during heel lance. Included neonates in this study were born at ≥ 37 weeks gestation, Apgar scores ≥ 7 at five minutes after childbirth, age ≥ 24 hours, weight between 2000 g and 4000 g, passed the hearing screen, undergoing heel lancing for metabolic screening between three and five days after childbirth, breastfed and had not been fed for the previous 30 minutes. This was an RCT and 250 neonates were divided into four groups.

- Group 1: Music therapy (n = 62)
- Group 2: Breastfeeding (n = 64)
- Group 3: Music therapy and breastfeeding (n = 63)
- Group 4: Control (n = 61)

In the music therapy group, classical music pieces were played on a loop at least five minutes before heel lance and maintained during blood sampling. The music speakers were placed bilaterally and kept 20 cm from the neonates' heads. In the breastfeeding group, neonates were breastfed in their mothers' arms, starting five minutes before the procedure and continuing throughout. Mothers were allowed to speak to their neonates in the breastfeeding group. In the breastfeeding with music therapy group, neonates were breastfed, and classical music was played to them at the same time. In the control group, neonates received routine care. Outcomes reported in this study were Neonatal Infant Pain Scale score, latency to first cry in seconds and duration of first cry in seconds.

### Excluded studies

We excluded nine studies.

We excluded Bilgen 2001 from the review because it is a duplicate publication of the same data reported by Ors 1999 (Heel lance). We excluded three reports because they were not RCTs (Iturriaga 2009; Osinaike 2007; Hsieh 2018), and excluded a further report because it studied infants between two and four months of age (Efe 2007a).

We excluded Cirik 2020 because the group used an oro-gastric tube for feeding. We excluded Erkul 2017 because it studied term infants up to two months of age. We excluded Shukla 2018 because all groups received expressed breast milk as a baseline pain control intervention. Wu 2020 was excluded because the study group used breast milk odour or taste, and it was unclear about consistent use of breast milk for taste.

See Characteristics of excluded studies for further details.

### Risk of bias in included studies

The methodological quality of the reviewed studies is shown in the Characteristics of included studies table, and risk of bias is reported in Figure 2 and Figure 3. We extracted the information from the published paper and by contacting the primary authors. The risk of bias for the individual included studies is described below.

**Figure 2. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.**

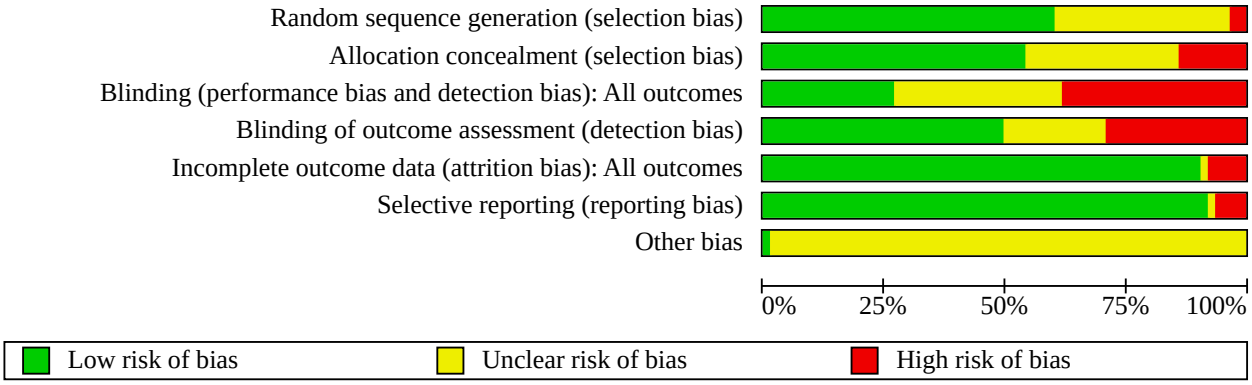


**Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.**

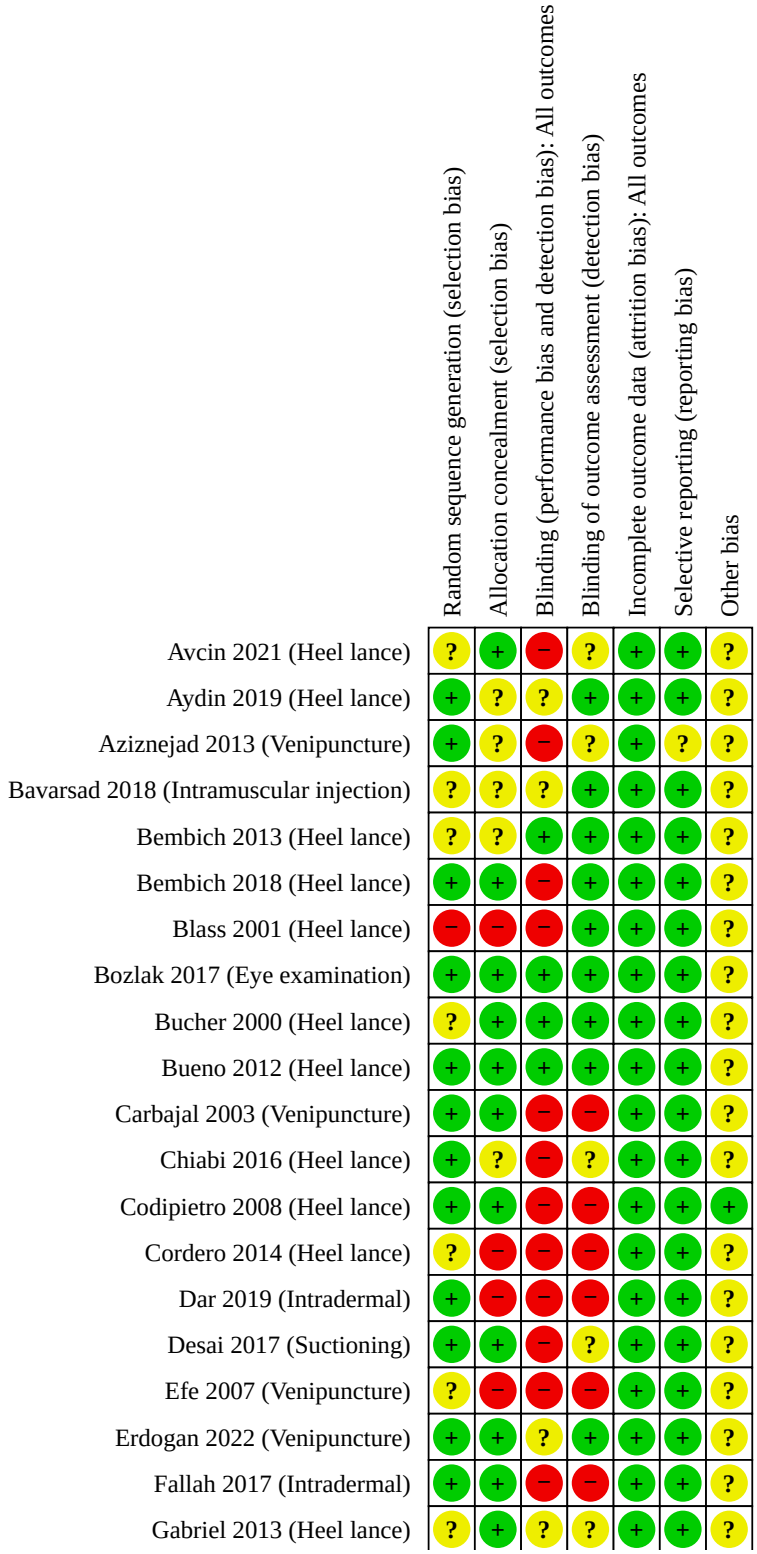


**Figure 3. (Continued)**

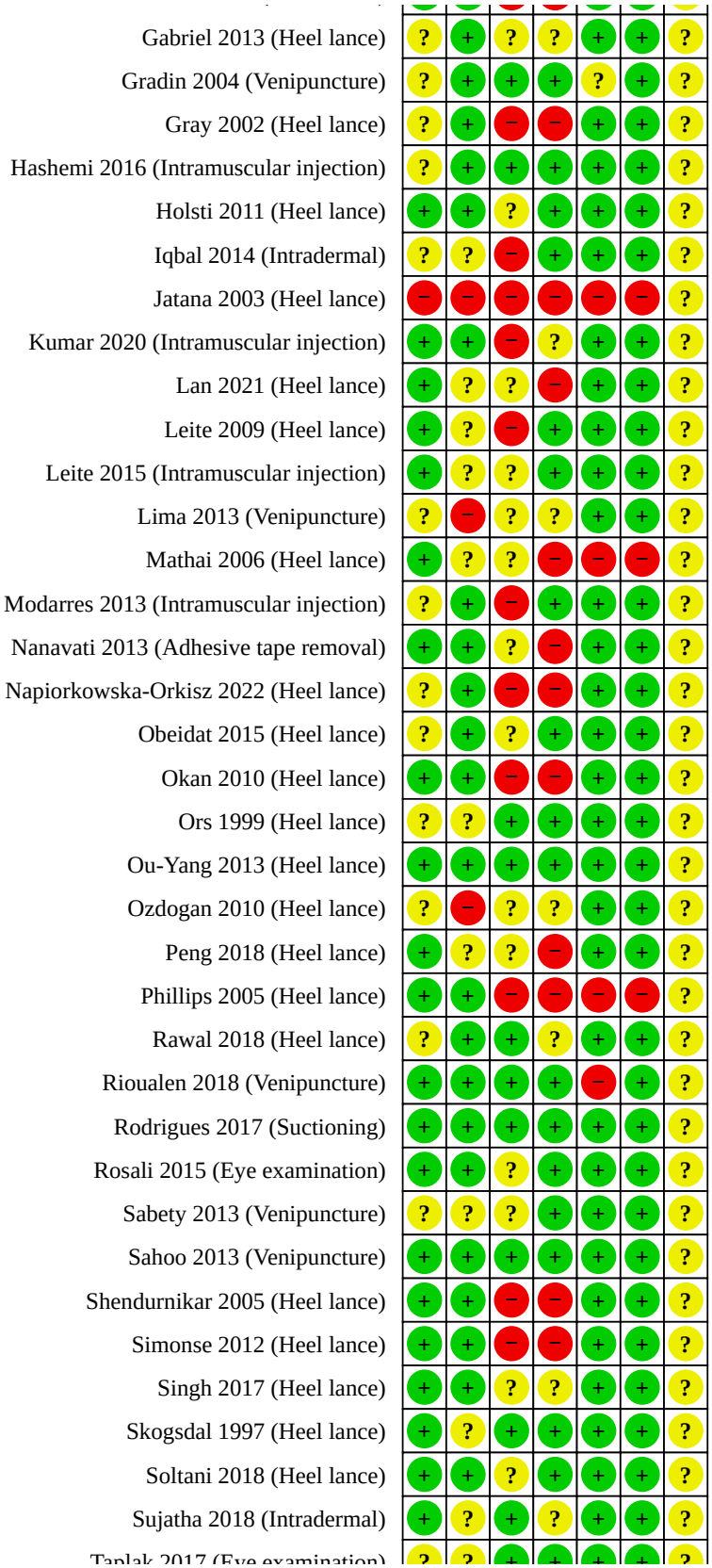
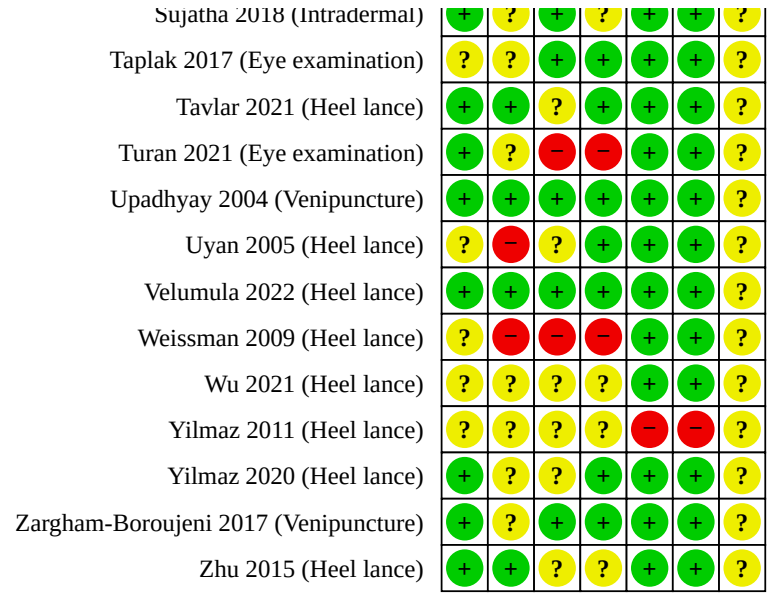


**Figure 3. (Continued)**




Avcin 2021 (Heel lance) was a quasi-randomised controlled trial. The group was determined by drawing lots. Cards with A (Foetus Position), B (Breastfeeding Group), C (Kangaroo Care) and D (Control Group) written on them were put into a bag and draw of a card determined which of the pain relief methods was to be used on the participant. Blinding was done for the parents and the researcher. Physiological parameters were measured by the researcher before and one minute after heel stick. Since the blinding expected to be done in randomised controlled studies could not be performed in this study, the study was expressed as quasi-experimental. It is unclear if intervention and outcome assessors were masked. All infants were included in the final analysis. All outcomes were reported.

Aydin 2019 (Heel lance): randomisation of the experimental and control groups was performed by the researcher using a computer-based random number table program. The researcher randomly picked one code for each neonate to ensure the 150 neonates were equally allocated into three groups based on the group number of each code. However, method of allocation concealment was not clear. Assessment of pain using NIPS was conducted independently by an observer nurse, who was blinded to the group allocation of the newborns. All infants were included in the final analysis. All outcomes were reported.

Aziznejad 2013 (Venipuncture): random allocation in blocks was carried out to assign samples to four groups. Simultaneously with inserting the needle, the DAN score was observed by a single trained nursing expert (a person other than the sampler) through three separate parameters of facial movements, body movements and noise level. Method of allocation concealment was unclear. It is not clear if other outcome assessments like heart rate and respiration per minute, percentage of arterial blood oxygen saturation and the duration of crying were masked. All infants were included in the final analysis. All outcomes were reported but the actual numbers of two outcomes (heart rate and oxygen saturation) are not available. The protocol was not available for comparison.

Bavarsad 2018 (Intramuscular injection): infants were randomised, but the method was not clear. We also cannot tell if allocation concealment was performed. Two nurses, who were blinded to the aim and methods of the study, were trained, and they measured the scores separately. It is unclear if intervention was masked. All infants were included in the final analysis. All outcomes were reported.

Bembich 2013 (Heel lance): infants were randomised, but the method was not clear. We also cannot tell if allocation concealment was performed. The assignment to a specific non-pharmacologic intervention was randomised. Assessments were performed by an investigator blinded to NIRS detection. All infants were included in the final analysis. All outcomes were reported.

Bembich 2018 (Heel lance): an independent statistician created a computer-generated, randomised treatment assignment list (simple randomisation). Treatment allocations were placed in opaque and sealed envelopes and sequentially numbered from 1 to 80. Both procedures were masked to investigators. Participants were recruited by a neonatologist after a full technical and procedural explanation. Three hours before performing the heel stick, the assigned envelope was opened by an investigator, and the treatment allocation was revealed to the nurse who was in charge of the blood sampling and to the participant's mother. Investigators, nurses and mothers were not blinded to the treatment allocation. NIPS scoring was performed by an investigator who was blinded to the NIRS data. All infants were included in the final analysis. All outcomes were reported.

Blass 2001 (Heel lance): the infants were initially assessed to determine whether they were successfully breastfed or not and then randomised into the colostrum groups and non-colostrum groups. Investigators initially planned the assignment of the infants based on a table of random numbers. Group assignment needed to be adjusted because some mothers were unable to obtain sufficient colostrum. After passing the exclusion criteria, investigators assessed mother's success regarding breastfeeding.

If the mother was unsuccessful, she was assigned to groups that did not involve breast milk (Groups 1, 3, 4 and 6). If breastfeeding was established the infant was assigned to Groups 2 or 5. The phlebotomist who performed the heel lance was unaware of allocation, study purpose or hypotheses. The authors did not define what constituted successful breastfeeding. The data collection for sucrose, water and pacifier groups was completed in June 1998, while colostrum data collection ended in March 1999.

Although the phlebotomist and the person who rated the video data were unaware of treatment allocation, this could have introduced a degree of bias. Masking of intervention was not possible in this study since it involved the use of a pacifier and a liquid (colostrum) that differed in colour from two other solutions. Masking of outcome assessment was possible with crying time and heart rate changes, but not so when assessing grimacing since the intervention involved the use of a pacifier. A number of infants in the water and colostrum groups were excused (data collection not continued and infant allowed to be comforted in other ways) after 90 seconds of recovery period due to excessive crying, although all infants were included in the final analysis with the assumption that these behaviours would have continued at the same level for the rest of the recovery period.

Bozlak 2017 (Eye examination): infants were randomised with no number repetition into three groups through the use of the Random Assignment computer program. This information was put in sealed envelopes that contained the private files of the infants. Only the research nurse responsible for the preparation of the nonpharmacologic solution for each infant group saw these envelopes. The nurses who assisted with the ROP examinations evaluated the pain level through the use of the PIPP scale. These nurses were blinded to the group assignments of the infants. All infants were included in the final analysis. All outcomes were reported.

Bucher 2000 (Heel lance): randomisation was completed, but the method was not clear. The allocation concealment was done through sealed envelopes. One nurse administered the solution in the absence of investigators and was not involved in heel prick or data collection. Masking of outcome assessment was done by blinding the observer as to the assignments to the study group.

Bueno 2012 (Heel lance): a statistician used the Statistical Analysis System (SAS), version 8.2 (SAS Institute, Inc, Cary, NC) to generate blocked randomisation lists. Allocation concealment was achieved by using numbered, opaque, sealed envelopes containing intervention codes. Envelopes were exclusively accessed by research assistants. Research assistants prepared syringes containing both solutions for all infants. Syringes were covered to mask the intervention and were labelled according to the envelope's codes. Infants' faces and the monitor screen were filmed in real time by using independent video cameras during the entire data collection procedure. The focus of the video camera was deviated from the infants' faces during solution administration to guarantee masking of the interventions during facial coding. Outcomes were assessed and coded by a trained coder who was masked to the intervention received. All infants were included in the final analysis. All outcomes were reported.

Carbajal 2003 (Venipuncture): the randomisation was done in blocks of 20 using a random number table. Allocation was concealed from the investigators. Masking of the intervention was not possible in this study since it involved breastfeeding, the use of a pacifier and cuddling before and throughout the procedure. The outcome assessment was masked as the observers who assessed the outcome measures were not aware as to the purpose and hypothesis of the study. However, personal bias on the part of the outcome observer could not be excluded.

Chiabi 2016 (Heel lance): newborns were randomly divided into the two groups by drawing of lots. Method of allocation concealment and masking of intervention and outcomes were not clear. All infants were included in the final analysis. All outcomes were reported. A protocol was not available for comparison.

Codipietro 2008 (Heel lance): randomisation was done by using a computer random number generator. Allocation was concealed using opaque, sealed envelopes, which were opened sequentially by the paediatric nurse who performed blood sampling. Masking of intervention was not possible since it involved breastfeeding before and throughout the procedure. There was incomplete blinding of outcome assessments. The PIPP scale was administered by a paediatric nurse. The paediatric nurses and mothers were not blinded to the treatment assignment. However, assessment of one of the outcomes (cry behaviour) was masked as it was assessed by two assistants who listened to tape recordings. All infants were accounted for in the analysis of outcomes.

Cordero 2014 (Heel lance): a random sample of newborns was taken from all the babies in the unit, who underwent the heel lance procedure. The population sample for the study was randomly divided into three groups. However, the method of randomisation was not clear. Allocation concealment was not performed. Masking of intervention or outcomes was not stated. All infants were included in the final analysis. All outcomes were reported. The protocol was not available for comparison.

Dar 2019 (Intradermal): a lottery method was used to randomise the neonates, but allocation concealment was not performed. Masking of intervention or outcomes was not done. All infants were included in the final analysis. All outcomes were reported. The protocol was not available for comparison.

Desai 2017 (Suctioning): the suctioning episodes in neonates on assisted ventilation were randomised by a computer-generated randomisation sequence. Randomisation was done in variable random blocks of three or six. Treatment allocations were inserted in sequentially numbered, opaque envelopes and were sealed. Just before suctioning, a senior resident opened the sequentially numbered envelope and allocated the group. Blinding of outcome measures was not done. All infants were included in the final analysis. All outcomes were reported.

Efe 2007 (Venipuncture): this was a quasi-randomised trial as allocation was done according to the mothers' preferences. The method of random sequence generation was unclear. There was no concealment of allocation or blinding of the intervention. All patients were accounted for in the analysis of outcomes.

Erdogan 2022 (Venipuncture): neonates were randomly assigned to the experimental and control groups by the urn method. In the case of an infant matching the sampling criteria in the urn method, the balls prepared by the researcher beforehand were put into a black bag, and the selection process was carried out blindly by one of the researchers. According to the colour of the selected ball,

the infant was assigned to the smell, taste, taste + smell or control group. Therefore, the infants were randomly distributed into four groups. Allocation concealment was adequate. A nurse drew blood in the clinic, and assessments and observations were made by another nurse who did not know the purpose and hypothesis of the study. The authors mentioned that this was a single-blind study and the interventions were started three or five minutes before blood collection. However, it is unclear if the interventions were masked. All infants were included in the final analysis. All outcomes were reported.

Fallah 2017 (Intradermal): the trial used computer-generated equal simple randomisation by random numbers and allocation ratio was 1:1 for the three groups. Blinding of parents of the participants, the hospital nurse, the data collector and the outcome assessor was not possible, and only data analysts were kept blinded to the allocation. However, concealment was done by writing down the intervention for each serially participating neonate in a numbered and sealed opaque envelope, which was opened by the paediatric neurologist immediately before intervention. Randomisation and concealment were done by a researcher with no clinical involvement in the trial. Blinding of outcome assessment was not performed. All infants were included in the final analysis. All outcomes were reported.

Gabriel 2013 (Heel lance): randomisation was by closed envelopes and nurses and parents were masked to the randomisation group but not blinded to the treatment assignment. Method of random sequence generation was not clear. Allocation concealment was performed by using opaque envelopes for the group assignment. Outcome was measured by three researchers who watched the videos: one expert neonatologist and two young paediatricians, but it is unclear if they were blinded to the study. It is also unclear if blinding of outcome assessment was performed. All infants were included in the final analysis. All outcomes were reported.

Gradin 2004 (Venipuncture): allocation concealment was achieved through sealed envelopes. The method of random sequence generation was not clear. The intervention involved the use of placebo to mask the solution in question. Masking of outcome assessment was done by blinding the observer as to the assignments to the study group.

Gray 2002 (Heel lance): the randomisation was done through sealed envelopes, but the method was unclear. The masking of the intervention was not possible since it involved breastfeeding before and throughout the procedure. Masking of outcome assessment was also not possible. All participants were accounted for in the analysis of outcomes.

Hashemi 2016 (Intramuscular injection): randomisation was done based on the sealed envelope. In all participants, blood oxygen saturation and heart rate were measured by a pulse oximeter: its probe was attached to the infant's left leg two minutes before the vaccination. These parameters were recorded by a research assistant who did not know the type of group. A trained research assistant recorded the neonate's face in a close-up view, using a digital video camera in such a way that the type of intervention was unknown. After interventions, the videotapes were reviewed and scored by another research assistant (blind) who was trained on how to score baby's face changes. All infants were included in the final analysis. All outcomes were reported.

Holsti 2011 (Heel lance): randomisation was done by generating randomly permuted sequential blocks of four and six allocation numbers by a statistician blind to study hypotheses. Treatment group assignments were placed in sequentially numbered envelopes and sealed. Two coders blind to the study hypotheses, clinical information about the infants and to the timing of the blood collection were trained to achieve inter-rater reliability on the BIIP. All analyses were conducted blind to treatment assignment and according to randomised treatment, following the intention-to-treat principle. In the non-nutritive sucking group, the soother was held in the infant's mouth by the research nurse to ensure that contact was maintained. In the breastfeeding group, if necessary, the research nurse provided quiet, verbal guidance to the mother to ensure that contact with the breast was maintained. However, the author did not provide information about whether intervention was blinded. All infants were included in the final analysis. All outcomes were reported.

Iqbal 2014 (Intradermal): randomisation was performed, however the method is not clear. It is also unclear if allocation concealment was done. The randomisation code was available only to a research fellow who was not connected to the study. The code was disclosed to the researchers when the statistical analysis was completed. The mothers and nurses were not blind to the group assignments. However, the outcome assessor did not know the purpose and hypothesis of the study. All infants were included in the final analysis. All outcomes were reported.

Jatana 2003 (Heel lance): there is no comment on how the randomisation was done or if allocation concealment was performed, although the authors comment that the groups were matched for gestational age, birth weight and sex distribution. There is also no comment on whether the intervention was masked or not, which could have been possible, given that all the solutions were administered in the same way. There are no comments on whether the outcome assessment was masked or not. One of the outcomes, neonatal facial scoring, was not published in the results.

Kumar 2020 (Intramuscular injection): the enrolled neonates were randomised using the sequentially numbered, opaque, sealed envelopes (SNOSE) method. The person performing randomisation was not involved in the study beyond this. The first observer opened one sealed envelope for each baby and recruited that baby to one of six groups depending upon the group mentioned in that envelope. However, it is unclear if blinding of intervention was performed. Outcome variables were recorded by the third observer. However, it is unclear if the outcome assessor was blinded. All infants were included in the final analysis. All outcomes were reported.

Lan 2021 (Heel lance): newborns were randomly assigned to one of the three multisensory conditions by a blinded statistician using a web-based blocked randomisation system. However, it is unclear if allocation concealment was done. Pain was assessed by a research assistant who was well-trained in observations of video recordings of heel sticks. The research assistant coded all the videotapes of heel sticks in random sequence, but it was not possible to maintain blinding to the conditions when evaluating the NIPS score, because the infants' behaviours during heel sticks for the three treatment conditions were easy to discern when analysing the videotapes. All infants were included in the final analysis. All outcomes were reported.

Leite 2009 (Heel lance): randomisation was done by a computer random number generator. There was inadequate information to decide whether allocation concealment was effective. Masking of the intervention was not possible since it involved breastfeeding before and throughout the procedure. Two digital cameras were used to record the newborns' behaviour; one focused on the newborns' face and the second camera on the neonates' body. Analysis of facial actions was carried out by a person who was blinded to the phase of the procedure (blood collection, compression or recovery). The observers obviously recognised the two groups when they were evaluating the recordings. However, they were not aware of the purpose of the study. It was not possible to blind to group assignment as the information about breastfeeding was easily determined in both body and face videos. All participants were accounted for in the analysis of outcomes.

Leite 2015 (Intramuscular injection): participants were randomly assigned to two groups by a computer randomisation program. It is unclear if allocation concealment was done. It is unclear if masking of randomisation or intervention was performed. For the outcome, independent observation of the recordings of newborns' faces made by the researcher and a research assistant was performed. All infants were included in the final analysis. All outcomes were reported.

Lima 2013 (Venipuncture): newborns were randomly divided into three groups. However, the method of randomisation was not stated. Allocation concealment was not done. Non-nutritive sucking and outcome evaluation procedures were performed by the same researcher who was previously trained. However, it is not clear if the researcher was blinded to the randomisation or the outcome of the study. All infants were included in the final analysis. All outcomes were reported.

Mathai 2006 (Heel lance): randomisation was done through a random number table. There was inadequate information to decide whether allocation concealment was effective. Masking of the intervention was not possible since some participants took oral solutions while others were held or rocked in different ways. A trained nurse gave the selected intervention two minutes before the heel prick. However, it is unclear if the nurse was blinded. Masking of cry behaviour was possible, as one of the investigators stood behind a screen during the assignment of the infant and during the procedure (this observer assessed the total duration of cry). Not all the study's prespecified outcomes were reported: no data were available for two of the outcomes, i.e. heart rate and saturations, although the authors commented that there was no significant difference between the groups. All infants were accounted for in the analysis of outcomes.

Modarres 2013 (Intramuscular injection): neonates were randomly assigned to the study groups, but the method of randomisation was not clear. A system of sealed envelopes was used for assignment of the eligible neonates. The randomisation code was available only to a research fellow who was not connected to the study. The code was disclosed to the researchers when the statistical analysis was completed. The mothers and nurses were not blind to the group assignments. However, outcome assessors did not know the purpose and hypothesis of the study and the main investigator was blind to when the statistical analysis had been completed. All infants were included in the final analysis. All outcomes were reported.

Nanavati 2013 (Adhesive tape removal): a computer-generated randomisation sequence was used to assign infants to two treatment groups in a 1:1 ratio. Randomisation was balanced in variable random blocks of two or four patients. Treatment allocations were inserted in sequentially numbered, opaque envelopes and sealed. Just prior to adhesive tape removal, a neonatal research nurse opened the sequentially numbered envelopes. It is unclear if masking of intervention was performed. Blinding of outcome measures was not done. All infants were included in the final analysis. All outcomes were reported.

Napiorkowska-Orkisz 2022 (Heel lance): infants were allocated randomly using envelopes that contained the three groups, i.e. I, II, III. Newborns were randomly assigned to one of three groups that differed in pain management methods. However, the method of randomisation was not clear. Blinding was not performed. All infants were included in the final analysis. All outcomes were reported.

Obeidat 2015 (Heel lance): following assessment of eligibility and recruitment, a research assistant blindly drew a card for each participant from an envelope containing equal numbers of cards representing each group and assigned each newborn to either group. The mothers in group I were instructed to continue breastfeeding and cuddling if the infants started to cry during or after the heel lance blood drawing. The mothers in group II were instructed to continue cuddling if the infants started to cry during and after the heel lance blood drawing. The same experienced skilful neonatal nurse performed all blood collections to reduce variability and human errors between the groups. However, the author does not mention if intervention was masked. The painful responses of all neonates were measured with the PIPP scale used simultaneously by two neonatal nurses who were blinded to the objectives of the study. The author also mentioned that two nurses who assessed the PIPP scores of infants were able to recognise the control and treatment groups, but the possibility of bias was minimised by blinding the assessing nurses to the purpose of the study. All infants were included in the final analysis. All outcomes were reported.

Okan 2010 (Heel lance): randomisation was done through a random number table. Allocation concealment was adequate. Masking of the intervention was not possible since it involved breastfeeding, skin-to-skin contact and no contact at all. Authors commented that NFCS was not assessed in breastfed infants, as the facial actions of these babies could not be evaluated. All infants were accounted for in the analysis.

Ors 1999 (Heel lance): the manner of randomisation was not discussed by the authors. It was unclear if allocation concealment was done. Masking of the intervention was made possible by using a placebo and by performing the heel prick one minute after giving the solutions. The two investigators who analysed the data were unaware of the treatment intervention, hence the outcome measure analysis was blinded. All infants were accounted for in the analysis.

Ou-Yang 2013 (Heel lance): participants were randomised into the three treatment groups using computer-generated numbers. Each number corresponded to a sealed, serially numbered, opaque envelope that contained the treatment to be administered. Each syringe used for treatment was covered such that the investigator was blinded to the contents. Heel lancing was performed by

another investigator who was not present during treatment administration. Pain scoring was performed by an investigator who was blinded to treatment and was not present during the experimental procedure. All infants were included in the final analysis. All outcomes were reported.

Ozdogan 2010 (Heel lance): this was a quasi-randomised trial. Participants were consecutively allocated to the different groups by order of admission. There is insufficient information to know if this study was blinded or not, as the authors do not comment on whether the syringe was wrapped or covered and whether the person watching the videotape could see the contents of it or not. The first three groups were probably masked (as the intervention occurred before the video was taken), but we do not know if the intervention was masked for the two-dose groups. All the babies were scored according to NFCS at zero, one, two and three minutes by two persons who were blind to the groups. The face and the crying of the baby were recorded by a second person with a video camera. It is unclear if this second person was blinded. All infants are accounted for in the analysis.

Peng 2018 (Heel lance): preterm infants needing heel sticks were randomly assigned by a blinded statistician using Clinstat block randomisation. However, it was unclear if allocation concealment was performed. 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. Additionally, videotapes were coded in random order. However, it was not possible for outcome assessors to be completely blind to all research processes. The author also states that outcome assessors were different from interveners, but it does not state if interveners or interventions were masked. All infants were included in the final analysis. All outcomes were reported. The protocol was not available for comparison.

Phillips 2005 (Heel lance): randomisation was done through envelopes containing allocation cards. A research assistant blindly drew a card for each participant from an envelope containing equal numbers of cards with letters representing each group. Masking of intervention was not possible since it involved breastfeeding before and throughout the procedure. Masking of outcome assessment (from video recordings) was not done; however, data from monitors (heart rate, saturation and blood pressure) were analysed in a masked manner. All participants were accounted for in the analysis of outcomes; however, for some analyses complete data were not available from all patients.

Rawal 2018 (Heel lance): the randomisation method was not clearly stated. Concealment was achieved by using sequentially numbered, opaque, sealed envelopes bearing serially numbered patient codes. The composition of these packets was decided by a consultant in the department of neonatology who had the access to the randomisation codes and was uninvolved in the study. The observer entered the room after the test solution was administered, and the face of the baby was cleaned to remove any solution residue and thus masked to the test solution given. However, it is unclear if outcome assessment was masked. All infants were included in the final analysis.

Rioualen 2018 (Venipuncture): an online computerised randomisation program was used that guaranteed allocation concealment. Two independent neonatal nurses, previously trained on the NFCS, who were not told the study's aim, scored the video recordings using the NFCS. Each set of sequential procedures, including randomisation, sampling and video recording, was carried out by a team of three experimenters: #1 performed the randomisation and instructed the others about the oral medication, sampling method and video recording; #2 performed the oral administration; and #3 performed the blood sampling. There were large amounts of missing data with regard to one of the outcomes, salivary cortisol concentrations, because of the insufficient amounts of saliva. All outcomes were reported.

Rodrigues 2017 (Suctioning): randomisation was done using block randomisation in block sizes of 10, using a computer-generated random number table. Randomisation was done by one of the investigators. Allocation concealment was done using sequentially numbered, opaque, sealed envelopes. Patient enrolment and assignment were done by co-investigators. The primary investigator was blinded to the intervention. Blinding was ensured by preventing primary investigator from being in the room when the intervention was done. The primary investigator was not involved in the randomisation, video recording or noting of vital parameters. She was responsible for the scoring of the videos and thus determining the PIPP score. All infants were included in the final analysis. All outcomes were reported. The protocol was not available for comparison.

Rosali 2015 (Eye examination): eligible babies were randomly allocated to the intervention and control groups using computer-generated random numbers. Allocation concealment was done by using sequentially numbered, opaque, sealed envelopes containing the codes for intervention. The principal investigator videotaped the face of the baby, while another nurse observed the maximum heart rate and minimum saturations on a pulse oximeter. Both these observers were blinded. The author stated that the main strengths of the study were the use of PIPP scale, which is a very objective, validated and reliable tool for assessment of pain response in preterm neonates, and blinding of the investigators. However, it does not state if or how intervention was masked or the method used. All infants were included in the final analysis. All outcomes were reported. The protocol was not available for comparison.

Sabety 2013 (Venipuncture): neonates were randomly divided into four groups, but the method of randomisation is not mentioned. No information is provided regarding allocation concealment. Masking of randomisation was not done. Outcome assessment was performed by an experienced nurse who was blind to the group allocation of patients, but the masking of the intervention is not clear. All infants were included in the final analysis.

Sahoo 2013 (Venipuncture): eligible babies were randomised into three groups using computer-generated random numbers. Allocation concealment was achieved by using sequentially numbered, opaque, sealed envelopes containing the codes for intervention. The envelopes were exclusively accessed by the principal investigator. The observers entered the room after the test solution was administered, and thus were masked to the test solution given. The same observers assessed outcome measures.

Shendurnikar 2005 (Heel lance): the primary author provided this information. The randomisation was done by the primary author asking the mother to choose from a collection of randomisation cards. Allocation concealment was adequate. The masking of the

intervention was not possible since it involved breastfeeding before and throughout the procedure. Masking of outcome assessment was not done and the primary author collecting the data was aware of the allocation and hypothesis of the study. All participants were accounted for in the analysis of outcomes.

Simonse 2012 (Heel lance): neonates were allocated to one of the three groups according to the method of sequentially numbered and opaque sealed envelopes created by an independent employee and masked for the investigator. Allocation concealment is guaranteed by this method. Randomisation sequence was created by using a fixed block size of eight for a maximum of 75 neonates with a 1:1:1 allocation. It was not possible to blind patients and investigators for the allocated intervention. Masking of outcome assessors was not performed. All infants were included in the final analysis.

Singh 2017 (Heel lance): neonates were randomised into two groups using a system of sealed envelope randomisation. Allocation concealment was adequate. Two specially trained observers independently took part in sampling and assessed the recordings. However, it is unclear whether they were masked for the intervention and outcome measures. All infants were included in the final analysis.

Skogsdal 1997 (Heel lance): randomisation was done through a random digit table. However, it was unclear if allocation concealment was done. The heel prick and administration of allocated solution was done by the same nurse. Outcome data collection was done by a different nurse who was unaware of allocation. All participants were accounted for in the analysis of outcomes.

Soltani 2018 (Heel lance): infants were allocated randomly using envelopes that contained a pain management method amongst four groups, i.e. A, B, C and D. The double-blinded method was set up for this study. The nurse in charge of the heel prick sampling was not aware of the pre-sampling pain management method. The author also mentioned that infants were allocated randomly to four groups of interventions, i.e. breast milk feeding, oral 25% dextrose, KMCM and KMCM ointment, and all interventions were applied 15 minutes before heel prick. However, it is unclear if the intervention was masked. Pain score levels were measured by one medical student who was not aware of the pain management method in each infant according to the Neonatal Infant Pain Scale parameters. All infants were included in the final analysis.

Sujatha 2018 (Intradermal): eligible neonates were randomised and included in the study using computer-generated random numbers. The envelopes were exclusively accessed by the research assistants, but it was unclear if allocation concealment was done. The excess amount of breast milk in the neonatal mouth and the syringe was cleared before the entry of a blinded researcher into the vaccination room and thus the intervention was masked. However, it is unclear whether outcome assessment was masked. All infants were included in the final analysis. All outcomes were reported. The protocol was not available for comparison.

Taplak 2017 (Eye examination): assignment of the preterm infants meeting the inclusion criteria to groups was performed by a nurse employed in the service, away from the researchers. The preterm infants were randomly assigned to the breast milk, sucrose and control groups. However, method of randomisation is not clear. It was not clear if allocation concealment was performed. Only the researcher providing breast milk, sucrose or distilled water to the preterm infants before the examination (two minutes) knew the group assignment of the preterm infants. Hence, this researcher performed the video recordings. The researcher carrying out the physiological measurements five minutes before and five minutes after the examination also performed the video recordings without having any information on the group assignment of the preterm infants. Three independent specialists (two nurses specialised in paediatric nursing and a neonatologist who had no information about each other) analysed the video records of the ROP examination and scored the PIPP. All infants were included in the final analysis. All outcomes were reported.

Tavlar 2021 (Heel lance): randomisation was performed using the lottery method. The lottery bag was prepared by the researcher. The drawing of lots was provided by the midwife who performed the heel blood draw. The hospital registration protocol numbers of the infants whose heel blood was planned to be collected were written on pieces of paper and collected in a bag. Then, protocol numbers were respectively drawn from the bag for each of the breastfeeding, maternal heart sounds and breast milk scent application groups. A total of 30 protocol numbers were drawn from the bag for each group, including breastfeeding, mother's heartbeat sound and breast milk odour, respectively. In accordance with the groups formed, heartbeat sound recordings or breast milk samples were taken from the mothers. However, it is unclear if the intervention was masked. ALPS-Neo scale score, heart rate, oxygen saturation and crying time were evaluated for each newborn by the researcher. The data were evaluated by two observers. The observers were a researcher and a newborns midwife. The data were assessed by two independent observers. All infants were included in the final analysis. All outcomes were reported.

Turan 2021 (Eye examination): preterm infants who would undergo ROP examination were allocated to three groups according to a simple randomisation method. However, it is unclear if allocation concealment was achieved. Masking of intervention and outcome assessment was not performed. All infants were included in the final analysis. All outcomes were reported.

Upadhyay 2004 (Venipuncture): randomisation was performed using computer-generated numbers. Allocation was adequately concealed. The observers were blinded as to the intervention given to the infants. The outcome observers were blinded to the groups. For the NFCS, the two independent observers came in the room after the intervention had been completed, therefore they were blinded to the solution given. The data for 81 participants were available for analysis because in six infants either there was a technical problem or the infants were not fully awake.

Uyan 2005 (Heel lance): the authors provided further information on the method of randomisation, indicating that it was quasi-randomised (based on number or day of the procedure). Allocation concealment was inadequate. According to the authors the intervention was masked, but they did not comment on how the investigators were blinded. The two investigators who analysed the data and the person who recorded the video for the NFCS coding were unaware of the treatment allocation; hence, the outcome measure analysis was blinded. All infants were accounted for in the analysis.

Velumula 2022 (Heel lance): randomisation was done utilising a computer-generated sequence for simple randomisation by the pharmacist, who provided consecutively numbered, sealed, opaque envelopes. The two investigators independently recorded baseline heart rate, oxygen saturation and behavioural state of the neonate, after which they left the room, and the nurse administered the assigned drug. The investigators returned to the room two minutes after the drug was administered, at which time the nurse performed the procedure. The investigators independently assigned pain scores during and after the procedure every 30 seconds until 120 seconds. All infants were included in the final analysis. All outcomes were reported.

Weissman 2009 (Heel lance): this was a quasi-randomised trial, given that the allocation was done according to mothers' preferences. The method of randomisation was not clear. There was no blinding of the interventions or outcome assessors. All infants are accounted for in the analysis.

Wu 2021 (Heel lance): the authors stated that this was an RCT, but no information was provided with regard to method of randomisation, allocation concealment or blinding. All infants were included in the final analysis. All outcomes were reported.

Yilmaz 2011 (Heel lance): the authors stated that this was an RCT, but no information was given with regard to method of randomisation, allocation concealment or blinding. We do not have information on whether the investigators analysing the videotapes for the NIPS were blinded to the infants' intervention. There was a plan to assess saturation changes, which are reported as "no difference" without providing data. All infants were accounted for in the analysis.

Yilmaz 2020 (Heel lance): groups were randomised by the researcher using a computer-based random number table program. Amongst the four groups, the numbers from 1 to 160 were randomly distributed without repetition by a computer program in order to include the babies into the suitable groups. A random code was picked for each newborn by the researcher. To prevent pre-judgement, NIPS was used by an independent observer, blinded to the group allocations of newborns, to evaluate the pain. For all the groups, the sum of crying duration and soothing duration of the newborns was assessed through video records by the nurse who was serving as an independent observer. The author mentioned that in all groups the mothers were by their baby's side during the processes. Right after the procedure, the newborns were soothed in their mother's arms and following the procedure, the pain levels and durations of crying of the newborns were evaluated through recordings. However, it is unclear if the intervention was masked. All infants were included in the final analysis. All outcomes were reported.

Zargham-Boroujeni 2017 (Venipuncture): neonates meeting the inclusion criteria were assigned to each group (total of three groups) through random computation. However, it was not clear if allocation concealment was achieved. The nurse responsible for venipuncture was unaware of the assignment of neonates to research groups. All stages of venipuncture were recorded by a Panasonic handy cam. Then, the recorded films were observed and scored by a person who was blind to the assignment of neonates to the three different groups. To prevent bias and prejudice, this person was unaware of the group, so that scoring was made based on codes. All infants were included in the final analysis. All outcomes were reported.

Zhu 2015 (Heel lance): the main researcher used the online tool to randomly generate 72 sets of numbers, each set containing four numbers ranging from one to four with random order. A total of 288 unique codes were generated based on the 72 sets of randomly ordered numbers and were then placed in a box, where the randomised blocking was not retained. After consent-taking, the main researcher randomly picked one code for each neonate to ensure the 288 neonates were equally allocated into four groups based on the group number of each code. Two research assistants were trained to observe the video and recorded the outcome findings. However, it is unclear if there was any masking of intervention or of outcome assessment. All infants were included in the final analysis. All outcomes were reported.

#### Allocation

Reported in individual studies and Figure 2 and Figure 3.

Random sequence generation (selection bias): overall, out of 66 included studies, 40 had low risk, 24 had unclear risk and two had high risk bias.

Allocation concealment (selection bias): overall, out of 66 included studies, 36 had low risk, 21 had unclear risk and nine had high risk of bias.

#### Blinding

Reported in individual studies and Figure 2 and Figure 3.

Performance bias: overall, out of 66 included studies, 21 had low risk, 24 had unclear risk and 21 had high risk of bias.

Detection bias: overall, out of 66 studies, 33 had low risk, 14 had unclear risk and 19 had high risk of bias.

#### Incomplete outcome data

Reported in individual studies and Figure 2 and Figure 3.

Overall, out of 66 included studies, 59 had low risk, two had unclear risk and five had high risk of bias.

#### Selective reporting

Reported in individual studies and Figure 2 and Figure 3.

Overall, out of 66 included studies, 61 had low risk, one had unclear risk and four had high risk of bias.

#### Other potential sources of bias

Reported in individual studies and Figure 2 and Figure 3.

Out of 66 included studies, two had low risk of bias. The remaining 64 studies had unclear risk of other bias.

### Effects of interventions

See: **Summary of findings 1** Summary of findings - Breastfeeding versus control; **Summary of findings 2** Summary of findings - Supplemental breast milk versus control

**Breastfeeding versus control (comparison 1)**

Thirty-seven studies (which includes one study that compared breastfeeding and supplemental breast milk groups against each other (Bembich 2018 (Heel lance))), reported on this comparison (Avcin 2021 (Heel lance), Aydin 2019 (Heel lance); Bavarsad 2018 (Intramuscular injection); Bembich 2013 (Heel lance); Bembich 2018 (Heel lance); Carbajal 2003 (Venipuncture); Chiabi 2016 (Heel lance); Codipietro 2008 (Heel lance); Dar 2019 (Intradermal); Efe 2007 (Venipuncture); Fallah 2017 (Intradermal); Gabriel 2013 (Heel lance); Gradin 2004 (Venipuncture); Gray 2002 (Heel lance); Hashemi 2016 (Intramuscular injection); Holsti 2011 (Heel lance); Iqbal 2014 (Intradermal); Kumar 2020 (Intramuscular injection); Leite 2009 (Heel lance); Leite 2015 (Intramuscular injection); Lima 2013 (Venipuncture); Modarres 2013 (Intramuscular injection); Napiorkowska-Orkisz 2022 (Heel lance); Obeidat 2015 (Heel lance); Okan 2010 (Heel lance); Phillips 2005 (Heel lance); Rioualen 2018 (Venipuncture); Shendurnikar 2005 (Heel lance); Simonse 2012 (Heel lance); Singh 2017 (Heel lance); Soltani 2018 (Heel lance); Tavlar 2021 (Heel lance); Weissman 2009 (Heel lance); Wu 2021 (Heel lance); Yilmaz 2020 (Heel lance); Zargham-Boroujeni 2017 (Venipuncture); Zhu 2015 (Heel lance)).

***Primary outcomes***

**Pain**

**1. Physiological parameters**

**a. Heart rate change (beats per minute)** (Analysis 1.1)

Eight studies reported on heart rate change during various procedures (Codipietro 2008 (Heel lance); Efe 2007 (Venipuncture); Gray 2002 (Heel lance); Hashemi 2016 (Intramuscular injection); Okan 2010 (Heel lance); Phillips 2005 (Heel lance); Singh 2017 (Heel lance); Weissman 2009 (Heel lance)). The heart rate tended to increase in every group during the procedure. The comparator group varied widely, with eight different comparisons included. Since many studies included multiple arms with breastfeeding as the main comparator, we were not able to synthesise all interventions together. Individual interventions are compared to breastfeeding and reported.

Compared to breastfeeding, the difference in heart rate change was as follows.

No intervention/control

1. Control group: mean difference (MD) -12.89 (95% confidence interval (CI) -19.93 to -5.85 beats per minute; 2 studies, 120 participants; low-certainty evidence).

Non-pharmacologic interventions

1. Pacifier group: MD 2.10 (95% CI -4.78 to 8.98 beats per minute; 1 study, 61 participants; low-certainty evidence).
2. Swaddling group: MD -23.00 (95% CI -34.55 to -11.45 beats per minute; 1 study, 30 participants; low-certainty evidence).
3. Held by research assistant group: MD -7.10 (95% CI -15.50 to 1.30 beats per minute; 1 study, 54 participants; low-certainty evidence).
4. Held by mother group: MD -14.45 (95% CI -20.95 to -7.96 beats per minute; 2 studies, 125 participants; low-certainty evidence).

Feeding or pharmacologic interventions

1. Glucose group: MD -4.30 (95% CI -12.33 to 3.73 beats per minute; 1 study, 62 participants; low-certainty evidence).
2. Sucrose (moderate concentration (20% to 33%)) group: MD -9.00 (95% CI -14.41 to -3.59 beats per minute; 1 study, 101 participants; low-certainty evidence).
3. Formula group: MD -13.00 (95% CI -22.44 to -3.56 beats per minute; 1 study, 61 participants; low-certainty evidence).

Three other studies evaluated heart rate changes, but did not report on the standard deviation (SD) of change, so we were not able to meta-analyse these results (Efe 2007 (Venipuncture); Hashemi 2016 (Intramuscular injection); Okan 2010 (Heel lance)). These studies reported a reduction in mean heart rate change in the breastfeeding group compared to the control group (low-certainty evidence).

**b. Heart rate (beats per minute)** (Analysis 1.2)

Eight studies reported on heart rate (beats per minute) during procedures (Avcin 2021 (Heel lance); Bavarsad 2018 (Intramuscular injection); Gabriel 2013 (Heel lance); Holsti 2011 (Heel lance); Leite 2009 (Heel lance); Leite 2015 (Intramuscular injection); Tavlar 2021 (Heel lance); Wu 2021 (Heel lance)). The heart rate tended to increase in every group during the procedure. The comparator group varied widely, with 11 different comparisons included.

Compared to breastfeeding, the difference in heart rate was as follows.

No invention/control

1. No intervention group: MD -5.56 (95% CI -16.34 to 5.22 beats per minute; 2 studies, 166 participants; low-certainty evidence).

Non-pharmacologic interventions

1. Pacifier group: MD -2.00 (95% CI -4.47 to 0.47 beats per minute; 1 study, 57 participants; low-certainty evidence).
2. Positioning group: MD 2.00 (-0.59 to 4.59 beats per minute; 1 study, 70 participants; low-certainty evidence).
3. Held by mother group: MD -12.06 (95% CI -22.74 to -1.37 beats per minute; 5 studies, 295 participants; moderate-certainty evidence).
4. Breast milk odour group: MD -7.00 (95% CI -15.11 to 1.11 beats per minute; 1 study, 60 participants; low-certainty evidence).
5. Mother's heart beats group: MD -7.00 (95% CI -14.85 to 0.85 beats per minute; 1 study, 60 participants; low-certainty evidence).
6. Skin-to-skin contact group: MD -3.93 (95% CI -6.35 to -1.51 beats per minute; 2 studies, 115 participants; low-certainty evidence).

Feeding or pharmacologic intervention

1. Bottle-feeding mother’s milk group: MD -21.10 (95% CI -25.81 to -16.39 beats per minute; 1 study, 50 participants; low-certainty evidence).
2. Sucrose (moderate concentration (20% to 33%)) group: MD -8.00 (95% CI -15.59 to -0.41 beats per minute; 1 study, 61 participants; low-certainty evidence).
3. Sucrose (moderate concentration (20% to 33%)) with skin-to-skin group: MD -11.00 (95% CI -19.48 to -2.52 beats per minute; 1 study, 64 participants; low-certainty evidence).
4. Formula group: MD 29.80 (95% CI 27.02 to 32.58 beats per minute; 1 study, 50 participants; low-certainty evidence).

**c. Respiratory rate change**

None of the studies included in this review reported on this outcome.

**d. Oxygen saturation change** (Analysis 1.3)

Six studies reported on oxygen saturation change during procedures (Codipietro 2008 (Heel lance); Efe 2007 (Venipuncture); Hashemi 2016 (Intramuscular injection); Okan 2010 (Heel lance); Phillips 2005 (Heel lance); Singh 2017 (Heel lance)).

Compared to breastfeeding, the difference in oxygen saturation change was as follows.

No intervention/control

1. No intervention group: MD -0.04 (95% CI -1.32 to 1.24; 1 study, 60 participants; low-certainty evidence).

Non-pharmacologic interventions

1. Pacifier group (neonate held by mother): MD 0.30 (95% CI -2.79 to 3.39; 1 study, 64 participants; low-certainty evidence).
2. Pacifier group (neonate held by research assistant): MD 0.60 (95% CI -1.48 to 2.68; 1 study, 53 participants; low-certainty evidence).

Feeding or pharmacologic interventions

1. Sucrose (moderate concentration: 20% to 33%) group: MD 2.00 (95% CI 0.38 to 3.62; 1 study, 101 participants; low-certainty evidence).

Efe 2007 (Venipuncture), Hashemi 2016 (Intramuscular injection) and Okan 2010 (Heel lance) reported on oxygen saturation changes, but there was no information on the SD of the change, so we could not meta-analyse these data; however, overall these studies reported no difference in the mean change in oxygen saturation between the breastfeeding and control groups (low-certainty evidence).

**e. Oxygen saturation** (Analysis 1.4)

Four studies reported the actual oxygen saturation in percentages (Avcin 2021 (Heel lance); Bavarsad 2018 (Intramuscular injection); Tavlar 2021 (Heel lance); Wu 2021 (Heel lance)). The comparator group varied widely with seven different comparisons included.

Compared to breastfeeding, the difference in oxygen saturation was as follows.

No intervention/control

1. No intervention group: MD 0.64 (95% CI 0.21 to 1.08; 2 studies, 166 participants; low-certainty evidence).

Non-pharmacologic intervention

1. Positioning group: MD 1.00 (95% CI 0.53 to 1.47; 1 study, 70 participants; low-certainty evidence).
2. Held by mother group: MD 0.25 (95% CI -0.19 to 0.68; 2 studies, 120 participants; low-certainty evidence).
3. Breast milk odour group: MD 0.00 (95% CI -1.20 to 1.20; 1 study, 60 participants; low-certainty evidence).
4. Mother's heart beats group: MD -1.00 (95% CI -2.92 to 0.92; 1 study, 60 participants; low-certainty evidence).

Pharmacologic or feeding interventions

1. Bottle-feeding mother's milk group (neonate held by mother) was MD -0.80 (95% CI -1.94 to 0.34; 1 study, 50 participants; low-certainty evidence).
2. Formula group (neonate held by research assistant) was MD 0.10 (95% CI -1.08 to 1.28; 1 study, 50 participants; low-certainty evidence).

**f. Blood pressure change** (Analysis 1.5)

One study reported on blood pressure change during heel lance (Phillips 2005 (Heel lance)).

Compared to breastfeeding, the difference in blood pressure change was as follows.

Non-pharmacologic interventions

1. Pacifier group (neonate held by mother): MD -3.60 (95% CI -9.08 to 1.88 mmHg; 1 study, 62 participants; low-certainty evidence).
2. Pacifier group (neonate held by research assistant): MD 1.60 (95% CI -4.86 to 8.06 mmHg; 1 study, 48 participants; low-certainty evidence).

**g. Systolic blood pressure change** (Analysis 1.6)

One study reported on systolic blood pressure change during heel lance (Singh 2017 (Heel lance)). The difference in blood pressure change between the breastfeeding group compared with the no intervention group was MD -5.48 (95% CI -12.28 to 1.32 mmHg; 1 study, 60 participants; low-certainty evidence).

**f. Diastolic blood pressure change** (Analysis 1.7)

One study reported on diastolic blood pressure change during heel lance (Singh 2017 (Heel lance)). The difference in blood pressure change between the breastfeeding group compared with the no intervention group was MD -0.92 (95% CI -5.58 to 3.74 mmHg; 1 study, 60 participants; low-certainty evidence).

**2. Cry variables**

**a. Latency to first cry** (Analysis 1.8)

One study reported latency to first cry in seconds during heel lance (Zhu 2015 (Heel lance)). Compared to breastfeeding, the difference in latency to first cry in the no intervention group was MD 8.82 (95% CI 7.98 to 9.66 seconds earlier; 1 study, 125 participants; low-certainty evidence). The difference between the breastfeeding and music therapy group was MD 8.62 (95% CI 7.79 to 9.45 seconds earlier; 1 study, 126 participants; low-certainty evidence).

**b. Duration of first cry (seconds)** (Analysis 1.9)

One study reported on the duration of first cry (Codipietro 2008 (Heel lance)). Compared to breastfeeding, the duration of first cry in the sucrose (moderate concentration: 20% to 33%) group was MD -18.00 (95% CI -25.80 to -10.20 seconds; 1 study, 101 participants; low-certainty evidence).

**c. Duration of crying (seconds)** (Analysis 1.10)

Seventeen studies reported on the duration of crying (Avcin 2021 (Heel lance); Aydin 2019 (Heel lance); Bavarsad 2018 (Intramuscular injection); Dar 2019 (Intradermal); Efe 2007 (Venipuncture); Fallah 2017 (Intradermal); Gabriel 2013 (Heel lance); Gradin 2004 (Venipuncture); Gray 2002 (Heel lance); Kumar 2020 (Intramuscular injection); Leite 2015 (Intramuscular injection); Okan 2010 (Heel lance); Singh 2017 (Heel lance); Tavlar 2021 (Heel lance); Weissman 2009 (Heel lance); Wu 2021 (Heel lance); Zhu 2015 (Heel lance)).

The difference in the duration of crying in the breastfeeding group compared to the no intervention group was MD -36.23 seconds (95% CI -55.57 to -16.89 seconds; 10 studies, 790 participants; moderate-certainty evidence) (Avcin 2021 (Heel lance); Aydin 2019 (Heel lance); Dar 2019 (Intradermal); Efe 2007 (Venipuncture); Gradin 2004 (Venipuncture); Kumar 2020 (Intramuscular injection); Singh 2017 (Heel lance); Weissman 2009 (Heel lance); Wu 2021 (Heel lance); Zhu 2015 (Heel lance)). However, there was high statistical heterogeneity ($I^2$ = 99%) and clinical heterogeneity (different procedures). Post hoc analyses of six studies of heel lance procedure identified that the difference in the duration of crying in the breastfeeding group compared to the no intervention group was MD -33.75 seconds (95% CI -58.96 to -8.54 seconds). However, the high statistical heterogeneity persisted ($I^2$ = 99%).

Compared to breastfeeding, the difference in the duration of crying was as follows.

No intervention/control

1. No intervention group: MD -36.23 (95% CI -55.57 to -16.89 seconds; 10 studies, 790 participants; moderate-certainty evidence).

Non-pharmacological interventions

1. Lying on a table group: MD -136.00 (95% CI -180.45 to -91.55 seconds; 1 study, 71 participants; low-certainty evidence).
2. Positioning group: MD -31.46 (95% CI -69.39 to 6.48 seconds; 3 studies, 180 participants; low-certainty evidence).
3. Rocking group: MD -19.43 (95% CI -33.86 to -5.00 seconds; 1 study, 100 participants; low-certainty evidence).
4. Holding by mother group: MD -16.50 (95% CI -29.68 to -3.32 seconds; 4 studies, 260 participants; low-certainty evidence).
5. Skin-to-skin contact group: MD -23.63 (95% CI -29.30 to -17.95 seconds; 2 studies, 131 participants; low-certainty evidence).
6. Breast milk odour group: MD -7.50 (95% CI -18.57 to 3.57 seconds; 1 study, 60 participants; low-certainty evidence).
7. Mother's heart beat group: MD 0.00 (95% CI -5.22 to 5.22 seconds; 1 study, 60 participants; low-certainty evidence).
8. Heel warming group: MD -18.76 (95% CI -31.32 to -6.20 seconds; 1 study, 100 participants; low-certainty evidence).
9. Pacifier group: MD -12.22 (95% CI -25.78 to 1.34 seconds; 2 studies, 161 participants; low-certainty evidence).
10. Water group: MD -6.85 (95% CI -17.35 to 3.65 seconds; 1 study, 100 participants; low-certainty evidence).

Feeding or pharmacologic interventions

1. Bottle feeding mother's milk group: MD -6.80 (95% CI -9.08 to -4.52 seconds; 1 study, 50 participants; low-certainty evidence).
2. Glucose (moderate concentration: 20% to 33%) group: MD -12.79 (95% CI -19.21 to -6.37 seconds; 2 studies, 115 participants; low-certainty evidence).
3. Sucrose (moderate concentration: 20% to 33%) group: MD 6.13 (-12.07 to 24.32 seconds; 3 studies, 229 participants; low-certainty evidence).
4. Sucrose (moderate concentration: 20% to 33%) and skin-to-skin contact group: MD -3.00 (95% CI -6.13 to 0.13 seconds; 1 study, 64 participants; low-certainty evidence).
5. Formula group: MD -6.31 (95% CI -34.83 to 22.21 seconds; 1 study, 111 participants; low-certainty evidence).

Phillips 2005 (Heel lance) reported that 69% of infants in the breastfeeding group cried during the procedure compared to 81% of the infants in the group held by mothers with pacifier use, and 100% of infants in the group held by a research assistant with use of a pacifier (P < 0.01).

**d. Percentage of time crying** (Analysis 1.11)

Four studies reported percentage of time crying during procedure (Codipietro 2008 (Heel lance); Gabriel 2013 (Heel lance); Gray 2002 (Heel lance); Phillips 2005 (Heel lance)).

Compared to breastfeeding, the difference in percentage time crying was as follows.

1. Positioning group: MD -39.00 (95% CI -55.03 to -22.97 seconds; 1 study, 30 participants; low-certainty evidence).
2. Skin-to-skin contact group: MD -49.00 (95% CI -58.32 to -39.68 seconds; 1 study, 60 participants; low-certainty evidence).
3. Pacifier group (neonate held by mother): MD -11.80 (95% CI -27.95 to 4.35 seconds; 1 study, 71 participants; low-certainty evidence).
4. Pacifier group (neonate held by research assistant): MD -32.60 (95% CI -49.83 to -15.37 seconds; 1 study, 57 participants; low-certainty evidence).
5. Sucrose (moderate concentration: 20% to 33%) group: MD -31.00 (95% CI -52.06 to -9.94 seconds; 2 studies, 162 participants; low-certainty evidence).
6. Sucrose (moderate concentration: 20% to 33%) and skin-to-skin contact group: MD -1.00 (95% CI -6.86 to 4.86 seconds; 1 study, 64 participants; low-certainty evidence).

**e. Time to first calming** (Analysis 1.12)

Two studies reported time of first cry during heel lance procedure (Aydin 2019 (Heel lance); Yilmaz 2020 (Heel lance)).

Compared to breastfeeding, the difference in time to first calming was as follows.

1. No intervention group: MD -26.82 (95% CI -39.97 to -13.67 seconds; 1 study, 100 participants; moderate-certainty evidence).
2. Heel warming group: MD -9.30 (95% CI -19.63 to 1.03 seconds; 1 study, 100 participants; low-certainty evidence).

Compared to breastfeeding, swaddling and holding the difference in time to first calming was as follows.

1. No intervention group: MD -34.02 (95% CI -49.42 to -18.62 seconds; 1 study, 80 participants; low-certainty evidence).
2. Swaddling group: MD -29.75 (95% CI -43.63 to -15.87 seconds; 1 study, 80 participants; low-certainty evidence).
3. Swaddling and holding group: MD -39.50 (95% CI -57.00 to -22.00 seconds; 1 study, 80 participants; low-certainty evidence).

#### 3. Validated pain measures

**a. Neonatal Infant Pain Scale (NIPS) score** (Analysis 1.13)

Fifteen studies reported on this outcome (Avcin 2021 (Heel lance); Aydin 2019 (Heel lance); Bembich 2013 (Heel lance); Bembich 2018 (Heel lance); Chiabi 2016 (Heel lance); Efe 2007 (Venipuncture); Fallah 2017 (Intradermal); Gabriel 2013 (Heel lance); Lima 2013 (Venipuncture); Napiorkowska-Orkisz 2022 (Heel lance); Soltani 2018 (Heel lance); Wu 2021 (Heel lance); Yilmaz 2020 (Heel lance); Zargham-Boroujeni 2017 (Venipuncture); Zhu 2015 (Heel lance)).

Compared to breastfeeding, the difference in NIPS was as follows.

No intervention/control

1. No intervention group: MD -2.53 (95% CI -3.46 to -1.60; 5 studies, 459 participants; moderate-certainty evidence).

Non-pharmacologic interventions

1. Positioning group: MD -0.59 (95% CI -2.70 to 1.51; 2 studies, 150 participants; low-certainty evidence).
2. Heel warming group: MD -1.66 (95% CI -2.11 to -1.21; 1 study, 100 participants; low-certainty evidence).
3. Holding by mother group: MD -0.81 (95% CI -1.57 to -0.05; 3 studies, 230 participants; low-certainty evidence).
4. Music therapy group: MD -2.98 (95% CI -3.44 to -2.52; 1 study, 126 participants; low-certainty evidence).
5. Non-nutritive sucking: MD -1.10 (95% CI -2.39 to 0.19; 1 study, 60 participants; low-certainty evidence).

Feeding/pharmacologic interventions

1. EMLA cream group: MD -1.85 (95% CI -2.75 to -0.95; 1 study, 83 participants; low-certainty evidence).
2. Glucose (moderate concentration: 20% to 33%) group: MD -1.17 (95% CI -1.75 to -0.60; 4 studies, 272 participants; low-certainty evidence).
3. Sucrose (moderate concentration: 20% to 33%) group: MD -1.21 (95% CI -4.74 to 2.31; 2 studies, 129 participants; low-certainty evidence).
4. Sucrose (moderate concentration: 20% to 33%) and skin-to-skin contact group: MD -1.00 (95% CI -1.32 to -0.68; 1 study, 64 participants; low-certainty evidence).

Compared to breastfeeding, swaddling and holding, the difference in time to first calming was as follows.

No intervention/control

1. No intervention group: MD -1.93 (95% CI -2.40 to -1.46; 1 study, 80 participants; low-certainty evidence).

Non-pharmacologic interventions

1. Swaddling group: MD -1.38 (95% CI -1.84 to -0.92; 1 study, 80 participants; low-certainty evidence).
2. Swaddling and holding group: MD -1.10 (95% CI -1.63 to -0.57; 1 study, 80 participants; low-certainty evidence).

We could not analyse the NIPS outcomes of three studies (Bembich 2018 (Heel lance); Lima 2013 (Venipuncture); Zargham-Boroujeni 2017 (Venipuncture)). Zargham-Boroujeni 2017 (Venipuncture) reported the outcome but SD values were not given, and Bembich 2018 (Heel lance) did not report the interquartile range. Lima 2013 (Venipuncture) reported the NIPS outcome, but in the form of prevalence of pain and not the actual score.

**b. Premature Infant Pain Profile (PIPP) score** (Analysis 1.14)

Five studies reported on PIPP scores (Carbajal 2003 (Venipuncture); Codipietro 2008 (Heel lance); Gradin 2004 (Venipuncture); Obeidat 2015 (Heel lance); Simonse 2012 (Heel lance)).

Compared to breastfeeding, the difference in PIPP score was as follows.

No intervention/control

1. No intervention group: MD -0.49 (95% CI -2.39 to 1.41; 1 study, 29 participants; low-certainty evidence).
2. Placebo group: MD -5.95 (95% CI -7.42 to -4.48; 1 study, 89 participants; low-certainty evidence).

Non-pharmacologic interventions

1. Positioning group: MD -7.49 (95% CI -8.95 to -6.03; 1 study, 89 participants; low-certainty evidence).
2. Holding by mother group: MD -3.68 (95% CI -4.50 to -2.86; 1 study, 128 participants; low-certainty evidence).

Feeding/pharmacologic interventions

1. Glucose (moderate concentration: 20% to 33%) group: MD 1.30 (95% CI 0.05 to 2.56; 2 studies, 127 participants; low-certainty evidence).
2. Sucrose (moderate concentration: 20% to 33%) group: MD -1.95 (95% CI -8.88 to 4.98; 2 studies, 147 participants; low-certainty evidence).
3. Formula group: MD 1.93 (95% CI 1.43 to 2.43; 1 study, 46 participants; low-certainty evidence).

**c. Neonatal Facial Coding System (NFCS)** (Analysis 1.15)

Five studies reported on the NFCS score (Hashemi 2016 (Intramuscular injection); Leite 2009 (Heel lance); Leite 2015 (Intramuscular injection); Rioualen 2018 (Venipuncture); Weissman 2009 (Heel lance)).

Compared to breastfeeding, the difference in NFCS was as follows.

No intervention/control

1. No intervention group: MD -4.20 (95% CI -5.14 to -3.26; 1 study, 60 participants; low-certainty evidence).

Non-pharmacologic intervention

1. Holding by mother group: MD -1.90 (95% CI -3.12 to -0.68; 1 study, 60 participants; low-certainty evidence).
2. Skin-to-skin contact group: MD -0.92 (95% CI -2.96 to 1.12; 1 study, 55 participants; low-certainty evidence).
3. Pacifier group: MD -2.00 (95% CI -3.15 to -0.85; 1 study, 61 participants; low-certainty evidence).

Feeding/pharmacologic interventions

1. Glucose (moderate concentration: 20% to 33%) group: MD -3.90 (95% CI -4.80 to -3.00; 1 study, 62 participants; low-certainty evidence).
2. Formula group: MD 0.60 (95% CI -0.63 to 1.83; 1 study, 61 participants; low-certainty evidence).

Hashemi 2016 (Intramuscular injection), Leite 2009 (Heel lance) and Rioualen 2018 (Venipuncture) did evaluate NFCS scores, but Hashemi 2016 (Intramuscular injection) did not report the SD; Leite 2009 (Heel lance) reported the proportion of total score and not the actual NFCS score, and Rioualen 2018 (Venipuncture) provided the pain score in the form of prevalence of pain and not the actual values of the NFCS score. Therefore, we were not able to meta-analyse these results and did not include them in the analysis.

**d. Other pain scores as reported**

- **Douleur Aigue Nouveau-né score (DAN) scale** (Analysis 1.16)

Four studies reported on DAN score (Carbajal 2003 (Venipuncture); Iqbal 2014 (Intradermal); Kumar 2020 (Intramuscular injection); Modarres 2013 (Intramuscular injection)).

Compared to breastfeeding, the difference in DAN score was as follows.

No intervention/control

1. No intervention group: MD -1.87 (95% CI -4.61 to 0.86; 2 studies, 250 participants; low-certainty evidence).
2. Placebo group: MD -6.24 (95% CI -7.38 to -5.10; 1 study, 89 participants; low-certainty evidence).

Non-pharmacologic interventions

1. Positioning group: MD -6.77 (95% CI -7.78 to -5.76; 1 study, 89 participants; low-certainty evidence).
2. Rocking group: MD -0.04 (95% CI -0.80 to 0.72; 1 study, 100 participants; low-certainty evidence).
3. Pacifier group: MD -0.10 (95% CI -0.74 to 0.54; 1 study, 100 participants; low-certainty evidence).
4. Holding by mother group: MD -3.26 (95% CI -3.79 to -2.73; 1 study, 130 participants; low-certainty evidence).
5. Water group: MD -0.46 (95% CI -1.20 to 0.28; 1 study, 100 participants; low-certainty evidence).

Feeding/pharmacologic interventions

1. Glucose (moderate concentration: 20% to 33%) group: MD -0.75 (95% CI -1.97 to 0.47; 1 study, 89 participants; low-certainty evidence).
2. Sucrose (moderate concentration: 20% to 33%) group: MD 1.44 (95% CI 0.74 to 2.14; 1 study, 100 participants; low-certainty evidence).

- **COMFORTneo scale** (Analysis 1.17)

Simonse 2012 (Heel lance) used the COMFORTneo scale. The score was calculated using the following criteria.

- Alertness (1 = quiet sleep (eyes closed, no facial movement); 2 = active sleep (eyes closed, facial movement); 3 = quietly awake (eyes open, no facial movement); 4 = actively awake (eyes open, facial movement); 5 = awake and hyperalert
- Calmness/agitation (1 = calm (appears lucid and serene); 2 = slightly anxious (shows slight anxiety); 3 = anxious (appears agitated but remains in control); 4 = very anxious (appears very agitated, just able to control); 5 = panicky (severe distress with loss of control)
- Respiratory response (only in mechanically ventilated children): 1 = no spontaneous respiration; 2 = spontaneous respiration on ventilator; 3 = unrest or resistance to ventilator; 4 = actively breathes against ventilator or coughs regularly; 5 = fights ventilator
- Crying (only in spontaneously breathing children): 1 = no crying; 2 = faint crying; 3 = soft crying or moaning; 4 = hard crying; 5 = intense crying or screaming
- Body movement: 1 = no or minimal movement; 2 = up to three slight arm and/or leg movements; 3 = more than three slight arm and/or leg movements; 4 = up to three vigorous arm and/or leg movements; 5 = more than three vigorous arm and/or leg movements, or whole body
- Facial tension: 1 = facial muscles fully relaxed, relaxed open mouth; 2 = normal facial tension; 3 = intermittent eye squeeze and brow furrow; 4 = continuous eye squeeze and brow furrow
- Body muscle tone (observation only: 1 = muscles fully relaxed (open hands, dribbling, open mouth); 2 = reduced muscle tone; less resistance than normal; 3 = normal muscle tone; 4 = increased muscle tone (clenched hands and/or clenched, bent toes); 5 = extreme muscle tone (rigidity and flexion of fingers and/or toes)

Compared to breastfeeding, the difference in COMFORTNeo scale was as follows.

1. Sucrose (moderate concentration: 20% to 33%) group: MD 3.20 (95% CI 2.71 to 3.69; 1 study, 47 participants; low-certainty evidence).
2. Formula group: MD 2.70 (95% CI 2.21 to 3.19; 1 study, 46 participants; low-certainty evidence).

- **Composite score** (Analysis 1.18)

Shendurnikar 2005 (Heel lance) calculated the composite score. The composite score was calculated using the following criteria, and by combining the scores.

- Heart rate (0 ≤ 120/minute; 1 = 120 to 160/minute and 2 ≥ 160/minute)
- Breathing (0 = relaxed; 1 = changed)
- Facial expression (0 = relaxed; 1 = grimaced)
- Body movements (0 = relaxed; 1 = no gross movement; 2 = gross body movement)
- State of arousal (0 = sleepy; 1 = awake; 2 = fussy)
- Cry (0 = no; 1 = whimper; 2 = vigorous)

There was a decrease in the composite score in the breastfeeding group compared to the swaddled group (MD -2.90, 95% CI -3.51 to -2.29; 1 study, 100 participants; low-certainty evidence).

- **ALPS-Neo pain scale** (Analysis 1.19)

Tavlar 2021 (Heel lance) used the ALPS-Neo scale for pain score. The scale was developed multidimensionally by Lundqvist 2014 to assess pain and stress in premature and term newborns. It is a three-point Likert-type scale consisting of five items: facial expression, breathing pattern, arm/leg muscle tone, hand/foot activity and level of activity. Measurements were made by observation. A higher scale score refers to a greater stress and pain, where 0 to 2 = no pain and stress, 3 to 5 = mild pain and stress, and > 5 = high pain and stress. The total internal reliability score of the scale was reported as 0.91 and the internal consistency alpha coefficient as 0.95.

Compared to breastfeeding, the difference in ALPS-Neo scale was as follows.

1. Breast milk odour group: MD -3.83 (95% CI -5.12 to -2.54; 1 study, 60 participants; low-certainty evidence).
2. Mother's heart beat group: MD -1.60 (95% CI -2.66 to -0.54; 1 study, 60 participants; low-certainty evidence).

***Secondary outcomes***

1. Any clinically important outcome reported by authors: no authors reported on any other outcomes.
2. Any harmful effects reported by any author: no authors reported any harmful effects of the interventions.

**Supplemental breast milk versus control (comparison 2)**

Twenty-nine studies reported on this comparison (Aziznejad 2013 (Venipuncture); Blass 2001 (Heel lance); Bozlak 2017 (Eye examination); Bucher 2000 (Heel lance); Bueno 2012 (Heel lance); Cordero 2014 (Heel lance); Desai 2017 (Suctioning); Erdogan 2022 (Venipuncture); Jatana 2003 (Heel lance); Lan 2021 (Heel lance); Mathai 2006 (Heel lance); Nanavati 2013 (Adhesive tape removal); Ors 1999 (Heel lance); Ou-Yang 2013 (Heel lance); Ozdogan 2010 (Heel lance); Peng 2018 (Heel lance); Rawal 2018 (Heel lance); Rodrigues 2017 (Suctioning); Rosali 2015 (Eye examination); Sabety 2013 (Venipuncture); Sahoo 2013 (Venipuncture); Skogsdal 1997 (Heel lance); Sujatha 2018 (Intradermal); Taplak 2017 (Eye examination); Turan 2021 (Eye examination); Upadhyay 2004 (Venipuncture); Uyan 2005 (Heel lance); Velumula 2022 (Heel lance); Yilmaz 2011 (Heel lance)).

One study compared breastfeeding and supplemental breast milk groups against each other (Bembich 2018 (Heel lance)).

***Primary outcomes***

**Pain**

**1. Physiological parameters**

**a. Heart rate change (beats per minute)** (Analysis 2.1)

Ten studies reported on changes in the heart rate (Blass 2001 (Heel lance); Bucher 2000 (Heel lance); Cordero 2014 (Heel lance); Jatana 2003 (Heel lance); Ors 1999 (Heel lance); Sabety 2013 (Venipuncture); Skogsdal 1997 (Heel lance); Upadhyay 2004 (Venipuncture); Uyan 2005 (Heel lance); Yilmaz 2011 (Heel lance)).

The heart rate tended to increase in both groups during the procedure.

Compared to supplemental breast milk, the difference in heart rate change was as follows.

Control/no intervention

1. No intervention group: MD -4.81 (95% CI -6.28 to -3.35 beats per minute; 2 studies, 122 participants; low-certainty evidence).
2. Placebo group: MD -3.13 (95% CI -9.51 to 3.26 beats per minute; 5 studies, 300 participants; moderate-certainty evidence).

Feeding or pharmacologic interventions

1. Glycine group: MD 4.00 (95% CI -2.82 to 10.82 beats per minute; 1 study, 40 participants; low-certainty evidence).
2. Artificial sweetener group: MD 8.00 (95% CI -0.15 to 16.15 beats per minute; 1 study, 40 participants; low-certainty evidence).
3. Glucose (low concentration: < 20%) group: MD 0.62 (95% CI -1.79 to 3.03 beats per minute; 2 studies, 110 participants; low-certainty evidence).
4. Glucose (moderate concentration: 20% to 33%) group: MD 3.91 (95% CI -6.05 to 13.87 beats per minute; 3 studies, 172 participants; low-certainty evidence).
5. Glucose (high concentration: > 33%) group: MD 10.10 (95% CI 8.08 to 12.12 beats per minute; 1 study, 50 participants; low-certainty evidence).
6. Sucrose (moderate concentration: 20% to 33%) group: MD 13.80 (95% CI 4.23 to 23.37 beats per minute; 1 study, 68 participants; low-certainty evidence).

We identified statistical heterogeneity when pooling data from breast milk versus placebo studies ($I^2$ = 70%; P = 0.01) (Higgins 2003), which is concordant with the clinical heterogeneity observed between studies (population and dose of breast milk).

Blass 2001 (Heel lance) reported on mean heart rate change during and following the heel lance in the form of a bar graph. The mean heart rate changes in the group given colostrum via a pacifier and the groups given sucrose, either via syringe or pacifier, were less than that in the group given water, either by syringe or pacifier, and the group given colostrum via syringe. Yilmaz 2011 (Heel lance) also reported heart rate changes and showed no difference, but no measure of dispersion was provided, and so we were unable to meta-analyse the data. This study presented the results showing heart rate before, during and after the procedure; heart rate increased in the four groups, although the authors commented that there was no difference between them. Sabety 2013 (Venipuncture) reported the outcome but did not provide the number of participants in the study and control group (only the total number of participants in the study was provided). Hence, we could not analyse the results, and it was not included in the meta-analysis.

**b. Heart rate** (Analysis 2.2)

Eight studies reported on this outcome (Erdogan 2022 (Venipuncture); Ou-Yang 2013 (Heel lance); Rawal 2018 (Heel lance); Sahoo 2013 (Venipuncture); Sujatha 2018 (Intradermal); Taplak 2017 (Eye examination); Turan 2021 (Eye examination); Velumula 2022 (Heel lance)).

Compared to supplemental breast milk, the difference in heart rate was as follows.

No intervention/control group

1. No intervention: MD -20.00 (95% CI -28.74 to -11.26 beats per minute; 1 study, 60 participants; low-certainty evidence).

Non-pharmacologic interventions

1. Positioning group: MD 1.00 (95% CI 0.07 to 1.94 beats per minute; 1 study, 145 participants; low-certainty evidence).
2. Supplemental breast milk smell: MD -1.00 (95% CI -9.19 to 7.19 beats per minute; 1 study, 60 participants; low-certainty evidence).
3. Supplemental breast milk taste + smell: MD 2.00 (95% CI -5.08 to 9.08 beats per minute; 1 study, 60 participants; low-certainty evidence).

Feeding or pharmacologic interventions

1. Water group: MD -7.16 (95% CI -13.91 to -0.42 beats per minute; 4 studies, 276 participants; moderate-certainty evidence).
2. Glucose (moderate concentration: 20% to 33%) group: MD 0.59 (95% CI -3.33 to 4.51 beats per minute; 3 studies, 233 participants; moderate-certainty evidence).
3. Sucrose (moderate concentration: 20% to 33%) group: MD -2.48 (95% CI -6.44 to 1.48 beats per minute; 3 studies, 162 participants; low-certainty evidence).
4. Proparacaine eye drops group: MD -3.10 (95% CI -16.08 to 9.88 beats per minute; 1 study, 34 participants; low-certainty evidence).

**c. Oxygen saturation change** (Analysis 2.3)

Three studies reported on the change in oxygen saturation (Cordero 2014 (Heel lance); Jatana 2003 (Heel lance); Upadhyay 2004 (Venipuncture)).

Compared to supplemental breast milk, the difference in oxygen saturation change was as follows.

No intervention/control group

1. No intervention group: MD -4.90 (95% CI -5.98 to -3.82; 1 study, 62 participants; low-certainty evidence).
2. Placebo group: MD -0.92 (95% CI -2.47 to 0.63; 2 studies, 131 participants; low-certainty evidence).

Feeding or pharmacologic interventions

1. Glucose (low concentration: < 20%) group: MD 0.20 (95% CI -0.88 to 1.28; 1 study, 50 participants; low-certainty evidence).
2. Glucose (moderate concentration: 20% to 33%) group: MD -0.37 (95% CI -4.98 to 4.24; 2 studies, 112 participants; low-certainty evidence).
3. Glucose (high concentration: > 33%) group: MD 2.10 (95% CI 1.22 to 2.98; 1 study, 50 participants; low-certainty evidence).

Yilmaz 2011 (Heel lance) mentioned that they assessed for saturation differences, and did not find differences, but no actual numbers were provided in the article.

**d. Oxygen saturation** (Analysis 2.4)

Seven studies reported on this outcome (Erdogan 2022 (Venipuncture); Ou-Yang 2013 (Heel lance); Rawal 2018 (Heel lance); Sahoo 2013 (Venipuncture); Sujatha 2018 (Intradermal); Taplak 2017 (Eye examination); Turan 2021 (Eye examination)).

Compared to supplemental breast milk, the difference in oxygen saturation was as follows.

No intervention/control

1. No intervention: MD 6.00 (95% CI 4.07 to 7.93 beats per minute; 1 study, 60 participants; low-certainty evidence).

Non-pharmacologic interventions

1. Positioning group: MD -0.81 (95% CI -1.26 to -0.36; 1 study, 155 participants; low-certainty evidence).
2. Supplemental breast milk smell: MD 2.00 (95% CI 0.40 to 3.60 beats per minute; 1 study, 60 participants; low-certainty evidence).
3. Supplemental breast milk taste + smell: MD -2.00 (95% CI -4.61 to 0.61 beats per minute; 1 study, 60 participants; low-certainty evidence).

Feeding or pharmacologic interventions

1. Water group: MD 0.82 (95% CI 0.12 to 1.52; 4 studies, 276 participants; moderate-certainty evidence).
2. Glucose (moderate concentration: 20% to 33%) group: MD -0.31 (95% CI -2.76 to 2.15; 3 studies, 233 participants; low-certainty evidence).
3. Sucrose (moderate concentration: 20% to 33%) group: MD 2.01 (95% CI -0.98 to 5.01; 2 studies, 74 participants; low-certainty evidence).
4. Proparacaine eye drops group: MD -0.50 (95% CI -5.69 to 4.69; 1 study, 34 participants; low-certainty evidence).

**e. Respiratory rate** (Analysis 2.5)

Four studies reported on this outcome (Aziznejad 2013 (Venipuncture); Ou-Yang 2013 (Heel lance); Sabety 2013 (Venipuncture); Sujatha 2018 (Intradermal)).

Compared to supplemental breast milk, the difference in respiratory rate was as follows.

No intervention/control

1. No intervention group: MD -10.90 (95% CI -14.70 to -7.10 per minute; 1 study, 60 participants; low-certainty evidence).

Non-pharmacologic interventions

1. Positioning group: MD 12.09 (95% CI 10.97 to 13.21 per minute; 1 study, 145 participants; low-certainty evidence).
2. Water group: MD -3.20 (95% CI -8.93 to 2.53 per minute; 1 study, 84 participants; low-certainty evidence).

Feeding or pharmacologic interventions

1. EMLA cream group: MD -4.70 (95% CI -10.92 to 1.52 per minute; 1 study, 60 participants; low-certainty evidence).

2. Glucose (moderate concentration: 20% to 33%) group: MD -1.50 (95% CI -6.24 to 3.24 per minute; 1 study, 79 participants; low-certainty evidence).
3. Sucrose (moderate concentration: 20% to 33%) group: MD 0.50 (95% CI -4.61 to 5.61 per minute; 1 study, 60 participants; low-certainty evidence).

Sabety 2013 (Venipuncture) reported the outcome, but the numbers of participants included in the study and control groups were not provided (only the total number of participants in the study). Hence, we could not analyse the results, and they were not included in the meta-analysis.

**f. Systolic blood pressure change** (Analysis 2.6)

Only one study reported on this outcome (Ou-Yang 2013 (Heel lance)). Compared to supplemental breast milk, the difference in systolic blood pressure in the water group was MD 1.10 (95% CI -2.93 to 5.13 mmHg; 1 study, 84 participants; low-certainty evidence) and in the glucose (moderate concentration: 20% to 33%) group was MD 1.80 (95% CI -1.57 to 5.17; 1 study, 79 participants; low-certainty evidence).

**g. Diastolic blood pressure change** (Analysis 2.7)

Only one study reported on this outcome (Ou-Yang 2013 (Heel lance)). Compared to supplemental breast milk, the difference in diastolic blood pressure in the water group was MD -0.50 (95% CI -5.18 to 4.18 mmHg; 1 study, 84 participants; low-certainty evidence) and in the glucose (moderate concentration: 20% to 33%) group was MD 1.30 (95% CI -2.21 to 4.81; 1 study, 79 participants; low-certainty evidence).

#### 2. Cry variables

**a. Duration of first cry (in seconds)** (Analysis 2.8)

Only two studies assessed the duration of first cry (Jatana 2003 (Heel lance); Mathai 2006 (Heel lance)).

Compared to supplemental breast milk, the duration of first cry was as follows.

Non-pharmacologic interventions

1. Pacifier group: MD 4.00 (95% CI 0.18 to 7.82 seconds; 1 study, 38 participants; low-certainty evidence).
2. Massage group: MD -6.00 (95% CI -12.34 to 0.34 seconds; 1 study, 35 participants; low-certainty evidence).
3. Rocking group: MD 3.00 (95% CI -1.52 to 7.52 seconds; 1 study, 35 participants; low-certainty evidence).
4. Water group: MD -2.68 (95% CI -8.45 to 3.10 seconds; 2 studies, 83 participants; low-certainty evidence).

Feeding or pharmacologic interventions

1. Glucose (low concentration: < 20%) group: MD 1.92 (95% CI -1.38 to 5.22 seconds; 1 study, 50 participants; low-certainty evidence).
2. Glucose (moderate concentration: 20% to 33%) group: MD 12.78 (95% CI 9.36 to 16.20 seconds; 1 study, 50 participants; low-certainty evidence).
3. Glucose (high concentration: > 33%) group: MD 11.56 (95% CI 8.54 to 14.58 seconds; 1 study, 50 participants; low-certainty evidence).
4. Sucrose (moderate concentration: 20% to 33%) group: MD 6.00 (95% CI 2.50 to 9.50 seconds; 1 study, 35 participants; low-certainty evidence).

**b. Duration of crying (in seconds)** (Analysis 2.9)

Fourteen studies reported on the duration of crying (Blass 2001 (Heel lance); Bucher 2000 (Heel lance); Jatana 2003 (Heel lance); Mathai 2006 (Heel lance); Ors 1999 (Heel lance); Ou-Yang 2013 (Heel lance); Ozdogan 2010 (Heel lance); Sabety 2013 (Venipuncture); Sahoo 2013 (Venipuncture); Skogsdal 1997 (Heel lance); Sujatha 2018 (Intradermal); Upadhyay 2004 (Venipuncture); Uyan 2005 (Heel lance); Yilmaz 2011 (Heel lance)).

Compared to supplemental breast milk, the difference in duration of crying was as follows.

No intervention/control group

1. No intervention group: MD 36.70 (95% CI 0.60 to 72.80 seconds; 1 study, 60 participants; low-certainty evidence).
2. Placebo group: MD -8.67 (95% CI -12.32 to -5.02 seconds; 7 studies, 357 participants; moderate-certainty evidence).

Non-pharmacologic group

1. Positioning group: MD -7.43 (95% CI -8.55 to -6.31 seconds; 1 study, 145 participants; low-certainty evidence).
2. Rocking was MD 31.00 (95% CI 24.47 to 37.53 seconds; 1 study, 35 participants; low-certainty evidence).
3. Massage group: MD -9.00 (95% CI -16.97 to -1.03 seconds; 1 study, 35 participants; low-certainty evidence).
4. Pacifier group: MD 44.23 (95% CI 38.47 to 49.98 seconds; 2 studies, 98 participants; low-certainty evidence).
5. Water was MD -54.91 (95% CI -117.56 to 7.74 seconds; 3 studies, 238 participants; low-certainty evidence).

Feeding or pharmacologic interventions

1. Glycine group: MD 51.80 (95% CI 6.33 to 97.27 seconds; 1 study, 40 participants; low-certainty evidence).
2. Artificial sweetener group: MD 41.00 (95% CI -6.61 to 88.61 seconds; 1 study, 40 participants; low-certainty evidence).
3. Glucose (low concentration: < 20%) group: MD 3.58 (95% CI -1.52 to 8.68 seconds; 2 studies, 110 participants; low-certainty evidence).
4. Glucose (moderate concentration: 20% to 33%) group: MD 27.41 (95% CI 23.04 to 31.78 seconds; 4 studies, 301 participants; low-certainty evidence).
5. Glucose (high concentration: > 33%) group: MD 27.20 (95% CI 20.89 to 33.51 seconds; 1 study, 50 participants; low-certainty evidence).
6. Sucrose (low concentration: < 20%) group: MD 35.00 (95% CI 29.04 to 40.96 seconds; 1 study, 43 participants; low-certainty evidence).
7. Sucrose (moderate concentration: 20% to 33%) group: MD 34.61 (95% CI 1.36 to 67.86 seconds; 3 studies, 163 participants; low-certainty evidence).

8. Two doses of sucrose (low concentration: < 20%) group: MD 22.00 (95% CI 13.09 to 30.91 seconds; 1 study, 41 participants; low-certainty evidence).
9. Two doses of supplemental breast milk group: MD -11.00 (95% CI -21.22 to -0.78 seconds; 1 study, 41 participants; low-certainty evidence).

**c. Percentage of time crying** (Analysis 2.10)

Two studies reported this outcome (Blass 2001 (Heel lance); Bucher 2000 (Heel lance)). Blass 2001 (Heel lance) reported the mean time spent crying during the recovery period in the form of a linear graph, so the data below are from Bucher 2000 (Heel lance).

Compared to supplemental breast milk, the difference in percentage time crying was as follows.

No intervention/control group

1. Placebo group: MD 9.00 (95% CI -1.99 to 19.99 seconds; 1 study, 40 participants; low-certainty evidence).

Feeding or pharmacologic interventions

1. Artificial sweetener group: MD 15.00 (95% CI 2.38 to 27.62 seconds; 1 study, 40 participants; low-certainty evidence).
2. Glycine group: MD 1.00 (95% CI -4.61 to 6.61 seconds; 1 study, 40 participants; low-certainty evidence).

Blass 2001 (Heel lance) identified reduction in the proportion of time crying in the group given sucrose (via syringe or pacifier) compared to the control group and the group given colostrum (via syringe or pacifier) (P < 0.0015). There was no difference between the colostrum group and the control group. It was not possible to abstract data from the graphs.

#### 3. Validated pain measures

**a. Neonatal infant pain score (NIPS)** (Analysis 2.11)

Three studies reported on this outcome (Lan 2021 (Heel lance); Turan 2021 (Eye examination); Yilmaz 2011 (Heel lance)).

Compared to supplemental breast milk, the difference in NIPS was as follows.

No intervention/control group

1. No intervention group: MD -0.30 (95% CI -1.60 to 1.00; 1 study, 60 participants; low-certainty evidence).

Non-pharmacologic intervention group

1. Pacifier group: MD 1.20 (95% CI -0.14 to 2.54; 1 study, 60 participants; low-certainty evidence).
2. Gentle touch + verbal comfort: MD -0.19 (95% CI -0.58 to 0.20; 1 study, 80 participants; low-certainty evidence).
3. Breast milk odour + gentle touch + verbal comfort: MD -0.04 (95% CI -0.39 to 0.31; 1 study, 80 participants; low-certainty evidence).

Feeding or pharmacologic interventions

1. Sucrose (moderate concentration: 20% to 33%) group: MD 0.91 (95% CI -1.45 to 3.28; 2 studies, 94 participants; low-certainty evidence).
2. Proparacaine eye drops group: MD 0.05 (95% CI -0.29 to 0.39; 1 study, 34 participants; low-certainty evidence).

**b. Premature Infant Pain Profile (PIPP)** (Analysis 2.12)

Ten studies reported this outcome (Bozlak 2017 (Eye examination); Bueno 2012 (Heel lance); Desai 2017 (Suctioning); Nanavati 2013 (Adhesive tape removal); Peng 2018 (Heel lance); Rawal 2018 (Heel lance); Rodrigues 2017 (Suctioning); Rosali 2015 (Eye examination); Sahoo 2013 (Venipuncture); Taplak 2017 (Eye examination)).

Compared to supplemental breast milk, the difference in PIPP score was as follows.

Non-pharmacologic interventions

1. Swaddling group: MD -0.64 (95% CI -4.57 to 3.29; 2 studies, 112 participants; low-certainty evidence).
2. Held by mother group: MD 0.28 (95% CI -0.83 to 1.39; 1 study, 50 participants; low-certainty evidence).
3. Water group: MD -1.87 (95% CI -4.59 to 0.85; 4 studies, 250 participants; low-certainty evidence).

Feeding or pharmacologic interventions

1. Glucose (moderate concentration: 20% to 33%) group: MD 1.86 (95% CI 1.02 to 2.71; 4 studies, 307 participants; moderate-certainty evidence).
2. Sucrose (moderate concentration: 20% to 33%) group: MD -0.02 (95% CI -0.94 to 0.91; 3 studies, 170 participants; low-certainty evidence).

Peng 2018 (Heel lance) reported the PIPP score as an outcome, but scores were divided into mild and moderate to severe grading during eight different heel stick phases, and were only provided in a graph. Hence, we could not analyse the data, and the results were not included in the meta-analysis.

**c. Neonatal Facial Coding System (NFCS) score at three minutes** (Analysis 2.13)

Three studies reported on the NFCS (Bucher 2000 (Heel lance); Upadhyay 2004 (Venipuncture); Uyan 2005 (Heel lance)). Bucher 2000 (Heel lance) used five components of the NFCS, and Upadhyay 2004 (Venipuncture) modified the score and collected data on only part of the components.

Compared to supplemental breast milk, the difference in NFCS at three minutes was as follows.

No intervention/control group

1. Placebo group: MD -0.86 (95% CI -2.24 to 0.52; 3 studies, 183 participants; low-certainty evidence).

Feeding or pharmacologic interventions

1. Glycine group: MD -0.47 (95% CI -0.90 to -0.04; 1 study, 40 participants; low-certainty evidence).
2. Artificial sweetener group: MD -0.22 (95% CI -0.65 to 0.21; 1 study, 40 participants; low-certainty evidence).

**d. Neonatal Facial Coding System (NFCS) score at two minutes** (Analysis 2.14)

One study reported on the NFCS at two minutes (Ozdogan 2010 (Heel lance)).

Compared to supplemental breast milk, the difference in the NFCS at two minutes was as follows.

No intervention/control group

1. Water group: MD -0.84 (95% CI -1.09 to -0.59; 1 study, 45 participants; low-certainty evidence).
2. Two doses of water group: MD -0.59 (95% CI -0.83 to -0.35; 1 study, 44 participants; low-certainty evidence).

Feeding or pharmacologic interventions

1. Sucrose (low concentration: < 20%) group: MD 0.92 (95% CI 0.64 to 1.20; 1 study, 43 participants; low-certainty evidence).
2. Two doses of sucrose (low concentration: < 20%) group: MD 0.16 (95% CI -0.11 to 0.43; 1 study, 41 participants; low-certainty evidence).
3. Two doses of supplemental breast milk group: MD -1.14 (95% CI -1.37 to -0.91; 1 study, 41 participants; low-certainty evidence).

**e. Other pain scores as reported (non-validated)**

- **Douleur Aigue du Nouveau-ne (DAN)** (Analysis 2.15)

Three studies reported on DAN scores (Aziznejad 2013 (Venipuncture); Mathai 2006 (Heel lance); Sabety 2013 (Venipuncture)). Compared to supplemental breast milk, the difference in DAN score was as follows.

No intervention/control group

1. No intervention group: MD -1.00 (95% CI -2.15 to 0.15; 1 study, 60 participants; low-certainty evidence).

Non-pharmacologic interventions

1. Massage group: MD -0.50 (95% CI -0.91 to -0.09; 1 study, 35 participants; low-certainty evidence).
2. Rocking group: MD 1.10 (95% CI 0.65 to 1.55; 1 study, 35 participants; low-certainty evidence).
3. Pacifier group: MD 0.80 (95% CI 0.40 to 1.20; 1 study, 38 participants; low-certainty evidence).
4. Water group: MD -1.10 (95% CI -1.65 to -0.55; 1 study, 33 participants; low-certainty evidence).

Feeding or pharmacologic interventions

1. EMLA cream group: MD -0.64 (95% CI -1.92 to 0.64; 1 study, 60 participants; low-certainty evidence).
2. Sucrose (moderate concentration: 20% to 33%) group: MD 0.29 (95% CI -0.34 to 0.92; 2 studies, 95 participants; low-certainty evidence).

Sabety 2013 (Venipuncture) reported the outcome, but the numbers of participants included in the study and control group were not provided (only total number of participants in the study). Hence, we could not analyse the results, and it was not included in the meta-analysis.

- **Body pain score** (Analysis 2.16)

One study reported on the body pain score outcome (maximum score was 8 and minimum score was 0) (Bucher 2000 (Heel lance)).

Compared to supplemental breast milk, the difference in body pain score was as follows.

No intervention/control group

1. Placebo group: MD 0.48 (95% CI -0.38 to 1.34; 1 study, 40 participants; low-certainty evidence).

Feeding or pharmacologic interventions

1. Glycine group: MD 0.43 (95% CI -0.51 to 1.37; 1 study, 40 participants; low-certainty evidence).
2. Artificial sweetener group: MD 0.16 (95% CI -0.72 to 1.04; 1 study, 40 participants; low-certainty evidence).

- **N-PASS** (Analysis 2.17)

Two studies reported on the N-PASS outcome (Erdogan 2022 (Venipuncture); Ou-Yang 2013 (Heel lance)).

Compared to supplemental breast milk, the difference in N-PASS was as follows.

1. No intervention was MD -0.45 (95% CI -0.55 to -0.35 beats per minute; 1 study, 60 participants; low-certainty evidence).

Non-pharmacologic interventions

1. Supplemental breast milk smell: MD -0.43 (95% CI -0.53 to -0.33 beats per minute; 1 study, 60 participants; low-certainty evidence).
2. Supplemental breast milk taste + smell: MD 0.60 (95% CI 0.40 to 0.80 beats per minute; 1 study, 60 participants; low-certainty evidence).
3. Water group: MD -2.11 (95% CI -3.51 to -0.71; 1 study, 84 participants; low-certainty evidence).

Feeding or pharmacologic interventions

1. Glucose (moderate concentration: 20% to 33%) group: MD -0.28 (95% CI -1.62 to 1.06; 1 study, 79 participants; low-certainty evidence).

- **Premature Infant Pain Profile-Revised** (Analysis 2.18)

One study reported on PIPP-R outcome (Velumula 2022 (Heel lance)).

Compared to supplemental breast milk, the difference in PIPP-R with 24% oral sucrose was MD -1.10 (95% CI -1.86 to -0.34; 1 study, 88 participants; low-certainty evidence).

***Secondary outcomes***

1. Any clinically important outcome reported by authors: no authors reported on any other outcomes.
2. Any harmful effects reported by any author: Carbajal 2003 (Venipuncture) gathered information on infants' sucking behaviour 48 to 72 hours after venipuncture by interviewing mothers. There was no difference in the number of infants in whom the suck was the same or more effective amongst four groups (P = 0.14). The authors reported that infants who

underwent venipuncture while they were being breastfed did not suck less effectively after the procedure. Bueno 2012 (Heel lance) reported no difference in adverse events between groups (11.6% in experimental group and 8.9% in control group; P = 0.74). They reported on oxygen saturation below 80%, nausea/regurgitation or vomiting and heart rate below 100/minute.

#### *Subgroup analyses*

We did not perform any planned subgroup analyses (based on gestational age, type of intervention and type of procedure) because subdividing the current data into these subgroups would have led to a very limited number of studies for each subgroup comparison. However, in future updates of this review we plan to carry out these subgroup analyses.

## DISCUSSION

### Summary of main results

Non-pharmacological interventions of breastfeeding or provision of supplemental breastfeeding as analgesic agents have been extensively studied in 66 randomised controlled trials involving 6821 neonates. The interventions against which these two methods were compared varied markedly between studies. Thus, the majority of the summary of results described below arise from comparisons of small numbers of studies. Of the 66 eligible studies, 36 evaluated breastfeeding, 29 evaluated supplemental breast milk and one study compared them against each other. The procedures conducted in the studies were: heel lance (39), venipuncture (11), intramuscular vaccination (nine), eye examination for retinopathy of prematurity (four), suctioning (two) and adhesive tape removal as procedure (one). We noted marked heterogeneity in the control interventions and pain assessment measures among the studies. We did not pool data for interventions that seemed similar, but which had minor differences in the delivery of the intervention (e.g. holding child, holding child by research assistant or by the child's mother), because the delivery method had the potential for a different effect.

Overall, the included studies were at low risk of bias except for masking of intervention and outcome assessment, where we identified nearly one-third of the studies to be at high risk of bias.

#### Breastfeeding versus control

Breastfeeding likely reduces heart rate increase during the procedure compared to being held by mother (mean difference (MD) -12 beats per minute, 95% confidence interval (CI) -20 to -4; $I^2$ = 97%; 5 studies, 295 participants; moderate-certainty evidence). Breastfeeding likely reduces the duration of crying compared to the no intervention group (MD -36 seconds, 95% CI -56 to -17; $I^2$ = 99%; 10 studies, 790 participants; moderate-certainty evidence). Breastfeeding likely reduces the Neonatal Infant Pain Scale (NIPS) score compared to the no intervention group (MD -2.5, 95% CI -3.5 to -1.6; $I^2$ = 96%; 5 studies, 459 participants; moderate-certainty evidence).

Due to the high number of comparator interventions, other measures of pain were assessed in a very small number of studies in each comparison, rendering the evidence of low certainty.

#### Supplemental breast milk versus control

Supplemental breast milk likely reduces heart rate increase during the procedure compared to water (MD -7 beats per minute, 95% CI -14 to -0.4; $I^2$ = 66%; 4 studies, 276 participants; moderate-certainty evidence). Supplemental breast milk likely reduces the duration of crying compared to a placebo group (MD -9 seconds, 95% CI -12 to -5; $I^2$ = 0%; 7 studies, 357 participants; moderate-certainty evidence). Supplemental breast milk results in little to no difference in the NIPS score compared to a no intervention group (MD -0.3, 95% CI -1.6 to 1; 1 study, 60 participants; low-certainty evidence).

Due to the high number of comparator interventions, other measures of pain were assessed in a very small number of studies in each comparison, rendering the evidence of low certainty.

The majority of studies did not report on adverse events, considering the benign nature of the intervention. Those that reported on adverse events identified none in any participants.

### Overall completeness and applicability of evidence

All studies evaluated in this review assessed the effects of breastfeeding or supplemental breast milk on single painful procedures only. Based on our exhaustive search, we are certain about overall completeness of evidence; however, none of the comparisons had a sufficient number of studies for us to investigate publication bias (because we only pooled similar interventions as subtotals rather than conducting an overall analysis).

Based on the available results of these studies, we suggest that term and late preterm neonates who can suck, swallow and co-ordinate with breathing, when undergoing a single painful procedure, be provided with breastfeeding for analgesia when possible. If the mother is not available or if it is not feasible to breastfeed the infant, because of immaturity or the clinical status of infant, alternatives such as other non-pharmacological interventions or glucose or sucrose can be considered. It should be noted that none of these agents eliminate pain completely. On the other hand, the efficacy of supplemental breast milk on physiological outcomes or pain scores was not convincing. The advantages of the provision of breastfeeding or supplemental breast milk for painful procedures provides further encouragement for mothers to breastfeed their infants, facilitating bonding and providing psychological advantages for maternal involvement in the care of their infants without any additional cost to the healthcare system.

The results of these studies are applicable to a large population, i.e. term infants requiring heel lance or venipuncture in their first days of life, intramuscular injection of vitamin K, or hepatitis B and BCG immunisations initiated during the neonatal period. Currently, in many countries around the world, all neonates are subjected to heel lance in the first week of life for metabolic screening, and even in those countries where metabolic screening is not yet available, healthy full-term neonates frequently require heel lance for simple tests such as glucose or bilirubin testing; therefore, the results of these studies are easily applicable to all settings.

The majority of the studies included in this review included healthy term neonates or stable late preterm neonates. A different population of interest who are subjected to a significantly higher number of interventions includes preterm or sick full-term neonates. These neonates are subjected to repeated painful procedures during hospitalisation and the ideal analgesic for this

population has not yet been identified. Johnston 2002 evaluated the effects of repeated administration of sucrose prior to painful procedures in infants < 31 weeks postmenstrual age. Use of sucrose was associated with reduced scores on motor development, vigour, alertness and orientation at 36 weeks, affected motor development and vigour at 40 weeks and had a higher Neuro-Biological Risk Score at two weeks postnatal age. Although unproven, breast milk may be an effective and safe alternative to sucrose, even for repeated use. Placing a small amount of solution in the oral cavity of small preterm infants was only associated with minor complications such as transient desaturation or transient choking, which did not require any intervention. As breast milk is the most natural/physiological substance available for oral stimulation, repeated exposure is not perceived to be associated with complications of oral aversion or repeated tongue thrusting. However, this needs to be studied.

Several methodological challenges were apparent during this review. First, there was marked heterogeneity in the interventions studied. A total of 22 different interventions were compared against breastfeeding or supplemental breast milk. The variety of interventions and their mechanisms of action did not allow us to pool estimates of 'any intervention' against breastfeeding or supplemental breast milk by combining them all. This was carefully considered in the review, and we only reported subgroup analyses as pooled estimates. Second, assessment of pain varied between studies. This has been a problem encountered in a previous review of sucrose for procedural pain in neonates (Stevens 2016). Behavioural and physiological parameters of pain or validated pain measures, or both, were used to assess pain at random in various studies. Standardisation by utilising only validated pain scales and at standardised assessment time points should be the framework for further research. Future studies of adequate sample size should only include validated measures of pain as outcomes. Third, all studies explored the effects of breastfeeding or supplemental breast milk following a single painful procedure. Future studies should include preterm or term neonates who require repeated painful stimuli to assess any side effects of repeated breastfeeding or oral administration of breast milk. Fourth, studies should also measure the future success of breastfeeding as an outcome, as repeated conditioning may prime infants to refuse breastfeeding at a later stage and lead to oral aversion. This is an important consideration, particularly for preterm neonates. Only one study that evaluated maternal perception regarding sucking after single venipuncture while breastfeeding found no differences; however, the effect of repeated exposure has not been studied. Fifth, it must be recognised that there was marked heterogeneity between studies in terms of the control intervention, amount/time of prior exposure to breastfeeding or breast milk, and the time interval between this exposure and the type of painful procedure. Sixth, studying the effects of pain, the condition causing pain and the effects of interventions for pain on neurodevelopment is difficult to tease out as all of these are associated with neurodevelopmental outcomes (Schneider 2018).

### Quality of the evidence

The evidence from the included studies is of low to moderate certainty, as some of the trials were associated with high or unclear risk of bias, even though we only included randomised or quasi-randomised controlled studies. Most studies were penalised under the domain of 'other bias', as we were not able to find protocols for comparison with the completed studies. Additionally, due to inherent limitations, masking is not possible for the breastfeeding group. Some investigators utilised independent assessors to score pain scales from video recordings; however, this may not be always feasible.

### Potential biases in the review process

There are no known potential biases in the review process. We performed an extensive literature search and applied no language restrictions. In addition, we reviewed the references in the articles included to make sure that we were not missing any other studies.

### Agreements and disagreements with other studies or reviews

There are multiple studies in the literature that have studied the effect of sucrose as a means to provide procedural pain relief in newborns. The Cochrane Review of sucrose included 74 studies (Stevens 2016), and they concluded that sucrose is also a safe and effective way of significantly reducing pain from a single painful procedure in newborns. Other studies have shown the benefits of other methods for procedural pain in newborns. In a meta-analysis, Warnock 2010 reported that maternal kangaroo care significantly reduced pain from a single pain procedure in term infants and stable preterm infants (of more than 26 weeks of gestational age). Another Cochrane Review of 25 studies reported that maternal skin-to-skin contact significantly reduced pain from a single painful procedure (Johnston 2017). Thus, a plethora of pharmacological and non-pharmacological interventions is available for procedural pain management in neonates. It is important that non-pharmacological measures are given first preference, especially when there is a need for repeated analgesia.

## AUTHORS' CONCLUSIONS

### Implications for practice

Where feasible and socially acceptable, breastfeeding or breast milk may be considered as a way to alleviate procedural pain in neonates undergoing a single painful procedure such as heel lance, venipuncture, or intramuscular or subcutaneous injection, compared to placebo, positioning or no intervention. Moderate- to low-certainty evidence suggests that breastfeeding or supplemental breast milk likely reduces pain in neonates undergoing painful procedures compared to no intervention/positioning/holding neonates, placebo or non-pharmacological interventions.

Although some benefits are reported, in general low-certainty evidence suggests that breastfeeding may make little or no difference in reducing pain compared to moderate concentration (20% to 33%) of glucose/sucrose.

When repeated painful procedures are needed, neither the safety nor the effectiveness of breastfeeding or supplemental breast milk has been established.

### Implications for research

Further definitive randomised controlled studies are needed to assess the efficacy and safety of breastfeeding and breast milk for repeated painful procedures in neonates, especially for preterm neonates. Safety should be studied with respect to the effect on

successful breastfeeding, oral aversion and neurodevelopmental issues, especially when repeated administration in preterm neonates is contemplated.

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## CHARACTERISTICS OF STUDIES

### Characteristics of included studies *[ordered by study ID]*

**Avcin 2021 (Heel lance)**

| ***Study characteristics*** | |
|---|---|
| Methods | Quasi-randomised controlled trial **(Heel lance)** |
| Participants | Term neonates (37 to 40 weeks of gestation) who underwent heel stick blood drawing for Guthrie test |
| Interventions | Neonates were randomised by the researcher into 4 groups: breastfeeding (n = 35), kangaroo care (n = 35), facilitated tucking position (n = 35) and control (n = 35).<br><br>Breastfeeding group (n = 35, mean GA in weeks 38.5, mean BW in grams 3184). Breastfeeding started 5 minutes before blood collection. The nurse performed the heel stick procedure at the end of the 5 minutes when the participant was in the breastfeeding position on its mother's lap.<br><br>Kangaroo care group (n = 35, mean GA in weeks 38.6 mean BW in grams 3293): the baby was undressed and the mother unbuttoned her shirt and placed her baby on her chest according to the kangaroo care guideline specified by WHO (World Health Organization (WHO), 2003). The room temperature was set at 24 °C to 26 °C. The mother was placed in the semi-Fowler's position, with her back supported by a pillow. The baby was facing the mother to ensure the maternal bond. The baby was wrapped in a blanket, with constant contact with its mother. The mother and the baby were in this position for 15 min, after which a blood sample was collected while the baby was still in the kangaroo care position.<br><br>Facilitated Tucking Position Group (n = 35, mean GA in weeks 38.4, mean BW in grams 3261): each participant received facilitated tucking 1 minute before blood collection with its lower and upper extremities held in lateral flexion and close to the midline. The facilitated tucking position was maintained during and for 1 minute after blood collection.<br><br>Control (n = 35, mean GA in weeks 38.3, mean BW in grams 3361): the heel stick procedure was conducted using the standard method, and the neonates received no intervention during the procedure. |
| Outcomes | Outcome 1: Neonatal Infant Pain Scale during procedure<br><br>Outcome 2: total crying time, seconds<br><br>Outcome 3: oxygen saturation<br><br>Outcome 4: heart rate |

**Avcin 2021 (Heel lance)** *(Continued)*

| | |
|---|---|
| | Outcome 5: respiratory rate |
| Notes | The study was conducted between March 2015 and July 2017 in 3 family health centres in Turkey<br><br>There was no funding or there were no conflicts of interest |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | It is unclear how the random sequence generation was performed |
| Allocation concealment (selection bias) | Low risk | The group was determined by drawing lots from cards put into a bag and drawing of a card determined which of the pain relief methods was used on the participant |
| Blinding (performance bias and detection bias)<br>All outcomes | High risk | The group was determined by drawing lots, and then parental consent was obtained. Interventions could not be masked. |
| Blinding of outcome assessment (detection bias) | Unclear risk | Unclear if outcome assessors were masked |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants included in the analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes were reported. No missing outcome data. |
| Other bias | Unclear risk | Protocol not available for comparison |

**Aydin 2019 (Heel lance)**

***Study characteristics***

| | |
|---|---|
| Methods | Randomised controlled trial **(Heel lance)** |
| Participants | Term neonates (38 to 42 weeks of gestation) who underwent heel stick blood drawing for routine metabolic screening; age 2 to 4 days |
| Interventions | Neonates were randomised by the researcher into 3 groups: breastfeeding (n = 50), heel warming (n = 50) and control (n = 50)<br><br>Breastfeeding group (n = 50, mean GA in weeks 39.3, mean BW in grams 3394): breastfeeding started just before the procedure (1 minute before) and continued for a minimum of 2 minutes during and after the procedure<br><br>Heel warming (n= 50, mean GA in weeks 39.1, mean BW in grams 3344): a thermal bag was used to heat the heel area before the heel stick procedure. Water was put at 40 °C in the thermal bag and applied against the puncture point for 3 to 5 minutes before the heel stick procedure.<br><br>Control (n = 50, mean GA in weeks 38.98, mean BW in grams 3241): the heel stick procedure was conducted using the standard method, and the neonates received no intervention during the procedure |
| Outcomes | Outcome 1: Neonatal Infant Pain Scale during procedure |

**Aydin 2019 (Heel lance)** *(Continued)*

| | |
|---|---|
| | Outcome 2: total crying time, seconds<br><br>Outcome 3: time to first calming |
| Notes | This study was conducted in the baby's nursery of Bandirma State Hospital, Turkey in 2016<br><br>This study did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors. The authors do not have any conflict of interest to declare. |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Random sequence generation was performed by using computer-based random number table program. The researcher randomly picked one code for each neonate to ensure the 150 neonates were equally allocated into three groups based on the group number of each code. |
| Allocation concealment (selection bias) | Unclear risk | The method of allocation concealment was not clear |
| Blinding (performance bias and detection bias)<br>All outcomes | Unclear risk | It is unclear if masking of intervention performed |
| Blinding of outcome assessment (detection bias) | Low risk | Assessment of pain using NIPS was conducted independently by an observer nurse who was blinded to the group allocation of the newborns |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes were reported. No missing outcome data. |
| Other bias | Unclear risk | Protocol not available for comparison |

**Aziznejad 2013 (Venipuncture)**

***Study characteristics***

| | |
|---|---|
| Methods | Randomised controlled trial **(Venipuncture)** |
| Participants | Inclusion criteria were diagnosis of jaundice, no cardiovascular, respiratory, infectious or neural diseases, no congenital malformations or chromosomal syndromes confirmed by an attending physician, which required daily venipuncture to control bilirubin, the stability of clinical status, Apgar score below 7 at 5 minutes and consciousness of neonate before venipuncture |
| Interventions | Randomly divided 120 neonates into 4 groups of 30: control group, sucrose group, breast milk group and EMLA cream group. Venipuncture was carried out in the first group (control) without any specific treatment. In the second and third groups, venipuncture was performed 2 minutes after feeding neonates (dropping the substances on neonates' tongue using a syringe) with respectively 2 cc of 25% sucrose and 2 cc of breast milk. In the 4th group, a layer of 2.5% EMLA cream (1 g) was first topically applied in the antecubital area.<br><br>Control group (no intervention): n = 30, mean age in days 10, mean BW in grams 3106 |

**Aziznejad 2013 (Venipuncture)** *(Continued)*

| | |
|---|---|
| | Sucrose group: n = 30, mean age in days 7.8, mean BW in grams 3055<br><br>Breast milk group: n = 30, mean age in days 7.8, mean BW in grams 3053<br><br>EMLA cream group: n = 30, mean age in days 8.5, mean BW in grams 3078 |
| Outcomes | 1. Pain score via DAN scale<br>2. Duration of crying in seconds<br>3. Change in heart rate<br>4. Change in arterial blood oxygen saturation<br>5. Change in respiratory rate |
| Notes | This study was conducted in Iran<br><br>There was no information regarding when this study was conducted, any conflicts of interest or funding |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Random allocation in blocks was carried out to assign samples to 4 groups, meaning that the first 4 neonates were allocated to the groups and the process continued until 120 neonates were divided into 4 groups of 30 |
| Allocation concealment (selection bias) | Unclear risk | Method of allocation concealment was not clear |
| Blinding (performance bias and detection bias)<br>All outcomes | High risk | No information was provided about whether the intervention was masked |
| Blinding of outcome assessment (detection bias) | Unclear risk | The DAN score was observed by a fixed and trained nursing expert (a person other than the sampler) through 3 separate parameters of facial movements, body movements and noise level. However, it is not clear if other outcome assessments like heart rate and respiration per minute, percentage of arterial blood oxygen saturation and the duration of crying were masked. |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants included in the final analysis |
| Selective reporting (reporting bias) | Unclear risk | All outcomes reported but the actual numbers for two outcomes (heart rate and $SpO_2$) were not available |
| Other bias | Unclear risk | Protocol not available for comparison |

**Bavarsad 2018 (Intramuscular injection)**

***Study characteristics***

| | |
|---|---|
| Methods | Randomised controlled trial **(Intramuscular injection)** |
| Participants | Included participants: full-term neonates, weighing 2500 g, born via vaginal delivery, having an Apgar score > 7, lacking any disease or congenital disorder, breastfed at least once, at least 2 hours after birth and having a consent form completed |

**Bavarsad 2018 (Intramuscular injection)** *(Continued)*

| | |
|---|---|
| Interventions | The hepatitis B vaccine was injected in neonates in the control group routinely with no feeding. The vaccine was injected in other 3 groups during oral feeding in different conditions: feeding from the mother's breast, bottle feeding of mother's milk and feeding of powdered formula. A similar method was considered for 3 feeding groups, i.e. neonates in a calm environment started feeding for 2 min and were still feeding during the injection for at least 2 min.<br><br>Breastfeeding (n = 25, mean GA in weeks 39.3, mean BW in grams 3000)<br><br>Bottle feeding mother's milk (n = 25, mean GA in weeks 39.2, mean BW in grams 2972)<br><br>Bottle feeding powdered formula (n = 25, mean GA in weeks 39.0, mean BW in grams 3032)<br><br>Control (mother holding) (n = 25, mean GA in weeks 39.1, mean BW in grams 3202) |
| Outcomes | Outcome 1: pulse rate, beats/min<br><br>Outcome 2: arterial $O_2$ sats<br><br>Outcome 3: crying duration, seconds<br><br>Outcome 4: face grimaces<br><br>Outcome 5: limb movements<br><br>Outcome 6: vocal responses |
| Notes | This study was conducted in teaching hospital in Ilam city, Iran in 2016<br><br>The authors declare that they have no competing interest. Ilam University of Medical Sciences, Ilam, Iran, supported this study. |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Infants were randomised, but the method is not clear; we cannot tell if random sequence generation was performed |
| Allocation concealment (selection bias) | Unclear risk | It is unclear if allocation concealment was performed |
| Blinding (performance bias and detection bias)<br>All outcomes | Unclear risk | It is unclear if the investigator was blinded. |
| Blinding of outcome assessment (detection bias) | Low risk | Two nurses, who were blinded to the aim and methods of the study, were trained and they measured the scores separately |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

### Bembich 2013 (Heel lance)

| ***Study characteristics*** | |
|---|---|
| Methods | Randomised controlled trial **(Heel lance)** |
| Participants | 30 healthy full-term newborns (gestational age range: 38 to 41 weeks; 16 males and 14 females) were enrolled who underwent heel prick on third day of life for metabolic screening; none had previously experienced any painful procedure<br><br>15 (8 males; 7 females) were given an oral glucose solution before the heel prick and 15 were breastfed during the heel prick |
| Interventions | Oral glucose group (n = 15): neonate was first placed on his/her back on a baby changing-table and the fibres were positioned on the infant scalp; 2 minutes before starting the blood sampling procedure, a bolus of 2 mL of 20% oral glucose solution was administered<br><br>Breastfeeding group (n = 15): the neonate was tested in his/her mother's lap. After placing optical fibres on the infant's scalp and waiting for the newborn to get used to the equipment, breastfeeding was started 2 min before the blood sampling procedure. Mothers were required not to talk to their newborns. Breastfeeding lasted until the blood sampling procedure was completed. |
| Outcomes | 1. Increase of cortical oxy-haemoglobin ($HbO_2$) during the heel-prick procedure as an estimate of cortical activation by use of multichannel near infra-red spectroscopy (NIRS) device<br>2. Neonatal Infant Pain Scale (NIPS) scores |
| Notes | This study was conducted in the Institute for Maternal and Child Health-IRCCS 'Burlo Garofolo', Trieste, Italy<br><br>No information was provided regarding when this study was conducted<br><br>No comments on conflict of interest. This study was financed by grant No. 50/11 of the IRCCS 'Bur- lo Garofolo'. |

| ***Risk of bias*** | | |
|---|---|---|
| **Bias** | **Authors' judgement** | **Support for judgement** |
| Random sequence generation (selection bias) | Unclear risk | We cannot tell if random sequence generation was performed |
| Allocation concealment (selection bias) | Unclear risk | We also cannot tell if allocation concealment was performed |
| Blinding (performance bias and detection bias)<br>All outcomes | Low risk | Assessments were performed by an investigator blinded to NIRS detection |
| Blinding of outcome assessment (detection bias) | Low risk | Outcomes assessment was performed by an investigator blinded to NIRS detection |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

### Bembich 2018 (Heel lance)

| ***Study characteristics*** | |
|---|---|
| Methods | A randomised controlled trial **(Heel lance)** |
| Participants | 80 healthy term newborns (gestational age: 37 to 42 weeks) who were undergoing a heel stick for metabolic screening on<br>their third day of life (and who had started breastfeeding) were included |
| Interventions | Infants were randomised into 4 groups<br><br>Infants who were allocated to group 1 and group 2 were placed on a changing table, and fibres were positioned on the scalp. A waiting period was allowed for the infant to get used to the equipment. Two minutes before starting the heel stick procedure, a 2 mL bolus of 20% oral glucose solution (group 1) or a 2 mL bolus of the mother's breast milk (group 2) was administered directly into the infant's mouth with a syringe.<br>Infants in group 3 and group 4 were tested while in their mothers' arms (mother-infant relationship). Optical fibres were placed on the scalp, and a waiting period was allowed for the newborn to adapt. 2 minutes before the heel stick procedure, a 2 mL bolus of 20% oral glucose solution was given directly into the infant's mouth with a syringe (group 3) or breastfeeding was started (group 4). Breastfeeding lasted at least until the heel stick procedure was completed.<br><br>20% oral glucose solution (OGS) (group 1): n = 20, mean GA in weeks 39.6, mean BW in grams 3354<br><br>EBM (Group 2): n = 20, mean GA in weeks 39.8, mean BW in grams 3155<br><br>Maternal holding + OGS (Group 3): n= 20, mean GA in weeks 39.8, mean BW in grams 3429<br><br>Breastfeeding (Group 4): n= 20, mean GA in weeks 39.9, mean BW in grams 3298 |
| Outcomes | Outcome 1: NIPS score<br><br>Outcome 2: cortical activity using multichannel NIRS |
| Notes | This study was conducted in the Institute for Maternal and Child Health–IRCCS "Burlo Garofolo," Trieste, Italy between November 2016 and May 2017<br><br>Authors have indicated they have no potential conflicts of interest to disclose or funding |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | An independent statistician created a computer-generated, randomised treatment assignment list (simple randomisation) |
| Allocation concealment (selection bias) | Low risk | Treatment allocations were placed in opaque and sealed envelopes and sequentially numbered from 1 to 80. Both procedures were masked to investigators. |
| Blinding (performance bias and detection bias)<br>All outcomes | High risk | Investigators, nurses and mothers were not blinded to the treatment allocation |
| Blinding of outcome assessment (detection bias) | Low risk | NIPS scoring was performed by an investigator who was blinded to the NIRS data |
| Incomplete outcome data (attrition bias) | Low risk | All infants were included in the final analysis |

**Bembich 2018 (Heel lance)** *(Continued)*

All outcomes

| | | |
|---|---|---|
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Blass 2001 (Heel lance)**

***Study characteristics***

| | |
|---|---|
| Methods | Quasi-randomised controlled trial **(Heel lance)** |
| Participants | 60 stable full-term newborn infants undergoing routine newborn screening (heel lance) between 30 and 55 hours of age were randomly assigned to one of the 6 treatment groups (10 neonates in each group)<br>Mean (range) BW - 3200 (2400 - 4200) grams<br>Male:female - 27:33 |
| Interventions | Group 1: 2 mL water given over 2 minutes via syringe<br>Group 2: 2 mL colostrum given over 2 minutes via syringe<br>Group 3: 2 mL of 12% sucrose given over 2 minutes via syringe<br>Group 4: 2 mL water given on a pacifier dipped in water every 30 seconds for 2 minutes<br>Group 5: 2 mL of colostrum given on a pacifier dipped in colostrum every 30 seconds for 2 minutes<br>Group 6: 2 mL of sucrose given on a pacifier dipped in sucrose every 30 seconds for 2 minutes |
| Outcomes | Percentage of time crying during the procedure in relation to control<br>Percentage of time grimacing during the procedure<br>Mean crying time during the recovery phase<br>Mean changes in heart rate during and following the procedure |
| Notes | This study was conducted in Boston Medical Center, Boston, USA between June 1997 and March 1999<br><br>This research has been supported by Grant RO-1 MH51705-04A1 and Research Scientist Award KO-5, MH5 00524, from the National Institutes of Mental Health to E.M.B. No information regarding any conflicts of interest. |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | High risk | Random sequence generation was performed through a table of random numbers and 6 groups were formed, but the random generation was not followed adequately because of the difficulty of obtaining sufficient colostrum in many instances |
| Allocation concealment (selection bias) | High risk | Inadequate |
| Blinding (performance bias and detection bias)<br>All outcomes | High risk | The phlebotomist who performed the heel lance and the person who rated the video data were unaware of allocation<br><br>Masking of intervention was not possible in this study since it involved the use of a pacifier and a liquid (colostrum) that differed in colour from two other solutions |

**Blass 2001 (Heel lance)** *(Continued)*

| | | |
|---|---|---|
| Blinding of outcome assessment (detection bias) | Low risk | Masking of outcome assessment was possible with crying time and heart rate changes, but not so when assessing the grimacing since the intervention involved the use of a pacifier |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included |
| Selective reporting (reporting bias) | Low risk | All infants were included in the final analysis |
| Other bias | Unclear risk | Protocol not available to compare |

**Bozlak 2017 (Eye examination)**

| | |
|---|---|
| ***Study characteristics*** | |
| Methods | A randomised controlled trial (RCT) **(Eye examination)** |
| Participants | Inclusion criteria: gestational age less than 32 weeks and body weight less than 1500 g, parent approval on the consent form, no requirement for invasive or noninvasive mechanical ventilator support during the procedure, no intake of analgesic or sedative drug in the past 24 hours, no contraindications to oral feeding, and no congenital abnormalities |
| Interventions | The oral sucrose was prepared as a 24% sucrose solution in the hospital pharmacy; breast milk was obtained from the mothers and stored under appropriate conditions. Ready-to-use sterile ampules were used for the distilled water. The research nurse first swaddled the infants and, according to the particular study group, then put 0.2 mL of the solution, breast milk or distilled water into the injector.<br>Swaddling with sucrose administration (n = 29, mean GA in weeks 29.9, mean BW in grams 1045)<br>Swaddling with breast milk administration (n = 29, mean GA in weeks 30.2, mean BW in grams 1048)<br>Swaddling with distilled water administration (n = 29, mean GA in weeks 29.8, mean BW in grams 1042) |
| Outcomes | PIPP pain scale |
| Notes | This study was conducted in the NICU of a Level III university hospital in Istanbul, Turkey between June and September 2013<br>The authors report no conflict of interest or relevant financial relationships |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | For randomisation, numbers 1 to 87 were randomised with no number repetition into 3 groups through the use of the Random Assignment computer program. The infants were thus randomly assigned to 1 of 3 groups. |
| Allocation concealment (selection bias) | Low risk | The information about group assignment was put in sealed envelopes that contained the private files of the infants. Only the research nurse responsible for the preparation of the nonpharmacologic solution for each infant group saw these envelopes. |
| Blinding (performance bias and detection bias) | Low risk | The nurses who assisted with the ROP examinations evaluated the pain level through the use of the Premature Infant Pain Profile (PIPP) scale |

**Bozlak 2017 (Eye examination)** *(Continued)*

| | | |
|---|---|---|
| All outcomes | | |
| Blinding of outcome assessment (detection bias) | Low risk | These nurses who evaluated pain levels were blinded to the group assignments of the infants |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Bucher 2000 (Heel lance)**

| | |
|---|---|
| ***Study characteristics*** | |
| Methods | Randomised controlled trial **(Heel lance)** |
| Participants | 80 stable full-term newborn infants undergoing routine newborn screening (heel lance) on postnatal day 3 were randomly assigned to one of the 4 treatment groups<br>Group 1: 20 neonates<br>Mean (range) BW - 3420 (2650 to 5000) grams<br>Male:female - 10:10<br>Group 2: 20 neonates<br>Mean (range) BW - 3430 (2640 to 3960) grams<br>Male:female - 10:10<br>Group 3: 20 neonates<br>Mean (range) BW - 3350 (2720 to 4200) grams<br>Male:female - 8:12<br>Group 4: 20 neonates<br>Mean (range) BW - 3410 (2740 to 4170) grams<br>Male:female - 9:11 |
| Interventions | Group 1: 2 mL of artificial sweetener<br>Group 2: 2 mL of glycine<br>Group 3: 2 mL of breast milk<br>Group 4: 2 mL of sterile water |
| Outcomes | Heart rate change<br>Percentage time crying<br>Body pain score<br>Facial pain score<br>Combined pain score |
| Notes | This study was conducted in University Hospital, Zurich, Switzerland<br><br>There was no mention of when this study was conducted or conflicts of interest/funding |

| ***Risk of bias*** | | |
|---|---|---|
| **Bias** | **Authors' judgement** | **Support for judgement** |
| Random sequence generation (selection bias) | Unclear risk | It is unclear if random sequence generation was performed |

**Bucher 2000 (Heel lance)** *(Continued)*

| | | |
|---|---|---|
| Allocation concealment (selection bias) | Low risk | Adequate: allocation concealment was done through sealed envelopes |
| Blinding (performance bias and detection bias) All outcomes | Low risk | A nurse administered the solution in the absence of investigators and was not involved in heel prick or data collection |
| Blinding of outcome assessment (detection bias) | Low risk | Masking of outcome assessment was done by blinding the observer to the study group assignments |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | All outcomes reported |
| Selective reporting (reporting bias) | Low risk | All infants were included in the final analysis |
| Other bias | Unclear risk | Protocol not available to compare |

**Bueno 2012 (Heel lance)**

| | |
|---|---|
| ***Study characteristics*** | |
| Methods | A noninferiority randomised controlled trial (RCT) **(Heel lance)** |
| Participants | Eligible infants were between 34 and 36 completed weeks of GA at birth; were between 24 and 72 hours old; had 5-minute Apgar scores of > 7; were fed at least 1 hour before data collection; had no syndromes, congenital anomalies or previous surgery; were not born to mothers with hepatitis C or HIV infection; were born to mothers not known to be a user of illicit drugs; and had clinical indication for blood sampling |
| Interventions | Interventions investigated were 2 mL of EBM (experimental group (EG)) and 2 mL of 25% glucose (control group (CG)), applied via a needleless syringe to the anterior portion of the tongue 2 minutes before the lancing procedure<br><br>EG: n = 56, mean BW 2460.5 g, mean GA 35.5 weeks<br><br>CG: n = 57, mean BW 2235.7 g, mean GA 35.8 weeks |
| Outcomes | Outcome 1: pain intensity assessed with the PIPP<br><br>Outcome 2: crying incidence<br><br>Outcome 3: incidence of adverse events (e.g. nausea, regurgitation, vomiting, choking, desaturation, tachycardia and bradycardia) |
| Notes | This study was conducted in the neonatal unit of a university-affiliated Level III hospital University of São Paulo, São Paulo, Brazil from August 2009 to May 2010<br><br>No comment about conflict of interest/funding |
| ***Risk of bias*** | |
| **Bias** | **Authors' judgement** **Support for judgement** |

**Bueno 2012 (Heel lance)** *(Continued)*

| | | |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | A statistician used the Statistical Analysis System (SAS), version 8.2 (SAS Institute, Inc,Cary, NC) to generate blocked randomisation lists |
| Allocation concealment (selection bias) | Low risk | Allocation concealment was achieved by using numbered, opaque, sealed envelopes containing intervention codes. Envelopes were exclusively accessed by research assistants. |
| Blinding (performance bias and detection bias)<br>All outcomes | Low risk | Research assistants prepared syringes containing both solutions for all infants. Syringes were covered to mask the intervention and were labelled according to the envelope's codes. Infants' faces and the monitor screen were filmed in real time by using independent video cameras during the entire data collection procedure. The focus of the video camera was deviated from the infants' faces during solution administration to guarantee masking of the interventions during facial coding. |
| Blinding of outcome assessment (detection bias) | Low risk | Outcomes were assessed and coded by a trained coder who was masked to the intervention received |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Carbajal 2003 (Venipuncture)**

| ***Study characteristics*** | |
|---|---|
| Methods | Randomised controlled trial **(Venipuncture)** |
| Participants | 179 healthy term neonates<br>Inclusion criteria: healthy term (≥ 37 weeks GA) undergoing venipuncture for diagnostic evaluation<br>Exclusion criteria: medical instability, received naloxone in the last 24 hours, received sedative or major analgesic in the last 48 hours<br>Group 1: 44 neonates<br>Mean GA - 39.7 (1.15) weeks<br>Mean BW - 3306 (382.8) g<br>Group 2: 45 neonates<br>Mean GA - 39.8 (1.23) weeks<br>Mean BW - 3304 (483.0) g<br>Group 3: 45 neonates<br>Mean GA - 40.0 (1.14) weeks<br>Mean BW - 3420 (418.8) g<br>Group 4: 45 neonates<br>Mean GA - 39.6 (1.20) weeks<br>Mean BW - 3313 (401.2) g |
| Interventions | Group 1: breastfeeding 2 minutes before and throughout the procedure<br>Group 2: cuddled in mother's arms without breastfeeding starting 2 minutes prior to procedure<br>Group 3: 1 mL of placebo (sterile water) without pacifier 2 minutes before the procedure while lying supine on the table<br>Group 4: 1 mL of 30% glucose followed by pacifier 2 minutes prior to venipuncture while lying supine on the table |

**Carbajal 2003 (Venipuncture)** *(Continued)*

| | |
|---|---|
| Outcomes | DAN rating scale for pain in neonates<br>PIPP<br>Standardised questionnaires to mothers to determine the effect of venipuncture on breastfeeding at 48 to 72 hours after the venipuncture |
| Notes | This study was conducted in Poissy-Saint Germain Hospital, France<br><br>No conflicts of interest declared<br><br>No information regarding when the study was conducted or about funding |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | An assistant not involved in the study performed the randomisation in advance in blocks of 20 using a random number table |
| Allocation concealment (selection bias) | Low risk | Treatment allocations were placed in opaque, sealed envelopes numbered 1 to 180; investigators were blind to these allocations. Codes of allocation were kept secret by the assistant who performed the randomisation. |
| Blinding (performance bias and detection bias)<br>All outcomes | High risk | Masking was not possible since it involved breastfeeding, the use of a pacifier and cuddling before and throughout the procedure |
| Blinding of outcome assessment (detection bias) | High risk | The outcome assessment was masked as the observers who assessed the outcome measures were not aware as to the purpose and hypothesis of the study. Personal bias on the part of the outcome observer cannot be excluded |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | The authors comment that each group had 45 patients, but they only show characteristics and results for 44 patients in the breastfeeding group |
| Selective reporting (reporting bias) | Low risk | All the outcomes mentioned by the authors were reported on |
| Other bias | Unclear risk | Protocol not available to compare |

**Chiabi 2016 (Heel lance)**

***Study characteristics***

| | |
|---|---|
| Methods | Randomised controlled trial **(Heel lance)** |
| Participants | Healthy term neonates of at least 24 h life. They first had a complete physical examination, and then a heel prick on the latero-external or the posteromedial surface using a 23 gauge needle. Breastfeeding or 30% glucose solution was then given. The newborns were divided into the 2 groups by drawing of lots.<br><br>Breastfeeding group: 50 neonates; mean GA 39.2 weeks; mean weight 3234 g<br><br>30% glucose solution group: 50 neonates; mean weight 3316 g; mean GA 39.3 weeks |
| Interventions | Breastfeeding group: mothers were placed in a calm room and the neonates placed on the breast after a period of about 30 min without feeding; the neonates were held in the arms of their mothers, and the |

**Chiabi 2016 (Heel lance)** *(Continued)*

| | |
|---|---|
| | heel prick was done 2 min later, just time sufficient for a good latch and sucking before performing the heel prick.<br>For neonates in the 30% glucose solution group: received, 2 doses of 30% glucose solution in a 2-min interval on the anterior surface of the tongue. The 2 doses were administered 2 min before doing the heel prick. Newborns weighing between 2.5 kg and 3 kg received 2 mL of the solution and those weighing more than 3 kg received 4 mL.<br><br>Heel pricks performed to determine the blood sugar |
| Outcomes | Neonatal Infant Pain Scale (NIPS) to evaluate pain - at the baseline state, at the impact of the needle and 2 min after the capillary prick |
| Notes | Study was conducted in maternity of the Yaoundé Gynaeco-Obstetric and Paediatric Hospital (YGOPH), Cameron from January 02 to October 31, 2013<br><br>No comments on conflict of interest/funding |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Newborns were randomly divided into the 2 groups by drawing of lots |
| Allocation concealment (selection bias) | Unclear risk | Allocation concealment is not clear |
| Blinding (performance bias and detection bias)<br>All outcomes | High risk | Masking of intervention and outcomes is not clear |
| Blinding of outcome assessment (detection bias) | Unclear risk | Masking of outcome assessment is unclear |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Codipietro 2008 (Heel lance)**

***Study characteristics***

| | |
|---|---|
| Methods | Randomised controlled trial **(Heel lance)** |
| Participants | 101 full-term infants at more than 60 hours of age undergoing heel lance for metabolic screening<br>Exclusion criteria: at-risk pregnancy, medical instability, birth in general anaesthesia, maternal use of opioids, administration of naloxone or phenobarbital in the previous 48 hours and artificial feeding<br>Group 1: 51 neonates<br>Mean (range) GA - 39.3 (+/- 1.2) weeks<br>Mean (range) BW - 3318 (+/- 402) g<br>Group 2: 50 neonates<br>Mean (range) GA - 39.4 (+/- 1.1) weeks |

**Codipietro 2008 (Heel lance)** *(Continued)*

| | |
|---|---|
| | Mean (range) BW - 3308 (+/- 430) g |
| Interventions | Group 1: Breastfeeding<br>Group 2: 1 mL of 25% sucrose<br><br>Infants in Group 1 were held by mother and breastfed until there was a continuous active suction prior to heel lance. Group 2 infants were laid on a changing table and a bolus of 1 mL of 25% sucrose solution was administered through syringe in the mouth 2 minutes before the heel lance |
| Outcomes | PIPP scale, changes in heart rate and saturation 30 seconds after the procedure, duration of first cry, percentage of crying in the first 2 minutes after the procedure |
| Notes | The study was performed in the neonatal unit of Agnelli Hospital, Italy from January to April 2007<br><br>No funding or financial relationships, but no information regarding any conflicts of interest |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Randomisation was done by using a computer random number generator |
| Allocation concealment (selection bias) | Low risk | Allocation was concealed using opaque, sealed envelopes, which were opened sequentially by the paediatric nurse who performed blood sampling |
| Blinding (performance bias and detection bias) All outcomes | High risk | Masking of intervention was not possible since it involved breastfeeding before and throughout the procedure. Incomplete blinding as breastfeeding group was held by mother while group 2 infants were laid on a changing table. |
| Blinding of outcome assessment (detection bias) | High risk | Incomplete blinding. Assessment of one of the outcomes (cry behaviour) was masked as it was assessed by 2 assistants who listened to tape recordings. The PIPP scale was administered by a paediatric nurse. All the paediatric nurses and mothers were not blinded to the treatment assignment. |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | All infants were accounted for in the analysis of outcomes |
| Selective reporting (reporting bias) | Low risk | All prespecified outcomes were reported on |
| Other bias | Low risk | Protocol available; all prespecified outcomes addressed |

**Cordero 2014 (Heel lance)**

| | |
|---|---|
| ***Study characteristics*** | |
| Methods | A randomised controlled trial **(Heel lance)** |
| Participants | A random sample of 93 newborns were taken from all the babies in neonatal care unit at the San Cecilio University Hospital in Granada who were required to undergo the heel lance procedure<br><br>They were divided randomly into 3 groups:<br><br>Group 1: breast milk group (31 neonates, mean GA 37.1 weeks, mean BW 2738 g) |

**Cordero 2014 (Heel lance)** *(Continued)*

| | |
|---|---|
| | Group 2: oral 24% glucose group (31 neonates, mean GA 37.4 weeks, mean BW 2782 g)<br><br>Group 3: control group (received nothing) (31 neonates, mean GA 36.9, mean BW 2781 g) |
| Interventions | The first group of newborns received breast milk; the second group was given a 24% oral glucose solution; and the third group, which was the control group, received nothing at all |
| Outcomes | 1. Change in heart rate before and after heel lance procedure<br>2. Change in oxygen saturation |
| Notes | Study was conducted in 2010 in the neonatal care unit at the San Cecilio University Hospital in Granada<br><br>No comments on conflict of interest or funding |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | A random sample of 93 newborns was taken from all the babies in the unit, who were required to undergo the heel lance procedure. The population sample for the study was randomly divided into 3 groups. However, method of random sequence generation is not clear. |
| Allocation concealment (selection bias) | High risk | Allocation concealment was not done |
| Blinding (performance bias and detection bias)<br>All outcomes | High risk | Masking of intervention or outcomes was not performed |
| Blinding of outcome assessment (detection bias) | High risk | Masking of outcome assessment was not performed |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes were reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Dar 2019 (Intradermal)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial (RCT)**(Intradermal)** |
| Participants | Healthy full-term neonates who were partially or exclusively breastfed and who came for BCG vaccination to outpatient department |
| Interventions | Infants randomly divided into 2 groups:<br><br>Breastfeeding group (partial or exclusive): n = 30 (male 14, female 16)<br><br>Control group (only routine care; no breastfeeding) n = 30 (male 15, female 15) |

**Dar 2019 (Intradermal)** *(Continued)*

| | |
|---|---|
| Outcomes | Crying time in seconds |
| Notes | This study was conducted in Combined Military Hospital, Pakistan between 1 June and 30 November 2015<br><br>No information available regarding any conflicts of interest or funding |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Lottery method was used to randomise the neonates |
| Allocation concealment (selection bias) | High risk | Allocation concealment was not performed |
| Blinding (performance bias and detection bias)<br>All outcomes | High risk | Masking of intervention or outcomes was not performed |
| Blinding of outcome assessment (detection bias) | High risk | Masking of outcome assessment was not performed |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Desai 2017 (Suctioning)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial (RCT)**(Suctioning)** |
| Participants | Preterm neonates on assisted ventilation requiring suction |
| Interventions | Randomised into 3 groups:<br><br>Breast milk group (n = 36, mean GA in weeks 32.76, mean BW in grams 1358): 2 cc EBM was administered to the neonate 2 min before suctioning<br><br>Swaddling group (n = 36, mean GA in weeks 33.1, mean BW in grams 1516): neonates swaddled for 10 to 15 min before suctioning<br><br>Oral sucrose group (n = 36, mean GA in weeks 32.9, mean BW in grams 1430) : 2 cc sucrose was administered to the neonate for 2 min before suctioning |
| Outcomes | Premature Infant Pain Profile Score |
| Notes | This study was carried out from March to August 2016 in Seth GS Medical College and KEM Hospital, Mumbai, India |

**Desai 2017 (Suctioning)** *(Continued)*

No conflicts of interest or funding declared

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | The suctioning episodes in neonates on assisted ventilation were randomised by a computer-generated randomisation sequence. Randomisation was done in variable random blocks of 3 or 6. |
| Allocation concealment (selection bias) | Low risk | Treatment allocations were inserted in sequentially numbered, opaque envelopes and were sealed. Just before suctioning, a senior resident opened the sequentially numbered envelope and allocated the group. |
| Blinding (performance bias and detection bias)<br>All outcomes | High risk | Masking of intervention was not done |
| Blinding of outcome assessment (detection bias) | Unclear risk | It is unclear if masking of outcome assessment was performed |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Efe 2007 (Venipuncture)**

***Study characteristics***

| | |
|---|---|
| Methods | Quasi-randomised controlled trial **(Venipuncture)** |
| Participants | 102 full-term infants undergoing venipuncture for metabolic screening or hyperbilirubinaemia<br>Group 1: 34 neonates<br>Mean GA - 38.9 (0.9) weeks<br>Mean BW - 3327.5 (409) g<br><br>Group 2: 34 neonates<br><br>Mean GA - 38.9 (1.1) weeks<br>Mean BW - 3202.5 (360) g<br>Group 3: 34 neonates<br>Mean GA - 39.2 (1.1) weeks<br>Mean BW - 3381.6 (434.3) g |
| Interventions | Infants in group 1 (breastfeeding group) were held in skin-to-skin contact with their mothers during the entire procedure. 3 minutes after the first jaw movements were observed, the venous blood sample was taken. Infants continued to breastfeed during and after the venipuncture. Group 2 infants received 2 mL of 25% sucrose solution put into pacifiers. The infants started to suck the pacifier with sucrose 3 minutes before the venipuncture and continued to suck during and after sampling. The control group infants were wrapped in a blanket with only the hand that would be used for sampling outside |

**Efe 2007 (Venipuncture)** *(Continued)*

| | |
|---|---|
| | the blanket. The mother stayed next to the infant trying to soothe him verbally. After the sample was collected, the infant was cuddled by the mother and could be given a pacifier |
| Outcomes | NIPS, heart rate, saturation levels and crying time |
| Notes | This study was conducted in Akdeniz University Hospital, Turkey between June and July 2002<br>This study received external funding from Akdeniz University Scientific Research Project Unit<br>No information regarding any conflicts of interest |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | The method of random sequence generation was unclear |
| Allocation concealment (selection bias) | High risk | The allocation was done according to mothers' preference |
| Blinding (performance bias and detection bias)<br>All outcomes | High risk | No blinding |
| Blinding of outcome assessment (detection bias) | High risk | No blinding |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | No missing outcome data |
| Selective reporting (reporting bias) | Low risk | The article includes all the outcomes that were prespecified |
| Other bias | Unclear risk | Protocol not available to compare |

**Erdogan 2022 (Venipuncture)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial **(Venipuncture)** |
| Participants | Term neonates (37 to 40 weeks of gestation) who underwent venipuncture for blood drawing; postnatal age 5 to 8 days |
| Interventions | Neonates were randomised by the researcher into 4 groups: breast milk taste (n = 30), breast milk smell (n = 30), breast milk taste + smell (n = 30) and control (n = 30)<br>Breast milk taste group (n = 30, mean GA in weeks 38.6, mean BW in grams 3185): 5 mL of milk was dripped into the infant's mouth to give the infant a taste of the breast milk 3 or 5 minutes before blood collection<br>Breast milk smell group (n = 30, mean GA in weeks 38.4, mean BW in grams 3221): 3 or 5 minutes before blood collection, 5 mL of milk-soaked cotton was placed at a 5 cm to 10 cm distance to the infant's nose for the infant to smell it |

**Erdogan 2022 (Venipuncture)** *(Continued)*

| | |
|---|---|
| | Breast milk taste + smell group (n = 30, mean GA in weeks 38.4, mean BW in grams 3105): the procedures in both Experimental Group I and Experimental Group II were performed 3 or 5 minutes before blood collection<br><br>Control Group (n = 30, mean GA in weeks 38.8, mean BW in grams 3044): no intervention was performed |
| Outcomes | Outcome 1: Neonatal Pain, Agitation, and Sedation Scale (N-PASS)<br><br>Outcome 2: heart rate<br><br>Outcome 3: oxygen saturation |
| Notes | This study was conducted in Turkey<br><br>No conflicts of interest or funding reported<br><br>However, no information regarding when this study was conducted |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | These 120 infants were randomly assigned to the experimental and control groups by the urn method. In the case of an infant matching the sampling criteria in the urn method, the balls prepared by the researcher beforehand were put into a black bag, and the selection process was carried out blindly by one of the researchers. |
| Allocation concealment (selection bias) | Low risk | According to the colour of the selected ball, the infant was assigned to the smell, taste, taste + smell or control group. Therefore, the infants were randomly distributed into 4 groups. |
| Blinding (performance bias and detection bias) All outcomes | Unclear risk | The author mentioned that this was a single-blind study and the interventions were started 3 or 5 minutes before blood collection. However, it is unclear if interventions were masked. |
| Blinding of outcome assessment (detection bias) | Low risk | A nurse drew blood in the clinic, and assessments and observations were made by another nurse who did not know the purpose and hypothesis of the study. |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | All infants were included in the analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes were reported. No missing outcome data. |
| Other bias | Unclear risk | Protocol not available for comparison |

**Fallah 2017 (Intradermal)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised, single-blind, clinical, parallel-group trial **(Intradermal)** |
| Participants | Healthy term neonates who received routine BCG vaccination in the first day of their life were enrolled in the study |

**Fallah 2017 (Intradermal)** *(Continued)*

| | |
|---|---|
| | Eligible participants included term neonates (gestation age of 37 to 42 weeks) who were the product of normal vaginal delivery, awake and alert before vaccination, without systemic illness, in a healthy medical condition and with birth weight of 2500 g to 4000 g |
| Interventions | The neonates were randomly distributed into 3 groups (40 neonates in each group)<br>Group 1 (n = 40; mean BW 3160 g) - breastfeeding started 2 minutes before vaccination and continued during and 1 minute after BCG vaccination<br>Group 2 (n = 40; mean BW 3150 g) - neonates received KMC 10 minutes before, during and 1 minute after BCG vaccination<br>Group 3 (n = 40; mean BW 3110 g) - neonates were swaddled 10 minutes before, during and 1 minute after vaccination |
| Outcomes | 1. Neonatal/Infant Pain Scale (NIPS) score<br>2. Duration of neonate crying during BCG vaccination |
| Notes | This study was conducted in Shahid Sadoughi Hospital, Yazd, Iran from March to June 2015<br>No conflicts of interest declared. This study was funded by a grant from the Deputy for Research of Shahid Sadoughi University of Medical Sciences, Yazd, Iran. |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | The trial used computer-generated equal simple randomisation by random numbers and the allocation ratio was 1:1 for the 3 groups |
| Allocation concealment (selection bias) | Low risk | Allocation concealment was done by writing down the intervention for each serially participating neonate in a numbered and sealed opaque envelope, which was opened by the paediatric research neurologist immediately before intervention. Randomisation and concealment were done by a researcher with no clinical involvement in the trial. |
| Blinding (performance bias and detection bias) All outcomes | High risk | Blinding of parents of the participants, the hospital nurse, the data collector and the outcome assessor was not possible, and data analysts were only kept blinded to the allocation. However, concealment was done by writing down the intervention for each serially participating neonate in a numbered and sealed opaque envelope, which was opened by the paediatric research neurologist immediately before intervention. Randomisation and concealment were done by a researcher with no clinical involvement in the trial. |
| Blinding of outcome assessment (detection bias) | High risk | Blinding of outcome assessment was not performed |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

### Gabriel 2013 (Heel lance)

| ***Study characteristics*** | |
|---|---|
| Methods | A randomised controlled trial **(Heel lance)** |
| Participants | Inclusion criteria: healthy term neonates (37 to 41 weeks of gestation) confirmed through a routine physical examination during the first 24 hours of life, wish to breastfeed and absence of feeding during the previous 60 min |
| Interventions | Participating neonates were randomly assigned to four groups:<br>1. Breastfeeding and skin-to-skin contact (BF + SSC Group - n = 35; mean GA 40 weeks, mean BW 3289 g) - neonates dressed with a diaper were held in prone, in SSC with the mother; BF was started at least 5 min before heel lance and maintained during sampling<br>2. Sucrose + SSC group (n = 35, mean GA 40 weeks, mean BW 3349 g): neonates were held in prone between the mothers' breast at least 5 min before sampling and 2 mL 24% sucrose was given with a sterile syringe in the mouth 2 min before heel lance.<br>3. SSC group (n = 33, mean GA 39 weeks, mean BW 3359 g): neonates were held between the mother's breast as in Sucrose + SSC group, but no sucrose was given<br>4. Sucrose group (n = 33, mean GA 39 weeks, mean BW 3215 g): 2 mL 24% sucrose was administered through a sterile syringe in the mouth 2 min before heel lance to neonates laid supine on a cot; the procedure was done in the presence of the mother<br>Mothers were allowed to speak or touch their babies in all the groups |
| Outcomes | 1. Pain score by Neonatal Infant Pain Scale (NIPS) score<br>2. Crying time (in seconds)<br>3. Percentage of crying in blood sampling<br>4. Change in HR |
| Notes | This study was conducted in the maternity ward of Hospital Puerta de Hierro- Majadahonda, Madrid, Spain<br>Year of the study not provided<br>No conflicts of interest or funding |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Randomisation was by closed envelopes and nurses and parents were masked to the randomisation group. However, the method of random sequence generation was not clear. |
| Allocation concealment (selection bias) | Low risk | Allocation concealment was performed by using opaque envelopes for the group assignment |
| Blinding (performance bias and detection bias)<br>All outcomes | Unclear risk | Outcome was measured by 3 researchers who watched the videos: 1 expert neonatologist (Observer 1) and 2 young paediatricians (Observers 2 and 3) but it is unclear if they were blinded to the study |
| Blinding of outcome assessment (detection bias) | Unclear risk | It is unclear if blinding of outcome assessment was performed |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |

**Gabriel 2013 (Heel lance)** *(Continued)*

| | | |
|---|---|---|
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Gradin 2004 (Venipuncture)**

| ***Study characteristics*** | |
|---|---|
| Methods | Randomised controlled trial **(Venipuncture)** |
| Participants | 120 full-term infants at 3 to 5 days of age undergoing venipuncture for metabolic screening<br>Exclusion criteria: feeding problems or suspicion of illness<br>Group 1: 27 neonates<br>Mean (range) GA - 39.4 (37 to 42) weeks<br>Mean (range) BW - 3638 (2325 to 4425) g<br>Group 2: 29 neonates<br>Mean (range) GA - 39.5 (37 to 42) weeks<br>Mean (range) BW - 3637 (2700 to 4830) g<br>Group 3: 26 neonates<br>Mean (range) GA - 39.4 (37 to 42) weeks<br>Mean (range) BW - 3442 (2185 to 4560) g<br>Group 4: 29 neonates<br>Mean (range) GA - 39.4 (37 to 42) weeks<br>Mean (range) BW - 3660 (3025 to 4950) g |
| Interventions | Group 1: breastfeeding and 1 mL sterile water<br>Group 2: breastfeeding and 1 mL 30% glucose<br>Group 3: fasting and 1 mL sterile water<br>Group 4: fasting and 1 mL 30% glucose<br><br>For the breastfed group, breastfeeding was allowed for as long as the infant wanted within 45 minutes prior to blood sampling<br>For the fasting group, blood sampling was performed at least 2 hours after the last feed<br>Venipuncture was done 1 minute after giving 30% glucose or sterile water |
| Outcomes | PIPP<br>Visual analogue scale<br>Median crying time |
| Notes | The study was undertaken at the maternity ward at Orebro University Hospital, Sweden between March 2001 and September 2002<br><br>This study was supported by grants from the Research Committee of Orebro County Council, the Orebro University Hospital Research Foundation, and the Sven Johansson Memorial Foundation<br><br>No information regarding the conflicts of interest |

| ***Risk of bias*** | | |
|---|---|---|
| **Bias** | **Authors' judgement** | **Support for judgement** |
| Random sequence generation (selection bias) | Unclear risk | Method of random sequence generation is unclear |

**Gradin 2004 (Venipuncture)** *(Continued)*

| | | |
|---|---|---|
| Allocation concealment (selection bias) | Low risk | Allocation concealment was performed by using sealed envelopes |
| Blinding (performance bias and detection bias) All outcomes | Low risk | Masking was done by blinding the observer to the assignments to study groups |
| Blinding of outcome assessment (detection bias) | Low risk | Masking was done by blinding the observer to the assignments to study groups |
| Incomplete outcome data (attrition bias) All outcomes | Unclear risk | Some infants had to be excluded from the analysis because of missing data (problems with videotapes) |
| Selective reporting (reporting bias) | Low risk | All patients were accounted for |
| Other bias | Unclear risk | Protocol not available to compare |

**Gray 2002 (Heel lance)**

| ***Study characteristics*** | |
|---|---|
| Methods | Randomised controlled trial **(Heel lance)** |
| Participants | 30 term neonates<br>Inclusion criteria: healthy full-term neonates delivered by normal spontaneous vaginal delivery undergoing heel lance for newborn screening<br>Exclusion criteria: patients with evidence of congenital abnormalities, medical complications, drug exposure, history of oxygen administration or ventilatory support<br>Group 1: 15 neonates<br>Mean GA - 39.8 weeks<br>Mean BW - 3480 g<br>Group 2: 15 neonates<br>Mean GA - 39.9 weeks<br>Mean BW - 3524 g |
| Interventions | Group 1: breastfeeding during procedure<br>Group 2: swaddled in the bassinet during procedure |
| Outcomes | Changes in facial grimacing, crying time and heart rate before, during and after blood collection |
| Notes | This study was conducted in Boston Medical Center, Boston, Massachusetts, and Beverly Hospital, Beverly, Massachusetts, USA between January and November 1999<br><br>This research was supported by grant RO-1 MH51705– 04A1 and Research Scientist Award KO-5, MH5 00 524 from the National Institutes of Mental Health and was supported by a Maternal and Child Health Bureau Training grant MCJ-259169. No information regarding any conflicts of interest. |

| ***Risk of bias*** | | |
|---|---|---|
| **Bias** | **Authors' judgement** | **Support for judgement** |
| Random sequence generation (selection bias) | Unclear risk | It is unclear how the random sequence was generated |

**Gray 2002 (Heel lance)** *(Continued)*

| | | |
|---|---|---|
| Allocation concealment (selection bias) | Low risk | Allocation concealment performed using a system of sealed envelopes |
| Blinding (performance bias and detection bias) All outcomes | High risk | Not possible since it involved breastfeeding before and throughout the procedure |
| Blinding of outcome assessment (detection bias) | High risk | Masking of outcome assessment not possible |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | No missing outcome data; all participants were accounted for |
| Selective reporting (reporting bias) | Low risk | All prespecified outcomes have been reported |
| Other bias | Unclear risk | Protocol not available to compare |

**Hashemi 2016 (Intramuscular injection)**

| | |
|---|---|
| ***Study characteristics*** | |
| Methods | A randomised, double-blind intervention study **(Intramuscular injection)** |
| Participants | This study was performed on 140 full-term healthy infants<br>Inclusion criteria - full-term (37 to 42 weeks), postnatal age less than 3 days, no evidence of obvious abnormality or illness by the physician exam, Apgar score 7 to 10 in 5th minute after birth, no history of transfusions or invasive procedures except vitamin K injection and at least 1 time breastfed |
| Interventions | Breastfeed group - breastfed within 45 minutes prior to vaccination and were not swaddled (n = 33, mean GA 38.5 weeks, mean birth weight 3045 g)<br>Swaddled group: swaddled a few minutes before vaccination and a few minutes later, while more than 45 minutes had passed from being breastfed (n = 34, mean GA 38.7 weeks, mean birth weight 3236 g)<br>Combined group: swaddled a few minutes before vaccination and a few minutes later, and breast-fed within 45 minutes prior to vaccination (n = 31, mean GA 38.8 weeks, mean birth weight 3179 g)<br>Control group: vaccinated according to the hospital routine without any intervention (n = 33, mean GA 38.5 weeks, mean birth weight 3107 g) |
| Outcomes | Outcome 1: pain score using NFCS (Neonatal Facial Coding System)<br>Outcome 2: changes in heart rate<br>Outcome 3: changes in $O_2$ saturation |
| Notes | This study was conducted in Motahari Teaching Hospital affiliated to Jahrom University of Medical Sciences in Iran in 2010-2011<br>No conflict of interest declared. This study was approved by Shiraz University of Medical Sciences through grant no 89-5404. |
| ***Risk of bias*** | |

**Hashemi 2016 (Intramuscular injection)** *(Continued)*

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Unclear |
| Allocation concealment (selection bias) | Low risk | Allocation concealment was done based on sealed envelopes |
| Blinding (performance bias and detection bias) All outcomes | Low risk | In all participants, blood oxygen saturation and heart rate were measured by a pulse oximeter with the probe attached to the infant's left leg 2 minutes before the vaccination. These parameters were recorded by a research assistant who did not know the type of group. A fixed trained research assistant recorded the neonate's face in a close-up view with a digital video camera in such a way that the type of intervention was unknown. After interventions, the videotapes were reviewed and scored by another fixed research assistant (blind) who had been trained on how to score baby's face changes. |
| Blinding of outcome assessment (detection bias) | Low risk | Masking of outcome assessment was performed by another fixed research assistant (blind) who had been trained to score |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Holsti 2011 (Heel lance)**

| ***Study characteristics*** | |
|---|---|
| Methods | A randomised controlled trial **(Heel lance)** |
| Participants | 1. Infants born between 30 and 36 completed weeks of gestation<br>2. Normally breastfeeding infants<br>3. Blood collection required for clinical management on or after day 3 of life<br>4. Mother/infant pairs were medically stable |
| Interventions | Non-nutritive sucking group (n = 29; mean GA 33.9 weeks, mean BW 2143 g): 2 minutes before the first contact by the laboratory technician, each infant remained in their cot/isolette and was given a soother to suck on throughout the blood collection. The soother was held in the infant's mouth by the research nurse to ensure that contact was maintained.<br><br>Breastfeeding group (n = 28; mean GA 34 weeks, mean BW 2114 g): 5 minutes before blood collection, infants were given to their mothers. 2 minutes before the heel lance, the mothers began breastfeeding their infants and continued until the last contact by the laboratory technician. |
| Outcomes | 1. Behavioural Indicators of Infant Pain (BIIP)<br>2. Change in heart rate<br>3. Preterm infant breast-feeding behaviour scale (PIBBS) |
| Notes | This study was conducted between January 2008 and May 2010 in 3 NICUs in the Greater Vancouver Regional District, British Columbia, Canada |

**Holsti 2011 (Heel lance)** *(Continued)*

| | |
|---|---|
| | No conflict of interest declared. Funding was provided by the Canadian Institutes of Health Research, SickKids Foundation, the Faculty of Medicine, University of British Columbia, and the Child and Family Research Institute. |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Randomisation was done by generating randomly permuted sequential blocks of 4 and 6 allocation numbers by a statistician blind to study hypotheses |
| Allocation concealment (selection bias) | Low risk | Treatment group assignments were placed in sequentially numbered envelopes and sealed |
| Blinding (performance bias and detection bias) All outcomes | Unclear risk | In the non-nutritive sucking group, the soother was held in the infant's mouth by the research nurse to ensure that contact was maintained. In the breastfeeding group, if necessary, the research nurse provided quiet, verbal guidance to the mother to ensure that contact with the breast was maintained. However, the author did not provide information about whether the intervention was blinded. |
| Blinding of outcome assessment (detection bias) | Low risk | Two coders blind to the study hypotheses, clinical information about the infants and the timing of the blood collection were trained to achieve inter-rater reliability on the BIIP. All analyses were conducted blind to treatment assignment and according to randomised treatment, following the intention-to-treat principle. |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Iqbal 2014 (Intradermal)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial **(Intradermal)** |
| Participants | Included infants: full-term neonates having gestational ages between 38 and 42 weeks; had Apgar score of 7 and higher at 5 minutes after birth; delivered by spontaneous and vaginal delivery; were exclusively breastfed; postnatal age not more than 48 hours. Excluded infants with any evidence of congenital abnormalities, medical complications, drug exposure or if the infant was crying before vaccination and the mother could not calm the infant. |
| Interventions | The participants were randomly assigned to 2 groups of 75 participants each<br><br>Breastfeeding group (n = 75; mean GA 39.2 weeks, mean BW 2870 g): the mothers cradled their infants in a breastfeeding position allowing maintaining full-body, skin-to-skin contact during the entire procedure. A large amount of areola was placed into baby's mouth and 2 minutes after the first jaw movements were observed the BCG vaccine was given. Infants were breastfeeding during and after the vaccination. The mothers were asked to continue breastfeeding their infants even if they started to cry during and after the procedure. |

**Iqbal 2014 (Intradermal)** *(Continued)*

| | |
|---|---|
| | Control group (n= 75; mean GA 38.9 weeks, mean BW 2920 g): infant was placed on the treatment table. The mother stayed next to the infant and helped in holding the infant. She tried to soothe the infant verbally during and after the vaccination. The infant was cuddled by the mother just after the injection. |
| Outcomes | Pain score by Douleur Aiguë du Nouveau-né (DAN) scale |
| Notes | This randomised controlled trial was carried out at the Combined Military Hospital (CMH) Lahore, Pakistan between January and June 2013<br><br>No comments on conflict of interest/funding source |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Randomisation was performed, however the method is not clear |
| Allocation concealment (selection bias) | Unclear risk | It is unclear if allocation concealment was done |
| Blinding (performance bias and detection bias)<br>All outcomes | High risk | The randomisation code was available only to a research fellow who was not connected to the study. The code was disclosed to the researchers when the statistical analysis was completed. The mothers and nurses were not blind to the group assignments. However, the outcome assessor (observer) did not know the purpose and hypothesis of the study. |
| Blinding of outcome assessment (detection bias) | Low risk | Outcome assessor was blinded |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Jatana 2003 (Heel lance)**

***Study characteristics***

| | |
|---|---|
| Methods | Randomised controlled trial **(Heel lance)** |
| Participants | 125 term neonates<br>Inclusion criteria: healthy full-term neonates between 1 and 7 days old, undergoing heel lance for blood sampling<br>Exclusion criteria: age below 24 hours or above 7 days, GA below 37 weeks, Apgar score of less than 7 or 8 at 1 and 5 minutes respectively, neonates with oxygen requirement of more than 40%, analgesic or sedative drug given within 5 days, neurological symptoms like seizures, listlessness, altered sensorium, etc.<br><br>There is no report on the mean GA or weight in each group, although the authors say that the groups were matched for GA, BW and gender |
| Interventions | Group 1: control group, 1 mL of sterile water |

**Jatana 2003 (Heel lance)** *(Continued)*

| | |
|---|---|
| | Group 2: 1 mL of 10% glucose<br>Group 3: 1 mL of 25% glucose<br>Group 4: 1 mL of 50% glucose<br>Group 5: 1 mL of EBM |
| Outcomes | Crying time (total and first cry), change in heart rate, change in oxygen saturation and facial action score |
| Notes | This study was conducted in India<br>No information regarding when the study was conducted. No information about conflicts of interest/funding. |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | High risk | No comment on how random sequence generation was done |
| Allocation concealment (selection bias) | High risk | No comment on how allocation concealment was done |
| Blinding (performance bias and detection bias)<br>All outcomes | High risk | No comment on how blinding was done |
| Blinding of outcome assessment (detection bias) | High risk | No comment on how blinding was done |
| Incomplete outcome data (attrition bias)<br>All outcomes | High risk | There are no results for one of the outcomes that was supposed to be evaluated (facial action score) |
| Selective reporting (reporting bias) | High risk | There are no results for one of the outcomes that was supposed to be evaluated (facial action score) |
| Other bias | Unclear risk | Protocol not available to compare |

**Kumar 2020 (Intramuscular injection)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial (RCT) **(Intramuscular injection)** |
| Participants | Term newborns (0 to 28 days) receiving hepatitis B vaccination; parents consenting to take part in the study |
| Interventions | Randomised into 6 groups:<br>1. Group 1, breastfeeding (n = 50, mean age in hours 25.3, mean BW in grams 3060): breastfeeding started 2 minutes before the vaccination and continued until 120 seconds<br>2. Group 2, 25% sucrose (n = 50, mean age in hours 34.3, mean BW in grams 2950): 2 mL of 25% dextrose solution was given through mouth with sterile dropper 2 minutes prior to vaccination |

**Kumar 2020 (Intramuscular injection)** *(Continued)*

| | |
|---|---|
| | 3. Group 3, DW (n = 50, mean age in hours 28.5, mean BW in grams 3080): 2 mL of DW was given through mouth with sterile dropper 2 minutes prior to vaccination<br>4. Group 4, NNS (n = 50, mean age in hours 25.6, mean BW in grams 3110): a sterile silicon pacifier was held gently to stimulate sucking. Vaccination was given 2 minutes after the newborn started sucking, and it was continued until 120 seconds.<br>5. Group 5, rocking (n = 50, mean age in hours 23.8, mean BW in grams 2850): newborns in this group were given gentle rocking movement by lifting the head on the palm of the hand. Rocking started 2 minutes before vaccination and continued till 120 seconds.<br>6. Group 6, none (n = 50, mean age in hours 22.3, mean BW in grams 3000): in this group no intervention was used |
| Outcomes | 1. Total cry duration<br>2. DAN score at 30, 60 and 120 seconds |
| Notes | This study was conducted in the tertiary care hospital of Shimla, India from October 2016 to March 2017<br>No potential conflict of interest relevant to this article was reported<br>No information provided regarding any funding source |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Randomisation was done using sequentially numbered envelopes |
| Allocation concealment (selection bias) | Low risk | Opaque, sealed envelopes were used |
| Blinding (performance bias and detection bias)<br>All outcomes | High risk | The person performing randomisation was not involved in the study beyond this. The first observer opened 1 sealed envelope for each baby and recruited that baby to 1 of 6 groups depending upon the group mentioned in that envelope. However, it is unclear if blinding of intervention was performed. |
| Blinding of outcome assessment (detection bias) | Unclear risk | Outcome variables were recorded by the third observer. However, it is unclear if the outcome assessor was blinded. |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Lan 2021 (Heel lance)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial **(Heel lance)** |
| Participants | Term neonates (38 to 40 weeks of gestation) who underwent heel lance for blood drawing, postnatal age 2 to 3 days |

**Lan 2021 (Heel lance)** *(Continued)*

| | |
|---|---|
| Interventions | Neonates were randomised by the researcher into 3 groups: gentle touch and verbal comfort group (n = 40); breast milk odour, gentle touch and verbal comfort group (n = 40); and breast milk odour, breast milk taste, gentle touch and verbal comfort group (n = 40)<br><br>Gentle touch and verbal comfort group (routine care) (n = 40, mean gestational age in weeks 39.42, mean birth weight in grams 3121): a female nurse gently touched the head (GT) and spoke softly to comfort the newborn (VC) during and after the heel sticks for newborn screening<br><br>Breast milk odour + gentle touch + verbal comfort group (n = 40, mean gestational age in weeks 39.07, mean birth weight in grams 3109): breast milk was expressed manually immediately when the mother woke in the morning (to reduce the influences of diet on the breast milk flavour) and was then refrigerated and stored in the nursery. Before the newborn screening, the breast milk was warmed up. 3 minutes prior to the heel stick to the 5th minute of recovery, a cotton ball with the breast milk was put near the newborn's nostrils for breast milk odour. Gentle touch and verbal comfort were provided.<br><br>Breast milk odour + breast milk taste + gentle touch + verbal comfort group (n = 40, mean gestational age in weeks 38.91, mean birth weight in grams 3070): breast milk was expressed manually immediately when the mother woke in the morning (to reduce the influences of diet on the breast milk flavour) and was then refrigerated and stored in the nursery. Before the newborn screening, the breast milk was warmed up. 3 minutes prior to the heel stick to the 5th minute of recovery, a cotton ball with the breast milk was put near the newborn's nostrils for breast milk-odour. For breast milk taste, 3 millilitres of his/her own mother's breast milk were fed slowly through syringe dripping to the newborn's mouth 2 min before and during the heel stick. Gentle touch and verbal comfort were provided. |
| Outcomes | Neonatal Infant Pain Scale |
| Notes | This study was conducted in Taiwan<br><br>No information regarding when it was conducted<br><br>This study was supported by a research grant from the Ministry of Science and Technology (grant number: MOST 107-2314-B-016-015-MY3). The authors declare no conflict of interest. |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Newborns were randomly assigned to one of the 3 multisensory conditions by a blinded statistician using a web-based blocked randomisation system |
| Allocation concealment (selection bias) | Unclear risk | It is unclear if allocation concealment was done |
| Blinding (performance bias and detection bias) All outcomes | Unclear risk | It is unclear if masking of intervention was done |
| Blinding of outcome assessment (detection bias) | High risk | Pain was assessed by a research assistant (RA) who was well-trained in observations of video recordings of heel sticks. The RA coded all the videotapes of heel sticks in random sequence, but it was not possible to maintain blinding to the conditions when evaluating the NIPS score, because the infants' behaviours during heel sticks for the 3 treatment conditions were easy to discern when analysing the videotapes. |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | All infants were included in the analysis |

**Lan 2021 (Heel lance)** *(Continued)*

| | | |
|---|---|---|
| Selective reporting (reporting bias) | Low risk | All outcomes reported. No missing outcomes. |
| Other bias | Unclear risk | Protocol not available for comparison |

**Leite 2009 (Heel lance)**

| ***Study characteristics*** | |
|---|---|
| Methods | Randomised controlled trial **(Heel lance)** |
| Participants | 60 term neonates<br>Inclusion criteria: healthy full-term neonates being exclusively breastfed, GA at least 37 weeks, Apgar ≥ 7 at 5 minutes after birth, and postnatal age not more than 7 days<br>Exclusion criteria: congenital diseases of the nervous system, malformation or neurologic damage, stomatognathic disorders that would interfere with sucking mechanics, use of analgesics interfering in nociceptive responses in infants, use postdelivery analgesics for the mothers and newborns being admitted to the neonatal intensive care unit (NICU)<br>Group 1: 31 neonates<br>Mean BW - 3168 (± 517) g<br>Group 2: 29 neonates<br>Mean BW - 3300 (± 478) g |
| Interventions | Group 1: breastfeeding<br>Group 2: held by mother<br><br>Infants underwent heel lance for routine newborn screening. Infants in group 1 were held by mother and were breastfeeding with effective sucking movements 5 minutes prior to procedure. Group 2 infants were held by the mother for the same length of time |
| Outcomes | NFCS and change in heart rate |
| Notes | This study was conducted in Clinics Hospital at Ribeirao Preto School of Medicine, University of São Paulo (Brazil) between October 2004 and January 2005<br><br>Study was funded by the Brazilian National Council for Scientific and Technological Development (CNPq). No information regarding conflicts of interest.<br><br>The heart rate was reported as mean and SD with P value. Recalculation of all numbers indicated that what is reported as SD is most likely SE. We recalculated the SD values and those are used in the review. |

| ***Risk of bias*** | | |
|---|---|---|
| **Bias** | **Authors' judgement** | **Support for judgement** |
| Random sequence generation (selection bias) | Low risk | Randomisation was done by a computer random number generator |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information to decide whether allocation concealment was effective |
| Blinding (performance bias and detection bias) All outcomes | High risk | It was not possible to blind to group assignment as the information about breastfeeding was easily determined in both body and face videos |

**Leite 2009 (Heel lance)** *(Continued)*

| | | |
|---|---|---|
| Blinding of outcome assessment (detection bias) | Low risk | Two digital cameras were used to record the newborns' behaviour, one focused on the newborns' face and the second camera on the neonates' body. Analysis of facial actions was carried out by a person who was blinded to the phase of the procedure (blood collection, compression or recovery). The observers obviously recognised the 2 groups when they were evaluating the recordings. However, they were not aware of the purpose of the study. |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All participants were accounted for in the analysis of outcomes |
| Selective reporting (reporting bias) | Low risk | All prespecified outcomes were reported in the article |
| Other bias | Unclear risk | Protocol not available to compare |

**Leite 2015 (Intramuscular injection)**

| | |
|---|---|
| ***Study characteristics*** | |
| Methods | A randomised controlled trial **(Intramuscular injection)** |
| Participants | Inclusion criteria: full-term newborns of up to 12 hours of life admitted to collective rooms; Apgar ≥ 7 at the 5th minute of life; exclusive breastfeeding on demand, who suckled the breast at least once after birth, and whose mothers had physical conditions to breastfeed. |
| Interventions | Skin to skin contact (n = 28; mean hour of life 6.93; mean BW in grams 3170): newborns were maintained for 5 minutes in a common crib (baseline period), and soon after, positioned in maternal skin-to skin contact for 15 minutes (treatment period), followed by the period of antisepsis/injection and compression with cotton soaked in 70% alcohol, and in skin-to-skin contact until 5 minutes after the procedure (recovery period).<br><br>Breastfeeding with skin-to-skin contact (n = 27; mean hour of life 6.93; mean BW in grams 3113): newborns were kept in a common crib for 5 minutes (baseline period), and then put in skin-to-skin contact for 5 minutes, then combined with breastfeeding for 10 more minutes, and kept in this condition during periods of antisepsis/injection, compression and recovery (5 minutes after the end of compression). |
| Outcomes | 1. Neonatal Facial Coding System (NFCS) score<br>2. Change in HR |
| Notes | This is a randomised clinical trial conducted in a maternity ward in the countryside of the state of São Paulo, Brazil<br><br>No information provided regarding when this study was conducted, conflicts of interest or funding |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Participants were randomly assigned to 2 groups by a computer randomisation program |
| Allocation concealment (selection bias) | Unclear risk | It is unclear if allocation concealment was done. |

**Leite 2015 (Intramuscular injection)** *(Continued)*

| | | |
|---|---|---|
| Blinding (performance bias and detection bias) All outcomes | Unclear risk | It is unclear if masking of randomisation or intervention was performed |
| Blinding of outcome assessment (detection bias) | Low risk | For the outcome assessment, independent observation of the recordings of newborns' faces made by the researcher and a research assistant was performed |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Lima 2013 (Venipuncture)**

| | |
|---|---|
| ***Study characteristics*** | |
| Methods | A randomised controlled trial **(Venipuncture)** |
| Participants | A random sample of 64 rooming-in newborns with medical requests for venipuncture was chosen for the study<br><br>All newborns suffering from neurological damage, head and neck malformations, and heart diseases or with absence of sucking reflex or motion were excluded |
| Interventions | The newborns were randomly divided into 3 groups:<br><br>First group (Group 1) (n = 20; GA < 37 weeks = 0, > = 37 weeks = 20; BW < 2500 g = 2, ≥ 2500 g = 18): NS stimuli were started 2 minutes before the venipuncture, were continued through the puncture, and were maintained until 1 minute after the painful procedure completion. The NS stimulation was performed through maternal breastfeeding.<br><br>Second group (Group 2) (n = 21; GA < 37 weeks= 2, ≥ 37 weeks = 19; BW < 2500 g = 4, ≥ 2500 g = 17): 21 newborns who received NNS stimulation before and during the venipuncture The NNS stimulus was performed through the introduction of the researcher's little finger in the newborn's oral cavity. The finger was protected by nonsurgical examination gloves.<br><br>Third group or control group (Group 3) (n = 23; GA < 37 weeks = 4, ≥ 37 weeks = 19; BW < 2500 g = 9, ≥ 2500 g = 14): did not receive any sucking stimulation |
| Outcomes | Prevalence of pain by Neonatal Infant Pain Scale (NIPS) score |
| Notes | Study was conducted in Hospital das Clínicas of the Federal University of Minas Gerais, Brazil<br><br>Timing of the study not reported<br><br>No conflicts of interest declared. No comment on funding. |
| ***Risk of bias*** | |

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|

**Lima 2013 (Venipuncture)** *(Continued)*

| | | |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | The newborns were randomly divided into 3 groups, however the method of randomisation is not stated |
| Allocation concealment (selection bias) | High risk | Allocation concealment was not done. |
| Blinding (performance bias and detection bias)<br>All outcomes | Unclear risk | Non-nutritive sucking and outcome evaluation procedures were performed by the same researcher who was previously trained. However, it is not clear if the researcher was blinded to the randomisation or the outcome of the study. |
| Blinding of outcome assessment (detection bias) | Unclear risk | Not clear if blinding of outcome assessment was performed |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Mathai 2006 (Heel lance)**

| ***Study characteristics*** | |
|---|---|
| Methods | Randomised controlled trial **(Heel lance)** |
| Participants | 104 term neonates<br>Inclusion criteria: healthy full-term neonates who underwent heel prick at more than 24 hours of age for collection of blood for bilirubin estimation<br>Exclusion criteria: patients with BW less than 2 kg, sick babies with unstable vitals or on intravenous fluids, oxygen or any drugs requiring resuscitation at birth, with neurological abnormalities or having major congenital defects<br>Group 1: 18 neonates<br>Mean BW - 2992 (± 312.2) g<br>Group 2: 17 neonates<br>Mean BW - 2953 (± 289) g<br><br>Group 3: 15 neonates<br>Mean BW - 3027 (± 302) g<br>Group 4: 20 neonates<br>Mean BW - 2994 (± 290) g<br><br>Group 5: 17 neonates<br>Mean BW - 3123 (± 302) g<br>Group 6: 17 neonates<br>Mean BW - 2995 (± 300) g |
| Interventions | Group 1: 2 mL of EBM<br>Group 2: 2 mL of 20% sucrose<br>Group 3: 2 mL of distilled water<br>Group 4: non-nutritive sucking<br>Group 5: massage<br>Group 6: rocking |

**Mathai 2006 (Heel lance)** *(Continued)*

| | |
|---|---|
| | For groups 1 to 3, the solution was administered in the infants' mouth with a dropper. In the NNS group, a sterile pacifier was held gently in the babies' mouth and the palate tickled to stimulate sucking. This was continued during and up until 2 minutes after the heel prick. In the massaging group, neonates were subjected to firm, gentle stroking with bare fingers in a rhythmical manner starting from the forehead and going down to the chest, arms and legs, during and up until 2 minutes after the heel prick. In the rocking group, newborns were rocked by lifting the infants' head off the cot on the palm of the hand (without lifting the body off the cot) and making rocking movements in a gentle rhythmic manner up until 2 minutes after the heel prick |
| Outcomes | Duration of first cry, total crying time and DAN at 30 sec, 1 min, 2 min and 4 min after the prick. For the purpose of this review, we analysed DAN at 2 minutes. Other outcome variables were heart rate increase and saturation decrease |
| Notes | This study was conducted in India<br><br>No information regarding when the study was conducted<br><br>Funding: none. Competing interests: none. |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Randomisation was done with a random number table |
| Allocation concealment (selection bias) | Unclear risk | Method of allocation concealment was not described |
| Blinding (performance bias and detection bias) All outcomes | Unclear risk | A trained nurse gave the selected intervention 2 minutes before the heel prick, however it is unclear if the nurse was blinded |
| Blinding of outcome assessment (detection bias) | High risk | Partially blinded. The duration of first cry (time to first inspiration after commencement of cry) and total duration of cry were recorded by Observer 1 who was blinded to the intervention. Observer 2, who was not blinded, assessed the DAN score again at 30 sec, 1 min, 2 min and 4 min after the prick. |
| Incomplete outcome data (attrition bias) All outcomes | High risk | No actual data on heart rate and saturations were given |
| Selective reporting (reporting bias) | High risk | Not all the outcomes were reported completely |
| Other bias | Unclear risk | Protocol not available to compare |

**Modarres 2013 (Intramuscular injection)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial **(Intramuscular injection)** |
| Participants | Inclusion criteria: full term neonate; Apgar score of 7 and higher at 5 minutes after birth; delivered by spontaneous and vaginal delivery; exclusively breastfed; postnatal age not more than 24 hours |

**Modarres 2013 (Intramuscular injection)** *(Continued)*

| | |
|---|---|
| Interventions | Breastfeeding group (N = 65; mean GA in weeks 39.4, mean BW in grams 3590): neonates were breast-fed during 2 minutes before, during and after hepatitis B vaccination. At the end of the second minute of breastfeeding, while the infants were still sucking, an experienced nurse performed the immunisation injections.<br><br>Control group (N = 65; mean GA in weeks 39.1, mean BW in grams 3550): they were held in mothers' arms but not fed |
| Outcomes | Douleur Aiguë du Nouveau-né (DAN) pain scale |
| Notes | Study was carried out in Mirza Kochak Khan Hospital, Tehran, Iran<br><br>The year is not mentioned<br><br>No conflicts of interest or funding |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Neonates were randomly assigned to the study groups, but the method is not clear |
| Allocation concealment (selection bias) | Low risk | Allocation concealment was achieved by using sealed envelopes |
| Blinding (performance bias and detection bias)<br>All outcomes | High risk | The randomisation code was available only to a research fellow who was not connected to the study. The code was disclosed to the researchers when the statistical analysis was completed. The mothers and nurses were not blind to the group assignments. |
| Blinding of outcome assessment (detection bias) | Low risk | The outcome assessor (observer) did not know the purpose and hypothesis of the study and the main investigator was blind to when the statistical analysis had been completed |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Nanavati 2013 (Adhesive tape removal)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial **(Adhesive tape removal)** |
| Participants | VLBW infants requiring removal of adhesive tape (Micropore Medical Tape, 3M) during removal of intravenous cannula were included. Neonates with neurological abnormalities and major congenital defects and those receiving sedatives or analgesics were excluded from the study. |
| Interventions | Kangaroo mother care group (n = 25; mean GA in weeks 32.7, mean BW in grams 1352): the baby was kept in Kangaroo Mother Care for 15 minutes before the removal of the adhesive tape |

**Nanavati 2013 (Adhesive tape removal)** *(Continued)*

| | |
|---|---|
| | Expressed breast milk group (n = 25; mean GA in weeks 32.4, mean BW in grams 1235): a swab soaked in EBM was kept in the baby's mouth for 2 minutes before the removal of the adhesive tape and continued during the intervention |
| Outcomes | Premature Infant Pain Profile (PIPP) score and its components |
| Notes | This study was conducted in the Neonatal Intensive Care Unit of Department of Neonatology at Seth G S Medical College and KEM Hospital, Mumbai, India between June to August 2010<br><br>No conflicts of interest or funding reported |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | A computer-generated randomisation sequence was used to assign infants to 2 treatment groups in a 1:1 ratio. Randomisation was balanced in variable random blocks of 2 or 4 patients. |
| Allocation concealment (selection bias) | Low risk | Treatment allocations were inserted into sequentially numbered, opaque envelopes and sealed. Just prior to adhesive tape removal, a neonatal research nurse opened the sequentially numbered envelopes. |
| Blinding (performance bias and detection bias) All outcomes | Unclear risk | It is unclear if masking of intervention was performed |
| Blinding of outcome assessment (detection bias) | High risk | Blinding of outcome measure assessment was not done |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Napiorkowska-Orkisz 2022 (Heel lance)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial (**Heel lance)** |
| Participants | Term neonates (38 to 42 weeks of gestation) who underwent heel stick blood drawing for screening test |
| Interventions | Neonates were randomised by the researcher into 3 groups: breastfeeding (n = 30), oral 20% glucose group (n = 30) and non-nutritive sucking group (n = 30)<br><br>Breastfeeding group (n = 30): the mothers of the newborns assigned to this group were instructed in the correct technique of latching the newborn to the breast before starting the capillary blood collection procedure. Newborns were attached to the mother's breast during the painful procedure<br><br>Oral 20% glucose group (n = 30): neonates in this group were given 2 mL to 3 mL of 20% glucose orally<br><br>Non-nutritive sucking group (n = 30): neonates sucked a pacifier during the study |

**Napiorkowska-Orkisz 2022 (Heel lance)** *(Continued)*

| | |
|---|---|
| Outcomes | Outcome 1: Neonatal Infant Pain Scale<br>Outcome 2: heart rate variability |
| Notes | This study was conducted in Poland from 1 March to 15 May 2015<br>The authors declare no conflict of interest or funding |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Method of randomisation not clear |
| Allocation concealment (selection bias) | Low risk | The infants were allocated randomly using envelopes that contained the 3 groups, i.e. I, II, III. Newborns were randomly assigned to one of 3 groups that differed in pain management methods. |
| Blinding (performance bias and detection bias) All outcomes | High risk | Blinding was not performed |
| Blinding of outcome assessment (detection bias) | High risk | Blinding was not performed |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | All infants were included in the analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported; no missing outcomes |
| Other bias | Unclear risk | Protocol not available for comparison |

**Obeidat 2015 (Heel lance)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial**(Heel lance)** |
| Participants | Full term neonates (38 to 42 weeks of gestation) who underwent heel lance blood drawing for routine hypothyroidism screening, aged 4 to 6 days, no feeding occurred in the previous 30 minutes, and Apgar score ranged from “7 to 10” at “1 and 5” minutes included |
| Interventions | Breastfeeding with maternal holding (Group I) (n = 64, mean GA in weeks 38, mean BW in grams 3340): neonates underwent heel lance blood sampling were breastfed and held in their mothers’ lap while their mothers were seated reclining on a comfortable chair. The mothers were instructed to continue breastfeeding and cuddling if the infants started to cry during or after the heel lance blood drawing.<br>Only maternal holding (Group II) (n = 64, mean GA in weeks 38, mean BW in grams 3370): neonates underwent sampling under the same conditions except for breastfeeding. The mothers were instructed to continue cuddling if the infants started to cry during and after the heel lance blood drawing. |
| Outcomes | Pain score by PIPP scale |

**Obeidat 2015 (Heel lance)** *(Continued)*

| | |
|---|---|
| Notes | The study was conducted at the neonatal unit of a major teaching hospital of Mutah University in Amman, Jordan, between January and June 2013<br><br>No conflicts of interest or funding reported |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Method of randomisation is not clear |
| Allocation concealment (selection bias) | Low risk | Following assessment of eligibility and recruitment, a research assistant blindly drew a card for each participant from an envelope containing equal numbers of cards representing each group and assigned each newborn to either group I or group II. Thus, allocation concealment was achieved. |
| Blinding (performance bias and detection bias)<br>All outcomes | Unclear risk | The mothers in group I were instructed to continue breastfeeding and cuddling if the infants started to cry during or after the heel lance blood drawing. The mothers in group II were instructed to continue cuddling if the infants started to cry during and after the heel lance blood drawing. The same experienced skilful neonatal nurse performed all blood collections to reduce variability and human errors between the groups. However, author does not mention if intervention was masked. |
| Blinding of outcome assessment (detection bias) | Low risk | The painful responses of all neonates were measured using the PIPP scale simultaneously by 2 neonatal nurses who were blinded to the objectives of the study. The author also mentioned that the 2 nurses who assessed the PIPP scores of infants were able to recognise the control and treatment groups, but the possibility of bias was minimised by blinding the assessing nurses to the purpose of the study. |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Okan 2010 (Heel lance)**

***Study characteristics***

| | |
|---|---|
| Methods | Randomised controlled trial **(Heel lance)** |
| Participants | 107 term neonates<br>Inclusion criteria: healthy full-term neonates between 24 and 48 hours of age undergoing heel lance for metabolic newborn screening<br>Exclusion criteria: patients with evidence of congenital abnormalities, perinatal asphyxia, medical complications or drug exposure, history of oxygen or ventilatory support<br>Group 1: 35 neonates<br>Mean GA - 40 (± 0.7) weeks<br>Mean BW - 3350 (± 360) g<br><br>Group 2: 36 neonates |

**Okan 2010 (Heel lance)** *(Continued)*

| | |
|---|---|
| | Mean GA - 39.5 (± 0.5) weeks<br>Mean BW - 3300 (± 285) g<br><br>Group 3: 36 neonates<br>Mean GA - 39.9 (± 0.7) weeks<br>Mean BW - 3317 (± 235) g |
| Interventions | Group 1: breastfeeding with skin-to-skin contact<br><br>Group 2: held by mother with skin-to-skin contact<br><br>Group 3: lying on the table<br><br>Mothers and infants from groups 1 and 2 were left alone for 15 minutes to allow them to rest comfortably in skin-to-skin contact position. Mothers in group 1 were asked to begin to breastfeed their infants during this time. In the no-contact group, tests were performed with the infants lying on an examination table in a silent nursery. Infants were wrapped in blankets and placed supine on the examination table |
| Outcomes | Heart rate and saturation changes, total time of crying and NFCS in group 2 and 3 (done at the moment of heel lance, 1, 2, 3, 4 and 5 minutes) |
| Notes | This study was conducted in Turkey (May to December 2006)<br><br>No information regarding any conflicts of interest or funding |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Randomisation was done through a random number table |
| Allocation concealment (selection bias) | Low risk | Allocation concealment was adequate |
| Blinding (performance bias and detection bias)<br>All outcomes | High risk | Unblinded |
| Blinding of outcome assessment (detection bias) | High risk | Outcome assessor was not blinded |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | NFCS measurement was not done in breastfed infants as it was difficult to evaluate the facial actions of babies while breastfeeding |
| Selective reporting (reporting bias) | Low risk | All prespecified outcomes were reported on, but NFCS measurement was not done in breastfed infants as it was difficult to evaluate the facial actions of babies while breastfeeding |
| Other bias | Unclear risk | Protocol not available to compare |

**Ors 1999 (Heel lance)**

***Study characteristics***

**Ors 1999 (Heel lance)** *(Continued)*

| | |
|---|---|
| Methods | Randomised controlled trial **(Heel lance)** |
| Participants | 102 healthy term infants at median age of 1.6 days undergoing routine heel lance blood sampling<br>Exclusion criteria: infants < 24 hours of age, Apgar score < 7 at 1 minute and on any medication were excluded<br>Group 1: 35 neonates<br>Median (range) GA - 40.0 (37 to 42) weeks<br>Median (range) BW - 3220 (2445 to 4210) g<br>Group 2: 33 neonates<br>Median (range) GA - 39.5 (37 to 42) weeks<br>Median (range) BW - 3200 (2390 to 4200) g<br>Group 3: 34 neonates<br>Median (range) GA - 39.0 (37 to 42) weeks<br>Median (range) BW - 3380 (2450 to 4300) g |
| Interventions | All infants were fed 1 hour before the procedure<br>Group 1: 2 mL of 25 % sucrose<br>Group 2: 2 mL of human milk<br>Group 3: 2 mL of sterile water<br>The solutions were administered by syringe over 1 minute<br>Heel lance was performed 2 minutes after administration of the solution |
| Outcomes | Recovery time<br>Percentage change in heart rate at 1, 2 and 3 minutes<br>Median crying time |
| Notes | This study was conducted in Marmara University Hospital, Turkey between September 1996 and January 1997<br><br>No information regarding any conflicts of interest of funding |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Manner of randomisation was not discussed by the authors |
| Allocation concealment (selection bias) | Unclear risk | Unclear |
| Blinding (performance bias and detection bias)<br>All outcomes | Low risk | Masking was possible by using a placebo and performing the heel prick 1 minute after giving the solutions |
| Blinding of outcome assessment (detection bias) | Low risk | The investigators who analysed the data were unaware of the treatment intervention |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were accounted for in the analysis |
| Selective reporting (reporting bias) | Low risk | All prespecified outcomes were reported on |
| Other bias | Unclear risk | Protocol not available to compare |

### Ou-Yang 2013 (Heel lance)

| ***Study characteristics*** | |
|---|---|
| Methods | A randomised controlled trial **(Heel lance)** |
| Participants | Included infants: preterm neonates (< 37 gestational age); age < 7 days at the time of study<br><br>Participants were randomly divided into 3 groups - distilled water (water, placebo); expressed breast milk; 25% glucose water (glucose) |
| Interventions | All treatments were given by a single independent investigator as a 5 mL volume via a syringe tube inserted into the participant's oral cavity<br><br>Distilled water group (n = 44, mean GA in weeks 34.2, mean BW in grams 1959)<br><br>Expressed breast milk (n = 40, mean GA in weeks 33.8, mean BW in grams 1996)<br><br>25% glucose water (n = 39, mean GA in weeks 34.6, mean BW in grams 2138) |
| Outcomes | 1. Duration of the first cry after heel lancing<br>2. Pain scores at baseline and 1, 2 and 3 min after heel lancing (N-PASS: Neonatal Pain, Agitation and Sedation Scale)<br>3. Physiological parameters at baseline and 3 minutes after heel lance: heart rate (beats/min), respiratory rate (breaths/min), $O_2$ saturations (%), systolic blood pressure (mmHg) and diastolic blood pressure (mmHg) |
| Notes | This study was conducted in neonatal intensive care unit of Chang Gung Memorial Hospital, Taiwan from February 2009 to September 2011<br><br>This study received grant from Kaohsiung Chang Gung Memorial Hospital, Chang Gung University College of Medicine (CMRPG880121). No information regarding any conflicts of interest. |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Participants were randomised into the 3 treatment groups using computer-generated numbers |
| Allocation concealment (selection bias) | Low risk | Allocation concealment was achieved by using sealed, serially numbered, opaque envelopes |
| Blinding (performance bias and detection bias)<br>All outcomes | Low risk | Each syringe used for treatment was covered such that the investigator was blinded to the contents. Heel lancing was performed by another investigator who was not present during treatment administration |
| Blinding of outcome assessment (detection bias) | Low risk | Pain scoring was performed by an investigator who was blinded to treatment and was not present during the experimental procedure |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Ozdogan 2010 (Heel lance)**

| ***Study characteristics*** | |
|---|---|
| Methods | Quasi-randomised controlled trial **(Heel lance)** |
| Participants | 142 healthy term infants undergoing routine heel lance blood sampling for screening tests<br>Exclusion criteria: infants born before the gestational age of 37 weeks, birth weight below 2500 g, Apgar score < 7 at 5 minutes, infants younger than 48 hours, ill or on any medication were excluded<br>Group 1: 18 neonates<br>Median (range) GA - 39 (38 to 41) weeks<br>Median (range) BW - 3210 (3120 to 3400) g<br><br>Group 2: 27 neonates<br>Median (range) GA - 39 (38 to 41) weeks<br>Median (range) BW - 3372 (3100 to 3726) g<br><br>Group 3: 25 neonates<br>Median (range) GA - 39.0 (38 to 41) weeks<br>Median (range) BW - 3444 (3010 to 3512) g<br><br>Group 4: 23 neonates<br>Median (range) GA - 39.4 (38 to 41) weeks<br>Median (range) BW - 3523 (3328 to 3722) g<br><br>Group 5: 26 neonates<br>Median (range) GA - 38.7 (38 to 41) weeks<br>Median (range) BW - 3225 (3162 to 3388) g<br><br>Group 6: 23 neonates<br>Median (range) GA - 39 (38 to 41) weeks<br>Median (range) BW - 3227 (3212 to 3430) g |
| Interventions | All infants were fed 1 hour before the procedure<br>Group 1: single-dose breast milk<br>Group 2: single-dose sterile water<br>Group 3: single-dose 12.5% sucrose solution<br><br>Group 4: 2 doses of breast milk<br><br>Group 5: 2 doses of sterile water<br><br>Group 6: 2 doses of 12.5% sucrose solution<br><br>Infants underwent routine neonatal screening through heel lance. In all the groups, babies received 2 ml of the test solutions through syringe onto the anterior part of the tongue, and they were not allowed to suck the syringe tip.<br><br>In the single-dose groups, the test solution was given 2 minutes before the heel prick and in the repeated-dose groups the dose was repeated just prior to heel prick |
| Outcomes | Total crying time<br><br>NFCS at 0, 1, 2, 3 minutes. For the purpose of this review, we analysed the NFCS at 2 minutes |
| Notes | This study was conducted in the newborn nursery in the Department of Neonatology, Marmara University Hospital, Istanbul, Turkey, in 2001.<br><br>No information regarding conflicts of interest or funding. |
| ***Risk of bias*** | |

**Ozdogan 2010 (Heel lance)** *(Continued)*

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Unclear method of randomisation |
| Allocation concealment (selection bias) | High risk | Participants were consecutively allocated to the different groups by order of admission |
| Blinding (performance bias and detection bias) All outcomes | Unclear risk | The authors do not comment on whether the syringe was wrapped or covered |
| Blinding of outcome assessment (detection bias) | Unclear risk | All the babies were scored according to NFCS at 0, 1, 2 and 3 min by 2 persons who were blind to the groups. The face and the crying of the baby were recorded by a second person with a video camera. It is unclear if this second person was blinded. |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | No missing outcome data |
| Selective reporting (reporting bias) | Low risk | All prespecified outcome data were reported on |
| Other bias | Unclear risk | Protocol not available to compare |

**Peng 2018 (Heel lance)**

| ***Study characteristics*** | |
|---|---|
| Methods | A randomised controlled trial (RCT) **(Heel lance)** |
| Participants | Included participants: gestational age 27 to 37 weeks and postmenstrual age 27.4 to 38 weeks, postbirth age 3 to 28 days, and disease condition acceptable for observation (illness severity indicated by the Neonatal Therapeutic Intervention Scoring System score ≤ 20) |
| Interventions | 109 were randomly assigned to 3 conditions:<br>1. Routine care (n = 36, mean GA in weeks 31.1, mean BW in grams 1556)<br>2. Non-nutritive sucking + breast milk (n = 37, mean GA in weeks 31.2, mean BW in grams 1572)<br>3. Non-nutritive sucking + breast milk + tucking (n = 36, mean GA in weeks 31.3, mean BW in grams 1559) |
| Outcomes | PIPP pain score |
| Notes | This study was conducted in Level III neonatal intensive care unit at a medical centre in Taipei, Taiwan from 2013 to 2014<br>No information provided regarding any conflicts of interest or funding source |

| ***Risk of bias*** | | |
|---|---|---|
| **Bias** | **Authors' judgement** | **Support for judgement** |
| Random sequence generation (selection bias) | Low risk | Preterm infants needing heel sticks were randomly assigned by a blinded statistician using Clinstat block randomisation |

**Peng 2018 (Heel lance)** *(Continued)*

| | | |
|---|---|---|
| Allocation concealment (selection bias) | Unclear risk | It is unclear if allocation concealment was done |
| Blinding (performance bias and detection bias) All outcomes | Unclear risk | The author states that outcome assessors were different from interveners, but it does not state whether interveners or interventions were masked |
| Blinding of outcome assessment (detection bias) | High risk | Pain was scored by a well-trained research assistant from videotapes of infants' faces 10 minutes before, 2 minutes during and 10 minutes after heel stick procedures. The research assistant was blinded to the study purpose and infants' clinical information. All videotapes were scored in a quiet room to maintain consistency and accuracy. Additionally, videotapes were coded in random order. However, it was not possible for outcome assessors to be completely blind to all research processes. |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Phillips 2005 (Heel lance)**

| | |
|---|---|
| ***Study characteristics*** | |
| Methods | Randomised controlled trial **(Heel lance)** |
| Participants | 96 stable full-term newborn infants undergoing routine newborn screening (heel lance) were randomly assigned to one of the 3 treatment groups<br>Group 1: 32 neonates<br>Mean (range) age at procedure - 37 (9) hours<br>Male:female - 13: 19<br>Group 2: 39 neonates<br>Mean (range) age at procedure 36 (8) hours<br>Male:female - 13:26<br>Group 3: 25 neonates<br>Mean (range) age at procedure 38 (14) hours<br>Male:female - 12:13 |
| Interventions | Group 1: breastfeeding<br>Group 2: held by mother with use of pacifier<br>Group 3: held by research assistant with the use of pacifier |
| Outcomes | Percentage of infants who cried<br>Proportion of cry time<br>Heart rate, blood pressure and oxygen saturation change before and after the procedure |
| Notes | This study was conducted in the University of California Davis Medical Center, Sacramento, CA, USA between June 2001 and October 2001<br><br>No information provided regarding any conflicts of interest or funding |
| ***Risk of bias*** | |

**Phillips 2005 (Heel lance)** *(Continued)*

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Randomisation was done by blindly drawing a card from an envelope |
| Allocation concealment (selection bias) | Low risk | Adequate: a research assistant blindly drew a card for each participant from an envelope containing equal numbers of cards with letters representing each group |
| Blinding (performance bias and detection bias) All outcomes | High risk | Masking of intervention was not possible since it involved breastfeeding throughout the procedure |
| Blinding of outcome assessment (detection bias) | High risk | Masking of outcome assessment (from video recordings) was not done; however, data from monitors (heart rate, saturation and blood pressure) were analysed in a masked manner |
| Incomplete outcome data (attrition bias) All outcomes | High risk | Blood pressure measurements were not obtained in all infants; the authors comment that this was due to occasional malfunction of blood pressure equipment |
| Selective reporting (reporting bias) | High risk | Heart rate and oxygen saturation were secondary outcomes that were not reported on. The authors comment that there was no difference amongst the groups, but no data are given. Also, 5 babies were dropped from the study, according to the authors, due to either excessive difficulties with equipment or due to excessive physiologic instability (2) |
| Other bias | Unclear risk | Protocol not available to compare |

**Rawal 2018 (Heel lance)**

| ***Study characteristics*** | |
|---|---|
| Methods | A randomised controlled trial (RCT) **(Heel lance)** |
| Participants | Included infants - hemodynamically stable, had not received any painful stimulus in last 30 minutes, and had received feeds at least 1 hour before data collection |
| Interventions | The eligible babies were in 3 groups. Sterile water (group 1) as the control group and 25% dextrose (group 2) and EBM (group 3) as the intervention groups. The enrolled neonates were administered either 2 mL of test solution (25% dextrose or EBM) or sterile water orally depending on their randomisation code allocation.<br><br>Sterile water group - n = 21, mean GA in weeks 35.2, mean BW in grams 2180<br><br>25% dextrose group - n = 21, mean GA in weeks 35.1, mean BW in grams 2070<br><br>EBM group - n = 21, mean GA in weeks 35.2, mean BW in grams 2160 |
| Outcomes | 1. PIPP score<br>2. Heart rate and $SpO_2$ variation during the test |
| Notes | Study was conducted in the NICU of Jaipur Golden Hospital, Delhi, India from February 2015 to October 2015<br><br>The authors declared no potential conflicts of interest or funding |

**Rawal 2018 (Heel lance)** *(Continued)*

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | The randomisation method is not clearly stated |
| Allocation concealment (selection bias) | Low risk | Concealment was achieved by using sequentially numbered, opaque, sealed envelopes bearing serially numbered patient codes. The composition of these packets was decided by a consultant in the department of neonatology who had the access to the randomisation codes and was uninvolved in the study. |
| Blinding (performance bias and detection bias) All outcomes | Low risk | The observer entered the room after the test solution was administered, and the face of the baby was cleaned to remove any solution residue and thus masked to the test solution given |
| Blinding of outcome assessment (detection bias) | Unclear risk | It is unclear if outcome assessment was masked |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Rioualen 2018 (Venipuncture)**

| ***Study characteristics*** | |
|---|---|
| Methods | A randomised controlled trial (RCT) **(Venipuncture)** |
| Participants | Inclusion criteria: healthy, 3 day-old breastfed full term newborn infants (> 37 weeks of gestation) |
| Interventions | Newborn infants were randomly assigned to either an experimental breastfed group or a sucrose-administered group, which involved holding and non-nutritive sucking<br><br>Breastfed group (n = 57, mean GA in weeks 39.4, mean BW in grams 3473): placed on their mother's chest and breastfed 2 minutes before and throughout the procedure<br><br>Sucrose group (n = 56, mean GA in weeks 39.2, mean BW in grams 3266): placed on their mother's arms and were given 2 mL of 24% sucrose 2 minutes before blood sampling. Infants in this group were allowed to suckle their mother's finger throughout the procedure to avoid the confounding soothing effect of sucking in the breastfed group. |
| Outcomes | 1. Cortical responses to pain by recorded by near infrared spectroscopy (NIRS)<br>2. Pain score by Neonatal Facial Coding System (NFCS)<br>3. Salivary cortisol level (µg/dL)<br>4. Skin conductance (mean peak/second) |
| Notes | This study was conducted in maternity ward of a university hospital in France<br><br>No conflicts of interest |

**Rioualen 2018 (Venipuncture)** *(Continued)*

No information provided about when the study was conducted or any funding

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | An online computerised randomisation program (Capture System, Clinsight) was used that guaranteed allocation concealment |
| Allocation concealment (selection bias) | Low risk | Adequate |
| Blinding (performance bias and detection bias) All outcomes | Low risk | 2 independent neonatal nurses, previously trained on the Neonatal Facial Coding System (NFCS), who were not told the study's aim, scored the video recordings using the NFCS. Each set of sequential procedures, including randomisation, sampling and video recording, was carried out by a team of 3 experimenters: #1 performed the randomisation and instructed the others about the oral medication, sampling method and video recording; #2 performed the oral administration; and # 3 performed the blood sampling |
| Blinding of outcome assessment (detection bias) | Low risk | 2 independent neonatal nurses, previously trained on the Neonatal Facial Coding System (NFCS), who were not told the study's aim, scored the video recordings using the NFCS |
| Incomplete outcome data (attrition bias) All outcomes | High risk | Large amount of missing data with regard to one of the outcomes, salivary cortisol concentrations, because of the insufficient amounts of saliva |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Rodrigues 2017 (Suctioning)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial (RCT) **(Suctioning)** |
| Participants | Preterm babies (< 37 weeks of gestation) who required CPAP included<br><br>Neonates with perinatal asphyxia (Apgar score at 5 min < 5/10), neuromuscular disorders, receiving analgesics, sedatives or anticonvulsants and those who were nil per os were excluded from the study |
| Interventions | Eligible neonates were randomised to 1 of 2 groups (A and B). Babies in group A received 25% dextrose orally during the first suctioning while babies in group B received expressed breast milk (EBM) orally. No intervention was done during the second suctioning. There was a gap of at least 24 hours between the first and second suctioning. For the second suction, the same procedure was followed, except that the babies received standard care (no milk or 25% dextrose).<br><br>25% dextrose group (n = 20, mean GA in weeks 31.5, mean BW in grams 1415)<br><br>Expressed breast milk group (n = 20, mean GA in weeks 31.6, mean BW in grams 1481) |
| Outcomes | Pain score by PIPP |

**Rodrigues 2017 (Suctioning)** *(Continued)*

| | |
|---|---|
| Notes | In the intervention - interventions associated with first suctioning were included. We did not include groups during second suctioning. During second sectioning, babies received standard care, i.e. no milk or 25% dextrose. There was a gap of 24 hours between first and second suction. Thus, during second suctioning, babies served as their own controls.<br><br>This study was conducted in St. Johns Medical College Hospital in Bangalore, India<br><br>No information was provided regarding when the study was conducted, any conflicts of interest or funding |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Randomisation was done using block randomisation in block sizes of 10, using a computer-generated random number table |
| Allocation concealment (selection bias) | Low risk | Allocation concealment was done using sequentially numbered, opaque, sealed envelopes |
| Blinding (performance bias and detection bias)<br>All outcomes | Low risk | Patient enrolment and assignment were done by co-investigators (MF and SA). The primary investigator (LR) was blinded to the intervention. Blinding was ensured by preventing LR from being in the room when the intervention was done. She was not involved in the randomisation, video recording or noting of vital parameters. |
| Blinding of outcome assessment (detection bias) | Low risk | The outcome assessor who was responsible for the scoring of the videos and thus determining the Premature Infant Pain Profile (PIPP) score was blinded |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Rosali 2015 (Eye examination)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial (RCT) **(Eye examination)** |
| Participants | Inclusion criteria: babies with a gestational age of less than 35 weeks (as assessed by New Ballard Score), birth weight less than 2000 g, requiring an ROP screening and who were on at least partial oral feeds |
| Interventions | Groups were randomly divided into 2 groups: intervention (EBM + standard practice) and control group (only standard practices). Standard practices included nesting, swaddling and receiving topical proparacaine.<br><br>Intervention Group (n = 20, mean GA in weeks 31.5, mean BW in grams 1426): infants in the intervention group were given 2 mL of EBM orally by paladai (a small cup used to feed neonates) 2 min prior to the procedure along with standard practice |

**Rosali 2015 (Eye examination)** *(Continued)*

| | |
|---|---|
| | Control group: n = 20, mean GA in weeks 30.3, mean BW in grams 1285 |
| Outcomes | Pain as assessed by the Premature Infant Pain Profile (PIPP) |
| Notes | This study was conducted in the NICU of St John's Medical College hospital in Bangalore, India from June to October 2012<br><br>No information provided regarding conflicts of interest or funding |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | The eligible babies were randomly allocated to the intervention and control groups using computer-generated random numbers |
| Allocation concealment (selection bias) | Low risk | Allocation concealment was done by using sequentially numbered, opaque, sealed envelopes containing the codes for intervention |
| Blinding (performance bias and detection bias)<br>All outcomes | Unclear risk | The author stated that the main strengths of the study were the use of the PIPP scale, which is a very objective, validated and reliable tool for assessment of pain response in preterm neonates, and blinding of the investigators. However, it does not state if or how the intervention was masked or the method. |
| Blinding of outcome assessment (detection bias) | Low risk | The principal investigator videotaped the face of the baby, while another nurse observed the maximum heart rate and minimum saturations on a pulse oximeter. Both these observers were blinded. |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Sabety 2013 (Venipuncture)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial (RCT) **(Venipuncture)** |
| Participants | Inclusion criteria: term neonates (38 to 42 weeks); without evidence of poor feeding; stable condition; per oral feeding; 5-minute APGAR > 7; and no history of narcotic usage |
| Interventions | 121 term neonates were randomly divided in 4 groups:<br><br>Group I (glucose): 2 cc glucose 50% was administered orally, 2 minutes before procedure<br>Group II (lignocaine): 13 minutes before venipuncture, 1 g of lidocaine gel (2%) was administered topically<br>Group III (breast milk): 2 cc breast milk was administered orally via syringe at 2 minutes before venipuncture<br>Group IV: (control group): routine venipuncture was performed without adding anything |
| Outcomes | 1. Crying time in seconds |

**Sabety 2013 (Venipuncture)** *(Continued)*

| | |
|---|---|
| | 2. DAN score<br>3. Respiratory rate<br>4. Pulse rate |
| Notes | This study was conducted in Iran. No information was provided regarding when this study was conducted.<br><br>There was no conflict of interest. This study was supported by student research committee of Ahvaz Jundishapur University of Medical Sciences (Registration no. 89s71). |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Method of randomisation is not mentioned |
| Allocation concealment (selection bias) | Unclear risk | No information provided regarding allocation concealment |
| Blinding (performance bias and detection bias)<br>All outcomes | Unclear risk | Masking of intervention is not clear |
| Blinding of outcome assessment (detection bias) | Low risk | Outcome assessment was performed by an experienced nurse who was blind to the group allocation of participants |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Sahoo 2013 (Venipuncture)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial (RCT) **(Venipuncture)** |
| Participants | Inclusion criteria: babies ≥ 34 weeks gestations that required venipuncture for blood sampling and who were on oral feeds |
| Interventions | The eligible babies were randomised into 3 groups: 2 intervention groups - expressed breast milk group (EBM) and 25% dextrose group (25D), and a control group sterile water (SW)<br><br>Expressed breast milk group (EBM): n = 62, mean GA in weeks 37.6, mean BW in grams 2605<br><br>25% dextrose group (25D): n = 50, mean GA in weeks 37.94, mean BW in grams 2965<br><br>Sterile water group (SW): n = 48, mean GA in weeks 37.9, mean BW in grams 2720 |
| Outcomes | 1. Change in HR<br>2. Change is oxygen saturation |

**Sahoo 2013 (Venipuncture)** *(Continued)*

| | |
|---|---|
| | 3. Crying time in seconds<br>4. Pain score by PIPP |
| Notes | This study was conducted in the postnatal ward of a tertiary care hospital in India from April 2010 to September 2010<br><br>No funding or conflicts of interest reported |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | The eligible babies were randomised into 3 groups using computer-generated random numbers |
| Allocation concealment (selection bias) | Low risk | Allocation concealment was achieved by using sequentially numbered, opaque, sealed envelopes containing the codes for intervention. The envelopes were exclusively accessed by the principal investigator. |
| Blinding (performance bias and detection bias) All outcomes | Low risk | The observers entered the room after the test solution was administered and thus were masked to the test solution given |
| Blinding of outcome assessment (detection bias) | Low risk | Outcome assessors were masked |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Shendurnikar 2005 (Heel lance)**

***Study characteristics***

| | |
|---|---|
| Methods | Randomised controlled trial **(Heel lance)** |
| Participants | 100 full-term newborn infants who underwent heel lance were randomly assigned to one of the 2 treatment groups:<br><br>Group 1: 50 neonates<br>GA: 38.2 weeks<br>Male:female = 22:28<br>Group 2: 50 neonates<br>GA: 38.6 weeks<br>BW: 2865 g<br>Male:female = 31:19<br>Postnatal age 3.4 days<br>BW: 2910 g<br>Postnatal age 3.1 days<br><br>Inclusion criteria: full-term neonates > 2500 g BW |

**Shendurnikar 2005 (Heel lance)** *(Continued)*

| | |
|---|---|
| | Exclusion criteria: septicaemia, birth asphyxia, major congenital malformation |
| Interventions | Group 1: breastfeeding group<br>Group 2: swaddled group |
| Outcomes | Behavioural (state of arousal, cry, facial expression, body movements)<br>Physiological (heart rate, breathing pattern)<br>Composite score |
| Notes | This study was conducted in India between July 2003 and October 2003<br><br>No information regarding conflicts of interest or funding |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | The randomisation was done by the primary author asking mother to choose from a collection of randomisation cards |
| Allocation concealment (selection bias) | Low risk | Adequate |
| Blinding (performance bias and detection bias)<br>All outcomes | High risk | Masking of intervention was not possible since it involved breastfeeding during the procedure |
| Blinding of outcome assessment (detection bias) | High risk | Masking of outcome assessment was not done. The primary author was aware of the allocation and hypothesis of the study. |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All participants were accounted for in the analysis of outcomes |
| Selective reporting (reporting bias) | Low risk | All prespecified outcomes were reported |
| Other bias | Unclear risk | Protocol not available to compare |

**Simonse 2012 (Heel lance)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial (RCT) **(Heel lance)** |
| Participants | Inclusion criteria: born at a gestational age between 32 + 0 and 36 + 6 weeks, being nourished with breastfeeding, and had to have a clinical blood sample taken |
| Interventions | Neonates randomly divided into 3 groups:<br><br>Breastfed group: neonates were held in their mother's arms; n = 23, mean GA in weeks 34.7, mean BW in grams 2361<br><br>Bottle-fed group: neonates were held in the arms of an experienced nurse and were given supplemental breast milk by a sterile syringe; n = 23, mean GA in weeks 34.5, mean BW in grams 2117 |

**Simonse 2012 (Heel lance)** *(Continued)*

| | |
|---|---|
| | Sucrose solution group: neonates lied in their cot and received 1 mL to 2 mL of 24% sucrose solution 2 minutes before the heel lance, combined with non-nutritive sucking; n= 25, mean GA in weeks 34.9, mean BW in grams 2186 |
| Outcomes | 1. Pain assessed by the COMFORT neo scale<br>2. Pain score by PIPP |
| Notes | The trial was performed from January 2010 to May 2011 at the neonatal ward of Amphia Hospital in Breda, Netherlands<br><br>No external funding or conflicts of interest reported |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | The randomisation sequence was created by using a fixed block size of 8 for a maximum of 75 neonates with a 1:1:1 allocation |
| Allocation concealment (selection bias) | Low risk | Neonates were allocated to 1 of the 3 groups according to the method of sequentially numbered and opaque, sealed envelopes created by an independent employee and masked for the investigator. Allocation concealment is guaranteed by this method. |
| Blinding (performance bias and detection bias)<br>All outcomes | High risk | It was not possible to blind patients and investigators to the allocated intervention |
| Blinding of outcome assessment (detection bias) | High risk | Masking of outcome assessors was not performed |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Singh 2017 (Heel lance)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial (RCT) **(Heel lance)** |
| Participants | Inclusion criteria: healthy, full-term, non-asphyxiated neonates up to 7 days of life who were scheduled to receive heel sticks to collect blood for obligatory newborn screening; haemodynamically stable and were not receiving oxygen or any analgesia |
| Interventions | These neonates were randomised into 2 groups (30 neonates each). The neonates in the test group (Group I) received breastfeeding during the heel prick procedure. The neonates in the control group (Group II) were not breastfed during the heel prick procedure.<br><br>Breastfeeding group (n = 30, mean GA in weeks 38, mean BW in grams 2850): breastfeeding initiated 2 minutes before the procedure and continued throughout |

**Singh 2017 (Heel lance)** *(Continued)*

| | |
|---|---|
| | Control group (n = 30, mean GA in weeks 39, mean BW in grams 3100) |
| Outcomes | 1. Total duration of cry over 10-min period after heel prick (seconds)<br>2. Change in heart rate after heel prick<br>3. Mean fall in transcutaneous oxygen saturation after heel prick<br>4. Mean change in blood pressure of neonates after heel prick test - systolic and diastolic blood pressure |
| Notes | This study was conducted in Command Hospital (Air Force) Bangalore, India<br>No information provided regarding when this study was conducted<br>No conflicts of interest or funding reported |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Neonates were randomised into 2 groups (30 neonates each) using a sealed envelope randomisation system |
| Allocation concealment (selection bias) | Low risk | Adequate |
| Blinding (performance bias and detection bias)<br>All outcomes | Unclear risk | It is unclear if the intervention was masked |
| Blinding of outcome assessment (detection bias) | Unclear risk | It is unclear if masking was performed for outcome assessors |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Skogsdal 1997 (Heel lance)**

***Study characteristics***

| | |
|---|---|
| Methods | Randomised controlled trial **(Heel lance)** |
| Participants | 120 stable newborn infants (66 preterm and 54 full-term) undergoing heel lance for blood collection for their care between 1 and 30 days of age were randomly assigned to one of the 4 treatment groups (30 neonates in each group)<br>Exclusion criteria: age < 24 hours, analgesic or sedative drug given within last 5 days, gestational age < 30 weeks, ventilator or CPAP treatment, oxygen requirement > 40%, neurological symptoms, antibiotic therapy and age > 1 month<br>Mean (SD) GA - 35.5 (2.3) weeks<br>Mean (SD) age at testing - 5.4 (4.9) days |
| Interventions | Group 1: no treatment group<br>Group 2: 1 mL 30% glucose |

**Skogsdal 1997 (Heel lance)** *(Continued)*

| | |
|---|---|
| | Group 3: 1 mL 10% glucose<br>Group 4: 1 mL breast milk |
| Outcomes | Crying time<br>Heart rate change |
| Notes | This study was conducted in the Neonatal Unit at Orebro Medical Centre Hospital, Sweden between May 1994 and August 1995<br><br>No information regarding conflicts of interest or funding provided |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | The randomisation was done through a random digit table |
| Allocation concealment (selection bias) | Unclear risk | Unclear about allocation concealment |
| Blinding (performance bias and detection bias)<br>All outcomes | Low risk | The administration of the allocated solution and the heel prick was done by the same nurse, who did not participate in recording the outcomes. |
| Blinding of outcome assessment (detection bias) | Low risk | The study personnel involved in assessing the outcomes were blinded |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All participants were accounted for in the analysis of outcomes |
| Selective reporting (reporting bias) | Low risk | All prespecified outcomes were reported |
| Other bias | Unclear risk | Protocol not available to compare |

**Soltani 2018 (Heel lance)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial (RCT) **(Heel lance)** |
| Participants | Infants with gestational age of 37 to 42 weeks; Apgar score of ± 9 at birth; birth weight of 2500 g to 4000 g, and ages between 3 and 5 days who were candidates for heel prick sampling |
| Interventions | Infants were allocated randomly to 4 groups of interventions:<br><br>A: breast milk feeding (n = 42), B: oral 25% dextrose (n = 40), C: KMCM (n = 38) and D: KMCM ointment (n = 40). All interventions were applied 15 minutes before heel prick procedure.<br><br>1. Breast milk feeding group: n = 42, mean BW in grams 2880<br>2. 25% dextrose group: n = 40, mean BW in grams 3110<br>3. Skin contact (KMCM) group: n = 38, mean BW in grams 3260<br>4. EMLA ointment group: n = 41, mean BW in grams 3040 |

**Soltani 2018 (Heel lance)** *(Continued)*

| | |
|---|---|
| Outcomes | Outcome 1: Neonatal Infant Pain Scale score variables and total pain score |
| Notes | This study was conducted in Shahid Motahhari Hospital, Marvdasht, Shiraz Province, Iran, from March to December 2015<br><br>There was no conflict of interest to declare. This research was supported financially by the Research Council of the International Campus of Shiraz University of Medical Sciences. |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | The infants were allocated randomly using envelopes that contained a pain management method amongst 4 groups |
| Allocation concealment (selection bias) | Low risk | Adequate |
| Blinding (performance bias and detection bias) All outcomes | Unclear risk | A double-blind method was set up for this study. The nurse in charge of the heel prick sampling was not aware of the pre-sampling pain management method. The author also mentioned that infants were allocated randomly to 4 groups of interventions, i.e. breast milk feeding, oral 25% dextrose, KMCM and KMCM ointment; all interventions were applied 15 minutes before the heel prick. However, it is unclear if the intervention was masked. |
| Blinding of outcome assessment (detection bias) | Low risk | Pain score levels were measured according to the Neonatal Infant Pain Scale parameters by a certain medical student who was not aware of the pain management method in each infant |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Sujatha 2018 (Intradermal)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial (RCT) **(Intradermal)** |
| Participants | Newborns of 37 weeks of gestation receiving BCG vaccination |
| Interventions | Infants randomly divided into 2 groups:<br><br>Breast milk (n = 45, weight in between 2.5 kg and 3 kg 90% of infants): 1 mL of breast milk was administered to the neonates of the study group through a sterile disposable syringe from one corner of the mouth<br><br>Control group of facilitated tucking (n = 100, weight in between 2.5 kg and 3kg 83% of infants): routine care |
| Outcomes | 1. Change in oxygen saturation |

**Sujatha 2018 (Intradermal)** *(Continued)*

| | |
|---|---|
| | 2. Change in HR<br>3. Pain score by NIPS<br>4. Change in respiratory rate<br>5. Duration of cry in seconds |
| Notes | This study was conducted in the tertiary care hospital in Puducherry, India during January 2013 to January 2014<br><br>No information regarding any conflicts of interest or funding |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | The eligible neonates were randomised and included in the study using computer-generated random numbers |
| Allocation concealment (selection bias) | Unclear risk | The envelopes were exclusively accessed by the research assistants, but it is unclear if allocation concealment was done |
| Blinding (performance bias and detection bias)<br>All outcomes | Low risk | The excess amount of breast milk in the neonatal mouth and the syringe was cleared before the entry of the blinded researcher into the vaccination room and thus the intervention was masked |
| Blinding of outcome assessment (detection bias) | Unclear risk | It is unclear whether outcome assessment was masked |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Taplak 2017 (Eye examination)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial (RCT) **(Eye examination)** |
| Participants | Included infants: preterm infants with a birth weight < 1500 g and gestational age of < 32 weeks<br><br>Exclusion criteria: preterm infants diagnosed with a congenital anomaly, hydrocephalus, necrotising enterocolitis, indirect hyperbilirubinaemia, and receiving ventilatory support or analgesic drug treatment |
| Interventions | Infants randomly divided into 3 groups:<br><br>Group 1 was provided with 1 mL breast milk; Group 2 was provided with 1 mL 33% sucrose; and Group 3 was provided with 1 mL distilled water via nipples + injector<br><br>Breast milk group (n = 20; mean GA ≤ 27.6 (1), > 28 weeks (19); mean BW in grams ≤ 1000 g (2), > 1000 g (18))<br><br>Sucrose group (n = 20; mean GA ≤ 27.6 (5), > 28 weeks (15); mean BW in grams ≤ 1000 g (7), > 1000 g (13)) |

**Taplak 2017 (Eye examination)** *(Continued)*

| | |
|---|---|
| | Control group (n = 20; mean GA ≤ 27.6 (7), > 28 weeks (13); mean BW in grams ≤ 1000 g (9), > 1000 g (11)) |
| Outcomes | 1. Change in oxygen saturation<br>2. Change in HR<br>3. Pain score by PIPP |
| Notes | This study was conducted in Turkey between 10 August 2010 and 10 March 2011<br><br>No conflicts of interest reported. This study has received funding from Scientific Research Project Coordination Unit Erciyes University (Project Code: TSY-10-3381). |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Method of randomisation is not clear |
| Allocation concealment (selection bias) | Unclear risk | Not clear if allocation concealment was performed |
| Blinding (performance bias and detection bias)<br>All outcomes | Low risk | The researcher carrying out the physiological measurements 5 minutes before and 5 minutes after the examination also performed the video recordings without having any information on the group assignment of the preterm infants |
| Blinding of outcome assessment (detection bias) | Low risk | Three independent specialists (two nurses specialised in paediatric nursing and a neonatologist who had no information about each other) analysed the video records of the ROP examination and scored the PIPP |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Tavlar 2021 (Heel lance)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial **(Heel lance)** |
| Participants | Term neonates (38 to 41 weeks of gestation) who underwent heel stick blood drawing for metabolic screening; age 1 to 2 days |
| Interventions | Neonates were randomised by the researcher into 3 groups: breastfeeding group (n = 30), breast milk odour group (n = 30) and mother's heartbeat sounds group (n = 30):<br><br>Breastfeeding group (n = 30, mean GA 39.3 in weeks, mean BW in grams 3191): newborns in this group were breastfed starting from 3 min before to 3 min after the procedure. The right breast was preferred so that the newborn would not be affected by the mother's heartbeat.<br><br>Breast milk odour group (n =30, mean GA in weeks 39.3, mean BW in grams 3219): a total of 5 mL of breast milk sample was extracted from each baby's own mother before the procedure and poured onto |

**Tavlar 2021 (Heel lance)** *(Continued)*

| | |
|---|---|
| | a sterile odourless cloth. Then, the cloth was placed 3 cm from the newborn's nose and remained there starting from 3 min before to 3 min after the procedure.<br><br>Mother's heartbeat sounds group (n = 30, mean GA in weeks 39.3, mean BW in grams 3259): before the procedure, the maternal heartbeat sounds of each newborn was recorded from their mothers by means of a foetal hand Doppler. The mother's heartbeat sound was played to the newborn starting from 3 min before to 3 min after the procedure (max. sound level: 60 dB) |
| Outcomes | Outcome 1: ALPS-Neo score<br><br>Outcome 2: total crying time, seconds<br><br>Outcome 3: oxygen saturation<br><br>Outcome 4: heart rate |
| Notes | ALPS-Neo score: the scale was developed multidimensionally by Lundqvist et al (2014) to assess pain and stress in premature and term newborns. It is a 3-point Likert-type scale consisting of 5 items: facial expression, breathing pattern, arm/leg muscle tone, hand/foot activity and level of activity. Measurements were made by observation. A higher scale score refers to a greater stress and pain, where 0 to 2 = no pain and stress, 3 to 5 = mild pain and stress, and > 5 = high pain and stress. The total internal reliability score of the scale was reported as 0.91 and the internal consistency alpha coefficient as 0.95.<br><br>This study was conducted in Marmara University, Turkey between 15 February 2019 and 15 July 2019<br><br>This study was not financial supported. No conflicts of interest among authors. |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Randomisation was performed using the lottery method |
| Allocation concealment (selection bias) | Low risk | Adequate. The hospital registration protocol numbers of the infants whose heel blood was planned to be collected were written on pieces of paper and collected in a bag. Then, the protocol numbers were respectively drawn from the bag for each of the breastfeeding, maternal heart sounds and breast milk scent application groups. |
| Blinding (performance bias and detection bias)<br>All outcomes | Unclear risk | A total of 30 protocol numbers were drawn from the bag for each group, including breastfeeding, mother's heartbeat sound and breast milk odour, respectively. In accordance with the groups formed, heartbeat sound recordings or breast milk samples were taken from the mothers. However, it is unclear if the intervention was masked. |
| Blinding of outcome assessment (detection bias) | Low risk | ALPS-Neo scale score, heart rate, oxygen saturation and crying time were evaluated for each newborn by the researcher and recorded 3 min before, during and 3 min after the heel lance procedure. The data were evaluated by 2 observers. The observers were a researcher and a newborns midwife. The data were assessed by 2 independent observers. |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported; no missing outcomes |
| Other bias | Unclear risk | Protocol not available for comparison |

### Turan 2021 (Eye examination)

| ***Study characteristics*** | |
|---|---|
| Methods | A randomised controlled trial (RCT) **(Eye examination)** |
| Participants | Hospitalised in the neonatal intensive care unit and whose gestational week was < 32 weeks and birth weight was < 1500 g |
| Interventions | The preterm infants who would undergo ROP examination were allocated to 3 groups according to simple randomisation method as follows: group 1 (n = 17): only local anaesthetic eye drops, proparacaine hydrochloride ophthalmic solution 0.5%; group 2 (n = 17): proparacaine hydrochloride ophthalmic solution 0.5% plus breast milk, and group 3 (n = 17): proparacaine hydrochloride ophthalmic solution 0.5% plus sucrose 24%.<br><br>The pupils of the infants were dilated using 2.5% phenylephrine and 0.5% tropicamide 3 times in 5-min intervals. Preterm infants in all groups were administered 1 drop of proparacaine hydrochloride 30 seconds prior to an eye examination. Preterm infants in group 2 were given 2 mL of breast milk 2 min before the eye examination and the preterm infants in group 3 were administered 0.3 mL of sucrose 24% onto the anterior part of the tongue 2 min before the eye examination.<br><br>Only proparacaine group: n = 17, mean GA in weeks 30.4, mean BW in grams 1384<br><br>Proparacaine + breast milk group: n = 17, mean GA in weeks 30.1, mean BW in grams 1449<br><br>Proparacaine + sucrose group: n = 17, mean GA in weeks 28.4, mean BW in grams 1144 |
| Outcomes | Outcome 1: $SpO_2$<br><br>Outcome 2: HR<br><br>Outcome 3: Perfusion Index (PI)<br><br>Outcome 4: NIPS |
| Notes | This study was conducted in Baskent University, Ankara, Turkey<br><br>No potential conflicts of interest was reported by the authors<br><br>No information available about when the study was conducted or regarding funding |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | The preterm infants who would undergo ROP examination were allocated to 3 groups according to a simple randomisation method |
| Allocation concealment (selection bias) | Unclear risk | It is unclear if allocation concealment was achieved |
| Blinding (performance bias and detection bias) All outcomes | High risk | Masking of intervention was not performed |
| Blinding of outcome assessment (detection bias) | High risk | Masking of outcome assessment was not performed |
| Incomplete outcome data (attrition bias) | Low risk | All infants were included in the final analysis |

**Turan 2021 (Eye examination)** *(Continued)*

| | | |
|---|---|---|
| All outcomes | | |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Upadhyay 2004 (Venipuncture)**

| | |
|---|---|
| ***Study characteristics*** | |
| Methods | Randomised controlled trial **(Venipuncture)** |
| Participants | 81 neonates requiring venipuncture for clinical indication<br><br>Inclusion criteria: GA of 37 to 41 weeks who were ≤ 4 weeks of postnatal age and required venipuncture for clinical indication<br>Exclusion criteria: perinatal asphyxia (Apgar score < 7 at 1 min), major congenital malformations, admission to neonatal intensive care unit, maternal anaesthesia, opiates administration before delivery or within 48 hours of sampling, babies given naloxone or phenobarbitone<br><br>Group 1: 40 neonates<br>Mean (SD) GA - 38 (0.9) weeks<br>Mean (SD) BW - 2600 (300) grams<br>Group 2: 41 neonates<br>Mean (SD) GA - 38 (0.8) weeks<br>Mean (SD) BW - 2900 (300) grams |
| Interventions | Group 1: 5 mL of expressed breast milk<br>Group 2: 5 mL of distilled water<br>The solutions were administered over 2 minutes prior to venipuncture |
| Outcomes | Duration of crying after venipuncture<br>NFCS at 1 and 3 minutes after the venipuncture<br>Changes in heart rate and oxygen saturation |
| Notes | This study was conducted in a tertiary care institute, New Delhi, India during the period March to November 2001<br><br>No information regarding conflicts of interest or funding |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | The randomisation was performed using computer-generated numbers |
| Allocation concealment (selection bias) | Low risk | Adequate: allocation was adequately concealed |
| Blinding (performance bias and detection bias)<br>All outcomes | Low risk | A single independent investigator who was not involved in the observations and analysis administered the solution |

**Upadhyay 2004 (Venipuncture)** *(Continued)*

| | | |
|---|---|---|
| Blinding of outcome assessment (detection bias) | Low risk | The outcome observers were blinded to the groups. For the NFCS, the 2 independent observers came in the room after the intervention had been completed, therefore they were blinded to the solution. |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | The data for 81 participants were available for analysis; the authors explain why 6 infants were excluded |
| Selective reporting (reporting bias) | Low risk | All prespecified outcomes are reported |
| Other bias | Unclear risk | Protocol not available to compare |

**Uyan 2005 (Heel lance)**

| | |
|---|---|
| ***Study characteristics*** | |
| Methods | Quasi-randomised controlled trial **(Heel lance)** |
| Participants | 62 term infants undergoing heel lance blood sampling for screening tests<br>Exclusion criteria: preterm neonates, neonates with Apgar score < 7 at 5 minutes, neonates with low birth weight, sick neonates and neonates on any medication<br><br>Group 1: 20 neonates<br>Median (range) GA - 39 (38 to 41) weeks<br>Median (range) BW - 3300 (2800 to 4260) g<br>Group 2: 21 neonates<br>Median (range) GA - 39 (38 to 41) weeks<br>Median (range) BW - 3510 (2750 to 4030) g<br>Group 3: 21 neonates<br>Median (range) GA - 40 (38 to 41) weeks<br>Median (range) BW - 3300 (2800 to 4500) g |
| Interventions | All infants were fed 1 hour before the procedure<br>Group 1: 2 mL of foremilk<br>Group 2: 2 mL of hind milk<br>Group 3: 2 mL of sterile water<br>The solutions were administered by syringe<br>Heel lance was performed 2 minutes after administration of the solution |
| Outcomes | Crying time<br>Duration of first cry<br>Percentage change in heart rate at 1, 2 and 3 minutes<br>NFCS |
| Notes | This study was conducted in Marmara University Hospital, Turkey between November 2001 and April 2003<br><br>No information regarding any conflicts of interest or funding |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Method of randomisation is not clear |

**Uyan 2005 (Heel lance)** *(Continued)*

| | | |
|---|---|---|
| Allocation concealment (selection bias) | High risk | Inadequate |
| Blinding (performance bias and detection bias) All outcomes | Unclear risk | The authors do not comment on how the investigators were blinded, but they say that the intervention was masked |
| Blinding of outcome assessment (detection bias) | Low risk | The 2 investigators who analysed the data and the person who recorded video for the NFCS coding were unaware of the treatment allocation; hence, the outcome measure analysis was blinded |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | All infants were accounted for in the analysis |
| Selective reporting (reporting bias) | Low risk | All prespecified outcomes are reported |
| Other bias | Unclear risk | Protocol not available to compare |

**Velumula 2022 (Heel lance)**

| ***Study characteristics*** | |
|---|---|
| Methods | A randomised controlled trial **(Heel lance)** |
| Participants | Preterm neonates (30 to 36 6/7 weeks of gestation) who underwent heel stick blood drawing for scheduled blood draw; age 1 to 30 days |
| Interventions | Neonates were randomised by the researcher into 2 groups: breast milk (n = 44) and 24% sucrose (n = 44)<br>Breast milk group (n = 44, mean GA in weeks 33.1, mean BW in grams 1869): neonates received 2 mL expressed breast milk via syringe, combined with swaddling, 2 minutes prior to scheduled heel lance<br>24% oral sucrose group (n = 44, mean GA in weeks 32.7 mean BW in grams 1948): 0.5 mL of 24% sucrose via syringe was administered orally, combined with swaddling, 2 minutes prior to scheduled heel lance |
| Outcomes | Outcome 1: PIPP score<br>Outcome 2: heart rate |
| Notes | This study was conducted at Hutzel Women's Hospital and Children's Hospital of Michigan, Detroit, USA from October 2019 to April 2021<br>This work was supported by an institutional grant from Sarnaik Endowment Grant, Detroit Medical Center. The authors declared no competing interests. |

| ***Risk of bias*** | | |
|---|---|---|
| **Bias** | **Authors' judgement** | **Support for judgement** |
| Random sequence generation (selection bias) | Low risk | Randomisation was done by the pharmacist using a computer-generated sequence for simple randomisation |

**Velumula 2022 (Heel lance)** *(Continued)*

| | | |
|---|---|---|
| Allocation concealment (selection bias) | Low risk | Allocation concealment was achieved by using consecutively numbered, sealed, opaque envelopes |
| Blinding (performance bias and detection bias) All outcomes | Low risk | The 2 investigators independently recorded baseline heart rate, oxygen saturation and behavioural state of the neonate, after which they left the room, and the nurse administered the assigned drug. The investigators returned to the room 2 minutes after the drug was administered at which time the nurse performed the procedure. |
| Blinding of outcome assessment (detection bias) | Low risk | The investigators independently assigned pain scores during and after the procedure every 30 seconds until 120 seconds. The study completion was documented in the neonate's electronic medical record by the nurse. |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | All neonates were included in the analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes were reported; no missing outcomes |
| Other bias | Unclear risk | Protocol not available for comparison |

**Weissman 2009 (Heel lance)**

| ***Study characteristics*** | |
|---|---|
| Methods | Quasi-randomised controlled trial **(Heel lance)** |
| Participants | 180 term infants undergoing heel lance blood sampling for routine screening tests<br>Group 1: 31 neonates<br>Mean (SD) GA - 39.7 (1.2) weeks<br>Mean (SD) BW - 3398 (428) g<br>Group 2: 30 neonates<br>Mean (SD) GA - 39.1 (1.4) weeks<br>Mean (SD) BW - 3227 (417) g<br><br>Group 3: 31 neonates<br>Mean (SD) GA - 39.5 (1.3) weeks<br>Mean (SD) BW - 3157 (397) g<br><br>Group 4: 29 neonates<br>Mean (SD) GA - 39.4 (1.1) weeks<br>Mean (SD) BW - 3390 (356) g<br><br>Group 5: 30 neonates<br>Mean (SD) GA - 39.6 (1.2) weeks<br>Mean (SD) BW - 3364 (460) g<br><br>Group 6: 29 neonates<br>Mean (SD) GA - 39.8 (1.5) weeks<br>Mean (SD) BW - 3368 (382) g |
| Interventions | All infants underwent heel lancing for routine neonatal screening. Infants in group 1 were breastfed, infants in group 2 were fed formula while in their cribs. For infants in group 3, the solution was given orally 2 minutes before the procedure. Infants in group 4 were held by their mothers (mothers were free to choose how to hold their infant with no specific recommendations given) and infants in group 5 had |

**Weissman 2009 (Heel lance)** *(Continued)*

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|---|---|
| | non-nutritive sucking with a pacifier during heel lancing. Infants in group 6 (control group) had no pain relief. |
| Outcomes | NFCS, through video recording, duration of cry and heart rate increase |
| Notes | This study was conducted in Israel<br>No funding reported<br>No information regarding when this study was conducted or any conflicts of interest |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | It is unclear if random sequence generation was done |
| Allocation concealment (selection bias) | High risk | Quasi-randomised trial; allocation was done according to mothers' preference |
| Blinding (performance bias and detection bias) All outcomes | High risk | There was no blinding of the interventions |
| Blinding of outcome assessment (detection bias) | High risk | Masking of outcome assessor was not done |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | All infants are accounted for in the analysis; no missing outcome data |
| Selective reporting (reporting bias) | Low risk | Published report includes all expected outcomes |
| Other bias | Unclear risk | Protocol not available to compare |

**Wu 2021 (Heel lance)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial **(Heel lance)** |
| Participants | Preterm neonates who underwent heel stick blood drawing for newborn screening test; age 12 to 15 days |
| Interventions | Neonates were randomised by the researcher into 2 groups: embracing breast milk sucking (n = 48) and control group (n = 48):<br>Embracing breast milk sucking (EBMS) group (n = 48, mean BW in grams 3290): neonates were given EBMS. The room temperature and humidity were controlled before the blood collection. After the neonates were bathed, they were placed on the mother's chests for skin-to-skin contact. Medical staff guided the mothers to simulate the process of communicating with the newborn during pregnancy, and comforted the newborn.<br>Control group (n = 48, mean BW in grams 3215): prior to heel blood sampling, the medical staff controlled the indoor temperature to about 23 °C and about 60% humidity, dedicated medical staff bathed |

**Wu 2021 (Heel lance)** *(Continued)*

| | |
|---|---|
| | the newborns and disinfected and collected blood from the heels of the newborns after the bath; and the newborn was wrapped in a quilt during blood collection to keep them warm |
| Outcomes | Outcome 1: Neonatal Infant Pain Scale<br>Outcome 2: total crying time, seconds<br>Outcome 3: oxygen saturation<br>Outcome 4: heart rate |
| Notes | This study was conducted in Taiwan<br>No information provided regarding when this study was conducted or if any conflicts of interest or funding |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | No information provided/available |
| Allocation concealment (selection bias) | Unclear risk | No information provided/available |
| Blinding (performance bias and detection bias)<br>All outcomes | Unclear risk | No information provided/available |
| Blinding of outcome assessment (detection bias) | Unclear risk | No information provided/available |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported; no missing outcomes |
| Other bias | Unclear risk | No protocol available for comparison |

**Yilmaz 2011 (Heel lance)**

***Study characteristics***

| | |
|---|---|
| Methods | Randomised controlled trial **(Heel lance)** |
| Participants | 120 term infants undergoing heel lance blood sampling for screening tests<br>Exclusion criteria: preterm neonates, neonates with Apgar score < 7 at 1 and 5 minutes, neonates with low birth weight (< 2500 g), sick neonates, newborns with congenital anomalies and newborns born by vaginal delivery<br>Group 1: 30 neonates<br>Mean (SD) GA - 39.1 (+/- 1.03) weeks<br>Mean (SD) BW - 3363 (+/- 391) g<br>Group 2: 30 neonates |

**Yilmaz 2011 (Heel lance)** *(Continued)*

| | |
|---|---|
| | Mean (SD) GA - 39.1 (+/-0.71) weeks<br>Mean (SD) BW - 3400 (+/- 353) g<br><br>Group 3: 30 neonates<br>Mean (SD) GA - 39.2 (+/- 0.93) weeks<br>Mean (SD) BW - 3298 (+/- 406) g<br><br>Group 4: 30 neonates<br>Mean (SD) GA- 39.7 (+/- 0.8) weeks<br>Mean (SD) BW - 3391 (+/- 383) g |
| Interventions | All infants were fed half an hour before the procedure<br>Group 1: 2 mL of breast milk<br>Group 2: 2 mL of 20% sucrose<br>Group 3: non-nutritive sucking (pacifier)<br><br>Group 4: control (no intervention)<br>The solutions were administered by syringe avoiding contact of the syringe with the mouth and lips<br>Heel lance was performed 2 minutes after administration of the solution |
| Outcomes | NIPS<br><br>Change in heart rate, saturation of oxygen, respiratory rate and body temperature |
| Notes | Study was conducted in the Trabzon Delivery and Children's Diseases hospital, Turkey between February 2007 and January 2008<br><br>No funding reported but no information regarding conflicts of interest |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Unclear risk | Insufficient information in terms of randomisation |
| Allocation concealment (selection bias) | Unclear risk | Insufficient information in terms of allocation concealment |
| Blinding (performance bias and detection bias)<br>All outcomes | Unclear risk | No information is given regarding blinding |
| Blinding of outcome assessment (detection bias) | Unclear risk | No information on whether the investigators analysing the videotapes for the NIPS were blinded to the infants' intervention |
| Incomplete outcome data (attrition bias)<br>All outcomes | High risk | The authors mention saturation of oxygen as one of the outcomes, and they say there was no significant difference amongst the groups, but they do not show the data |
| Selective reporting (reporting bias) | High risk | There are no data for one of the prespecified outcomes, oxygen saturation |
| Other bias | Unclear risk | Protocol not available to compare |

**Yilmaz 2020 (Heel lance)**

| ***Study characteristics*** | |
|---|---|
| Methods | A randomised controlled trial (RCT) **(Heel lance)** |
| Participants | 2 to 4 days old newborns (38 to 42 weeks gestation) who needed a routine heel lance |
| Interventions | The researcher randomised the newborns into 4 groups:<br>Swaddling (n = 40, mean GA in weeks 38.8, mean BW in grams 3369): swaddling was carried out 1 min before the heel stick procedure and continued 2 min after the procedure<br>Swaddling and holding (n = 40, mean GA in weeks 39.0, mean BW in grams 3459): the mothers in this group sat down on a comfortable chair and following the safe swaddling of the newborns, the baby was placed in the mother's arms and the heel lance process was applied<br>Swaddling, holding and breastfeeding (n = 40, mean GA in weeks 39.3, mean BW in grams 3398): first infant was swaddled, held by mother and breastfeeding began immediately before the heel lance procedure (about 1 min before) and continued for a minimum of 2 min during and after the procedure<br>Control group (n = 40, mean GA in weeks 38.9, mean BW in grams 3248): no intervention was performed for the newborns during the procedure |
| Outcomes | Outcome 1: NIPS total score<br>Outcome 2: total crying time, seconds<br>Outcome 3: time to first calming |
| Notes | This study was conducted in a public hospital in Bandirma, Turkey<br>The authors do not have any conflicts of interest to declare<br>No information regarding when this study was conducted or any funding sources |

***Risk of bias***

| **Bias** | **Authors' judgement** | **Support for judgement** |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | The groups were randomised by the researcher using a computer-based random number table program. Amongst the 4 groups, the numbers from 1 to 160 were randomly distributed without repetition by a computer program in order to include the babies in the suitable groups. A random code was picked for each newborn by the researcher. |
| Allocation concealment (selection bias) | Unclear risk | It is unclear if allocation concealment was done |
| Blinding (performance bias and detection bias) All outcomes | Unclear risk | The author mentioned that in all the groups mothers were by their baby's side during the processes. Right after the procedure, the newborns were soothed in their mother's arms and following the procedure, the pain levels and durations of crying of the newborns were evaluated through recordings. However, it is unclear if intervention was masked. |
| Blinding of outcome assessment (detection bias) | Low risk | NIPS was used by an independent observer, blinded to the group allocations of the newborns, to evaluate pain. For all groups, the sum of crying duration and soothing duration of the newborns was assessed through video records by the nurse who was serving as an independent observer. |
| Incomplete outcome data (attrition bias) | Low risk | All infants were included in the final analysis |

**Yilmaz 2020 (Heel lance)** *(Continued)*

All outcomes

| | | |
|---|---|---|
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Zargham-Boroujeni 2017 (Venipuncture)**

***Study characteristics***

| | |
|---|---|
| Methods | A randomised controlled trial (RCT) **(Venipuncture)** |
| Participants | Inclusion criteria:<br>1. Conscious neonates<br>2. Aged > 34 weeks<br>3. Term and near-term<br>4. No limitation for breastfeeding<br>5. Being on mother's milk feeding<br>6. Having experience of being fed by mothers' breasts<br>7. No paralysis in limbs, or major congenital abnormalities such as Down syndrome or asphyxiation<br>8. Being relaxed<br>9. No cry before venipuncture<br>10. A need for venipuncture |
| Interventions | Randomly divided into 3 groups:<br>1. Breastfeeding group (first group) (n = 25; male 11, female 14): mother started breastfeeding until the researcher observed active sucking. This was continued for 3 minutes and then venipuncture was administrated<br>2. Massage group (second group) (n = 25; male 9, female 16): effleurage massage technique (stroking) was administrated on venipuncture site for 3 minutes and then venipuncture was administrated<br>3. Control group (third group) (n = 25, female 13, male 12): no intervention was administrated to manage pain and then venipuncture was done |
| Outcomes | Mean NIPS pain score |
| Notes | This study was conducted in Isfahan University of Medical Sciences, Iran in 2013<br>This research was funded by Isfahan University of Medical Sciences, project number 391294. There were no conflicts of interest declared. |

***Risk of bias***

| Bias | Authors' judgement | Support for judgement |
|---|---|---|
| Random sequence generation (selection bias) | Low risk | Neonates meeting the inclusion criteria were assigned to each group (total of 3 groups) through random computation |
| Allocation concealment (selection bias) | Unclear risk | It is unclear if allocation concealment was done |
| Blinding (performance bias and detection bias) | Low risk | The nurse responsible for venipuncture was unaware of the assignment of neonates to research groups. All stages of venipuncture were recorded by a |

**Zargham-Boroujeni 2017 (Venipuncture)** *(Continued)*

| | | |
|---|---|---|
| All outcomes | | Panasonic handy cam and the recorded films were observed and scored by a person who was blind to the assignment of neonates to the 3 different groups. |
| Blinding of outcome assessment (detection bias) | Low risk | To prevent bias and prejudice, a person who was unaware of the group assignments scored and the scoring was based on codes |
| Incomplete outcome data (attrition bias)<br>All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

**Zhu 2015 (Heel lance)**

| ***Study characteristics*** | |
|---|---|
| Methods | A randomised controlled trial (RCT) **(Heel lance)** |
| Participants | Inclusion criteria: born at ≥ 37 weeks gestation, APGAR scores ≥ 7 at 5 minutes after childbirth, age ≥ 24 hours, weight between 2000 g and 4000 g, passed the hearing screen, undergoing heel lancing for metabolic screening between 3 and 5 days after childbirth, breastfed and had not been fed for the previous 30 minutes, multiple births and operative deliveries |
| Interventions | Music therapy (MT) group (n = 62, mean GA in days 275, mean BW in grams 3280): classical music pieces were played on a loop at least 5 minutes before heel lance and maintained during blood sampling. The music speakers were placed bilaterally and kept 20 cm from the infants' heads.<br><br>Breastfeeding (BF) group (n = 64, mean GA in days 277, mean BW in grams 3290): neonates were breastfed in their mothers' arms, starting 5 minutes before the procedure and continuing throughout. Mothers were allowed to speak to their babies in the BF group.<br><br>BF + MT group (n = 63, mean GA in days 276, mean BW in grams 3180): neonates were breastfed, and classical music was played to them at the same time<br><br>Control group (n = 61, mean GA in days 273, mean BW in grams 3140): received routine care |
| Outcomes | 1. Neonatal Infant Pain Scale score<br>2. Latency to first cry in seconds<br>3. Duration of first cry in seconds |
| Notes | This study was conducted in the postpartum unit of a university-affiliated hospital in China from August 2013 to February 2014<br><br>No conflicts of interest or funding reported |

| ***Risk of bias*** | | |
|---|---|---|
| **Bias** | **Authors' judgement** | **Support for judgement** |
| Random sequence generation (selection bias) | Low risk | The main researcher used an online tool to randomly generate 72 sets of numbers, each set containing 4 numbers ranging from 1 to 4 with random order |
| Allocation concealment (selection bias) | Low risk | Once a total of 288 unique codes were generated based on the 72 sets of randomly ordered numbers (1, 2, 3, 4), they were placed in a box, where the ran- |

**Zhu 2015 (Heel lance)** *(Continued)*

| | | |
|---|---|---|
| | | domised blocking was not retained. After consent-taking, the main researcher randomly picked one code for each neonate to ensure the 288 neonates were equally allocated into 4 groups based on the group number of each code. |
| Blinding (performance bias and detection bias) All outcomes | Unclear risk | Two research assistants were trained to observe the video and recorded the outcome findings, but it is unclear if there was any masking of intervention |
| Blinding of outcome assessment (detection bias) | Unclear risk | It is unclear whether the outcome assessor was masked |
| Incomplete outcome data (attrition bias) All outcomes | Low risk | All infants were included in the final analysis |
| Selective reporting (reporting bias) | Low risk | All outcomes reported |
| Other bias | Unclear risk | Protocol not available for comparison |

ALPS-Neo score: Astrid Lindgren Children's Hospital Pain Scale; BCG: Bacillus Calmette–Guérin; BF: breastfeeding; BIIP: Behavioural Indicators of Infant Pain; BW: birth weight; CPAP: continuous positive airway pressure; CPAP: continuous positive airway pressure; DAN: Douleur Aigue Nouveau-né; DW: distilled water; EBM: expressed breast milk; GA: gestational age; GT: gentle touch; HR: heart rate; IM: intramuscular injection; KMCM: kangaroo mother care method; MT: music therapy; NFCS: Neonatal Facial Coding System; NICU: neonatal intensive care unit; NIPS: Neonatal Infant Pain Scale; NIRS: near infrared spectroscopy; NNS: non-nutritive sucking; N-PASS: Neonatal Pain, Agitation, and Sedation Scale; OGS: oral glucose solution; PI: perfusion index; PIBBS: Preterm Infant Breast-feeding Behaviour Scale; PIPP: Premature Infant Pain Profile; RA: research assistant; RCT: randomised controlled trial; ROP: retinopathy of prematurity; SD: standard deviation; SE: standard error; SNOSE: sequentially numbered, opaque, sealed envelopes; SSC: skin-to-skin contact; SW: sterile water; VC: verbal comfort; VLBW: very low birth weight; WHO: World Health Organization

## Characteristics of excluded studies *[ordered by study ID]*

| Study | Reason for exclusion |
|---|---|
| Bilgen 2001 | Article was retracted by the journal<br>We have not used any of the data from this report because the data were previously reported by Ors 1999 (Heel lance) |
| Cirik 2020 | Group used oro-gastric tube for feeding |
| Efe 2007a | Excluded because the age of patients studied was 2 to 4 months, not newborns |
| Erkul 2017 | Studied term infants up to 2 months of age |
| Hsieh 2018 | No randomisation |
| Iturriaga 2009 | Not a RCT |
| Osinaike 2007 | Not a RCT |
| Shukla 2018 | All groups received expressed breast milk as a baseline pain control intervention |
| Wu 2020 | Group used breast milk odour or taste; unclear about consistent use of breast milk for taste |

RCT: randomised controlled trial

**Characteristics of ongoing studies** *[ordered by study ID]*

**NCT00908401**

| | |
|---|---|
| Study name | Analgesic effect of breast milk for procedural pain in preterm infants (BMoS) |
| Methods | RCT |
| Participants | Preterm neonates between 27 and 29 + 6 weeks gestation |
| Interventions | Sucrose 0.2 mL or breast milk 0.2 mL |
| Outcomes | Pain evaluated by DAN score |
| Starting date | April 2009 |
| Contact information | Elodie Zana, MD - Centre Hospitalier Intercommunal Creteil |
| Notes | — |

**NCT01355640**

| | |
|---|---|
| Study name | 2 methods of analgesia for Chinese term infants receiving heel lance |
| Methods | RCT |
| Participants | Term infants |
| Interventions | Breastfeeding versus non-nutritive sucking versus no Intervention |
| Outcomes | Changes in heart rate value and oxygen saturation value, time length of grimacing and crying |
| Starting date | April 2008 |
| Contact information | Jingli Chen, Master - Peking Union Medical College |
| Notes | — |

DAN: Douleur Aigue Nouveau-né
GA: gestational age
RCT: randomised controlled trial

## DATA AND ANALYSES

### Comparison 1. Breastfeeding vs control

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1.1 Heart rate change (beats per minute) | 5 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1.1.1 Breastfeeding vs control | 2 | 120 | Mean Difference (IV, Random, 95% CI) | -12.89 [-19.93, -5.85] |
| 1.1.2 Breastfeeding vs pacifier | 1 | 61 | Mean Difference (IV, Random, 95% CI) | 2.10 [-4.78, 8.98] |
| 1.1.3 Breastfeeding vs swaddling | 1 | 30 | Mean Difference (IV, Random, 95% CI) | -23.00 [-34.55, -11.45] |
| 1.1.4 Breastfeeding vs held by research assistant | 1 | 54 | Mean Difference (IV, Random, 95% CI) | -7.10 [-15.50, 1.30] |
| 1.1.5 Breastfeeding vs held by mother | 2 | 125 | Mean Difference (IV, Random, 95% CI) | -14.45 [-20.95, -7.96] |
| 1.1.6 Breastfeeding vs glucose | 1 | 62 | Mean Difference (IV, Random, 95% CI) | -4.30 [-12.33, 3.73] |
| 1.1.7 Breastfeeding vs sucrose (moderate concentration: 20% to 33%) | 1 | 101 | Mean Difference (IV, Random, 95% CI) | -9.00 [-14.41, -3.59] |
| 1.1.8 Breastfeeding vs formula | 1 | 61 | Mean Difference (IV, Random, 95% CI) | -13.00 [-22.44, -3.56] |
| 1.2 Heart rate (beats per minute) | 8 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 1.2.1 Breastfeeding vs no intervention | 2 | 166 | Mean Difference (IV, Random, 95% CI) | -5.56 [-16.34, 5.22] |
| 1.2.2 Breastfeeding vs pacifier use (held by research assistant) | 1 | 57 | Mean Difference (IV, Random, 95% CI) | -2.00 [-4.47, 0.47] |
| 1.2.3 Breastfeeding vs positioning | 1 | 70 | Mean Difference (IV, Random, 95% CI) | 2.00 [-0.59, 4.59] |
| 1.2.4 Breastfeeding vs held by mother | 5 | 295 | Mean Difference (IV, Random, 95% CI) | -12.06 [-22.74, -1.37] |
| 1.2.5 Breastfeeding vs breast milk odour | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -7.00 [-15.11, 1.11] |
| 1.2.6 Breastfeeding vs mother's heart beats | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -7.00 [-14.85, 0.85] |
| 1.2.7 Breastfeeding with skin-to-skin contact vs skin-to-skin contact | 2 | 115 | Mean Difference (IV, Random, 95% CI) | -3.93 [-6.35, -1.51] |
| 1.2.8 Breastfeeding vs bottle-feeding mother's milk | 1 | 50 | Mean Difference (IV, Random, 95% CI) | -21.10 [-25.81, -16.39] |
| 1.2.9 Breastfeeding with skin-to-skin contact vs sucrose (moderate concentration: 20% to 33%) | 1 | 61 | Mean Difference (IV, Random, 95% CI) | -8.00 [-15.59, -0.41] |

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1.2.10 Breastfeeding with skin-to-skin contact vs sucrose (moderate concentration: 20% to 33%) with skin-to-skin contact | 1 | 64 | Mean Difference (IV, Random, 95% CI) | -11.00 [-19.48, -2.52] |
| 1.2.11 Breastfeeding vs formula feeding | 1 | 50 | Mean Difference (IV, Random, 95% CI) | 29.80 [27.02, 32.58] |
| 1.3 Oxygen saturation change | 3 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 1.3.1 Breastfeeding vs no intervention | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -0.04 [-1.32, 1.24] |
| 1.3.2 Breastfeeding vs pacifier use (neonate held by mother) | 1 | 64 | Mean Difference (IV, Random, 95% CI) | 0.30 [-2.79, 3.39] |
| 1.3.3 Breastfeeding vs pacifier use (neonate held by research assistant) | 1 | 53 | Mean Difference (IV, Random, 95% CI) | 0.60 [-1.48, 2.68] |
| 1.3.4 Breastfeeding vs sucrose (moderate concentration: 20% to 33%) | 1 | 101 | Mean Difference (IV, Random, 95% CI) | 2.00 [0.38, 3.62] |
| 1.4 Oxygen saturation | 4 | | Mean Difference (IV, Fixed, 95% CI) | Subtotals only |
| 1.4.1 Breastfeeding vs no intervention | 2 | 166 | Mean Difference (IV, Fixed, 95% CI) | 0.64 [0.21, 1.08] |
| 1.4.2 Breastfeeding vs positioning | 1 | 70 | Mean Difference (IV, Fixed, 95% CI) | 1.00 [0.53, 1.47] |
| 1.4.3 Breastfeeding vs held by mother | 2 | 120 | Mean Difference (IV, Fixed, 95% CI) | 0.25 [-0.19, 0.68] |
| 1.4.4 Breastfeeding vs breast milk odour | 1 | 60 | Mean Difference (IV, Fixed, 95% CI) | 0.00 [-1.20, 1.20] |
| 1.4.5 Breastfeeding vs mother's heart beats | 1 | 60 | Mean Difference (IV, Fixed, 95% CI) | -1.00 [-2.92, 0.92] |
| 1.4.6 Breastfeeding vs bottle-feeding mother's milk | 1 | 50 | Mean Difference (IV, Fixed, 95% CI) | -0.80 [-1.94, 0.34] |
| 1.4.7 Breastfeeding vs formula feeding | 1 | 50 | Mean Difference (IV, Fixed, 95% CI) | 0.10 [-1.08, 1.28] |
| 1.5 Blood pressure changes (mmHg) | 1 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 1.5.1 Breastfeeding vs pacifier use (neonate held by mother) | 1 | 62 | Mean Difference (IV, Random, 95% CI) | -3.60 [-9.08, 1.88] |
| 1.5.2 Breastfeeding vs pacifier use (neonate held by research assistant) | 1 | 48 | Mean Difference (IV, Random, 95% CI) | 1.60 [-4.86, 8.06] |

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1.6 Systolic blood pressure change (mmHg) | 1 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 1.6.1 Breastfeeding vs no intervention | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -5.48 [-12.28, 1.32] |
| 1.7 Diastolic blood pressure change (mmHg) | 1 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 1.7.1 Breastfeeding vs no intervention | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -0.92 [-5.58, 3.74] |
| 1.8 Latency to first cry | 1 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 1.8.1 Breastfeeding vs no intervention | 1 | 125 | Mean Difference (IV, Random, 95% CI) | 8.82 [7.98, 9.66] |
| 1.8.2 Breastfeeding vs music therapy | 1 | 126 | Mean Difference (IV, Random, 95% CI) | 8.62 [7.79, 9.45] |
| 1.9 Duration of first cry (seconds) | 1 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 1.9.1 Breastfeeding vs sucrose (moderate concentration: 20% to 33%) | 1 | 101 | Mean Difference (IV, Random, 95% CI) | -18.00 [-25.80, -10.20] |
| 1.10 Duration of crying (seconds) | 16 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 1.10.1 Breastfeeding vs no intervention | 10 | 790 | Mean Difference (IV, Random, 95% CI) | -36.23 [-55.57, -16.89] |
| 1.10.2 Breastfeeding and skin-to-skin contact vs lying on a table | 1 | 71 | Mean Difference (IV, Random, 95% CI) | -136.00 [-180.45, -91.55] |
| 1.10.3 Breastfeeding vs positioning | 3 | 180 | Mean Difference (IV, Random, 95% CI) | -31.46 [-69.39, 6.48] |
| 1.10.4 Breastfeeding vs rocking | 1 | 100 | Mean Difference (IV, Random, 95% CI) | -19.43 [-33.86, -5.00] |
| 1.10.5 Breastfeeding vs holding by mother | 4 | 260 | Mean Difference (IV, Random, 95% CI) | -16.50 [-29.68, -3.32] |
| 1.10.6 Breastfeeding and skin-to-skin contact vs skin-to-skin contact | 2 | 131 | Mean Difference (IV, Random, 95% CI) | -23.63 [-29.30, -17.95] |
| 1.10.7 Breastfeeding vs breast milk odour | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -7.50 [-18.57, 3.57] |
| 1.10.8 Breastfeeding vs mother's heart beats | 1 | 60 | Mean Difference (IV, Random, 95% CI) | 0.00 [-5.22, 5.22] |

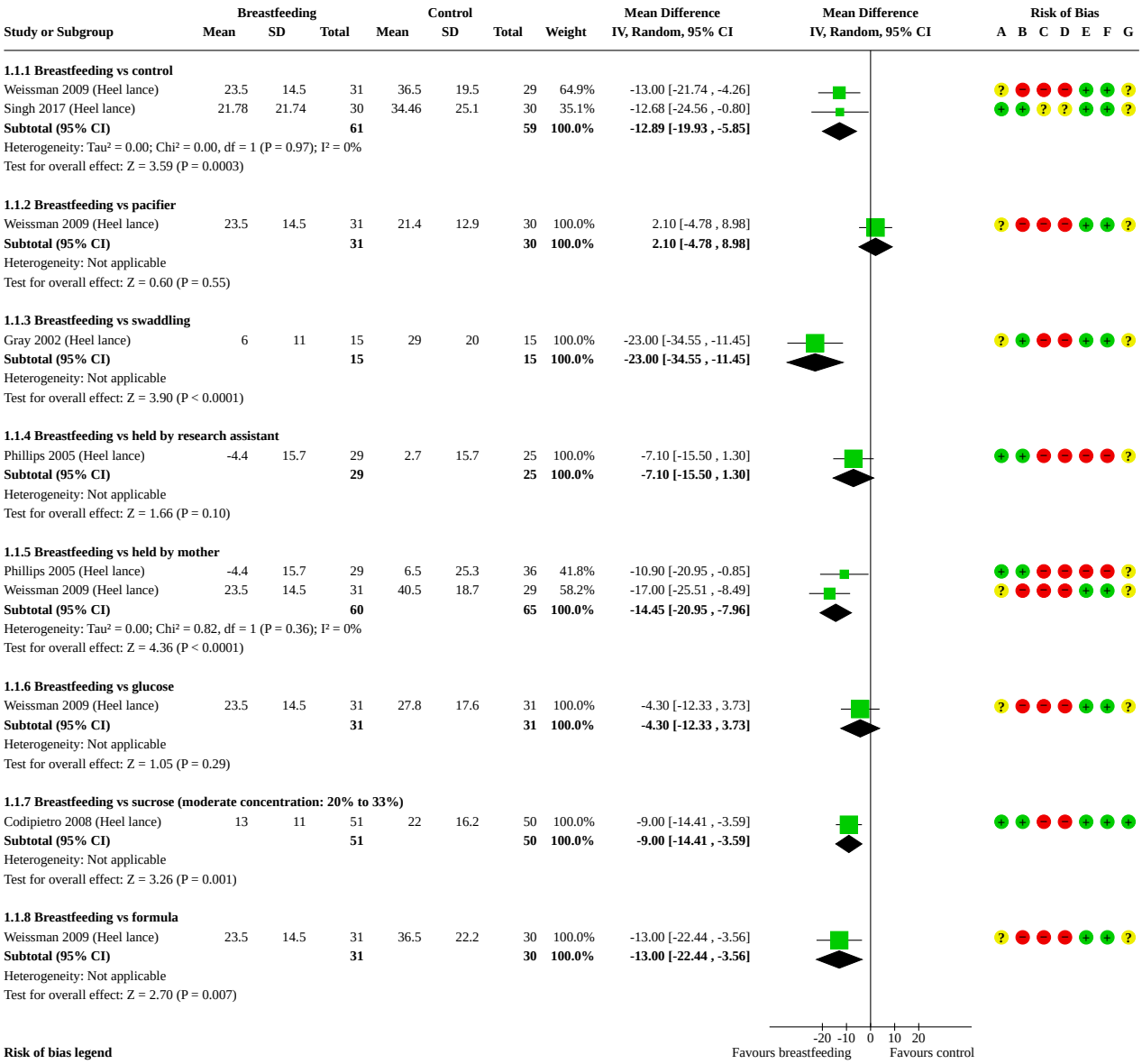
| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1.10.9 Breastfeeding vs heel warming | 1 | 100 | Mean Difference (IV, Random, 95% CI) | -18.76 [-31.32, -6.20] |
| 1.10.10 Breastfeeding vs non-nutritive sucking on pacifier | 2 | 161 | Mean Difference (IV, Random, 95% CI) | -12.22 [-25.78, 1.34] |
| 1.10.11 Breastfeeding vs water | 1 | 100 | Mean Difference (IV, Random, 95% CI) | -6.85 [-17.35, 3.65] |
| 1.10.12 Breastfeeding vs bottle feeding mother's milk | 1 | 50 | Mean Difference (IV, Random, 95% CI) | -6.80 [-9.08, -4.52] |
| 1.10.13 Breastfeeding vs glucose (moderate concentration: 20% to 33%) | 2 | 115 | Mean Difference (IV, Random, 95% CI) | -12.79 [-19.21, -6.37] |
| 1.10.14 Breastfeeding vs sucrose (moderate concentration: 20% to 33%) | 3 | 229 | Mean Difference (IV, Random, 95% CI) | 6.13 [-12.07, 24.32] |
| 1.10.15 Breastfeeding and skin-to-skin contact vs sucrose (moderate concentration: 20% to 33%) and skin-to-skin contact | 1 | 64 | Mean Difference (IV, Random, 95% CI) | -3.00 [-6.13, 0.13] |
| 1.10.16 Breastfeeding vs formula feeding | 2 | 111 | Mean Difference (IV, Random, 95% CI) | -6.31 [-34.83, 22.21] |
| 1.11 Percentage of time crying | 4 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 1.11.1 Breastfeeding vs positioning | 1 | 30 | Mean Difference (IV, Random, 95% CI) | -39.00 [-55.03, -22.97] |
| 1.11.2 Breastfeeding with skin-to-skin contact vs skin-to-skin contact | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -49.00 [-58.32, -39.68] |
| 1.11.3 Breastfeeding vs pacifier use (neonate held by mother) | 1 | 71 | Mean Difference (IV, Random, 95% CI) | -11.80 [-27.95, 4.35] |
| 1.11.4 Breastfeeding vs pacifier use (neonate held by research assistant) | 1 | 57 | Mean Difference (IV, Random, 95% CI) | -32.60 [-49.83, -15.37] |
| 1.11.5 Breastfeeding vs sucrose (moderate concentration: 20% to 33%) | 2 | 162 | Mean Difference (IV, Random, 95% CI) | -31.00 [-52.06, -9.94] |
| 1.11.6 Breastfeeding with skin-to-skin contact vs sucrose (moderate concentration: 20% to 33%) with skin-to-skin contact | 1 | 64 | Mean Difference (IV, Random, 95% CI) | -1.00 [-6.86, 4.86] |
| 1.12 Time to first calming (seconds) | 2 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1.12.1 Breastfeeding vs no intervention | 1 | 100 | Mean Difference (IV, Random, 95% CI) | -26.82 [-39.97, -13.67] |
| 1.12.2 Breastfeeding, swaddling and holding vs no intervention | 1 | 80 | Mean Difference (IV, Random, 95% CI) | -34.02 [-49.42, -18.62] |
| 1.12.3 Breastfeeding, swaddling and holding vs swaddling | 1 | 80 | Mean Difference (IV, Random, 95% CI) | -29.75 [-43.63, -15.87] |
| 1.12.4 Breastfeeding, swaddling and holding vs swaddling and holding | 1 | 80 | Mean Difference (IV, Random, 95% CI) | -39.50 [-57.00, -22.00] |
| 1.12.5 Breastfeeding vs heel warming | 1 | 100 | Mean Difference (IV, Random, 95% CI) | -9.30 [-19.63, 1.03] |
| 1.13 Neonatal Infant Pain Scale (NIPS) | 12 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 1.13.1 Breastfeeding vs no intervention | 5 | 459 | Mean Difference (IV, Random, 95% CI) | -2.53 [-3.46, -1.60] |
| 1.13.2 Breastfeeding vs positioning | 2 | 150 | Mean Difference (IV, Random, 95% CI) | -0.59 [-2.70, 1.51] |
| 1.13.3 Breastfeeding vs heel warming | 1 | 100 | Mean Difference (IV, Random, 95% CI) | -1.66 [-2.11, -1.21] |
| 1.13.4 Breastfeeding vs held by mother | 3 | 230 | Mean Difference (IV, Random, 95% CI) | -0.81 [-1.57, -0.05] |
| 1.13.5 Breastfeeding vs non-nutritive sucking | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -1.10 [-2.39, 0.19] |
| 1.13.6 Breastfeeding vs music therapy | 1 | 126 | Mean Difference (IV, Random, 95% CI) | -2.98 [-3.44, -2.52] |
| 1.13.7 Breastfeeding vs EMLA cream | 1 | 83 | Mean Difference (IV, Random, 95% CI) | -1.85 [-2.75, -0.95] |
| 1.13.8 Breastfeeding vs glucose (moderate concentration: 20% to 33%) | 4 | 272 | Mean Difference (IV, Random, 95% CI) | -1.17 [-1.75, -0.60] |
| 1.13.9 Breastfeeding vs sucrose (moderate concentration: 20% to 33%) | 2 | 129 | Mean Difference (IV, Random, 95% CI) | -1.21 [-4.74, 2.31] |
| 1.13.10 Breastfeeding and skin-to-skin contact vs sucrose (moderate concentration: 20% to 33%) and skin-to-skin contact | 1 | 64 | Mean Difference (IV, Random, 95% CI) | -1.00 [-1.32, -0.68] |
| 1.13.11 Breastfeeding, swaddling and holding vs no intervention | 1 | 80 | Mean Difference (IV, Random, 95% CI) | -1.93 [-2.40, -1.46] |

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1.13.12 Breastfeeding, swaddling and holding vs swaddling | 1 | 80 | Mean Difference (IV, Random, 95% CI) | -1.38 [-1.84, -0.92] |
| 1.13.13 Breastfeeding, swaddling and holding vs swaddling and holding | 1 | 80 | Mean Difference (IV, Random, 95% CI) | -1.10 [-1.63, -0.57] |
| 1.14 Premature Infant Pain Profile Score | 5 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 1.14.1 Breastfeeding vs no intervention | 1 | 29 | Mean Difference (IV, Random, 95% CI) | -0.49 [-2.39, 1.41] |
| 1.14.2 Breastfeeding vs placebo | 1 | 89 | Mean Difference (IV, Random, 95% CI) | -5.95 [-7.42, -4.48] |
| 1.14.3 Breastfeeding vs positioning | 1 | 89 | Mean Difference (IV, Random, 95% CI) | -7.49 [-8.95, -6.03] |
| 1.14.4 Breastfeeding vs held by mother | 1 | 128 | Mean Difference (IV, Random, 95% CI) | -3.68 [-4.50, -2.86] |
| 1.14.5 Breastfeeding vs glucose (moderate concentration: 20% to 33%) | 2 | 127 | Mean Difference (IV, Random, 95% CI) | 1.30 [0.05, 2.56] |
| 1.14.6 Breastfeeding vs sucrose (moderate concentration: 20% to 33%) | 2 | 147 | Mean Difference (IV, Random, 95% CI) | -1.95 [-8.88, 4.98] |
| 1.14.7 Breastfeeding vs formula feeding | 1 | 46 | Mean Difference (IV, Random, 95% CI) | 1.93 [1.43, 2.43] |
| 1.15 Neonatal Facial Coding System (NFCS) | 2 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 1.15.1 Breastfeeding vs no intervention | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -4.20 [-5.14, -3.26] |
| 1.15.2 Breastfeeding vs holding by mother | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -1.90 [-3.12, -0.68] |
| 1.15.3 Breastfeeding and skin-to-skin contact vs skin-to-skin contact | 1 | 55 | Mean Difference (IV, Random, 95% CI) | -0.92 [-2.96, 1.12] |
| 1.15.4 Breastfeeding vs non-nutritive sucking with pacifier | 1 | 61 | Mean Difference (IV, Random, 95% CI) | -2.00 [-3.15, -0.85] |
| 1.15.5 Breastfeeding vs glucose (moderate concentration: 20% to 33%) | 1 | 62 | Mean Difference (IV, Random, 95% CI) | -3.90 [-4.80, -3.00] |
| 1.15.6 Breastfeeding vs formula feeding | 1 | 61 | Mean Difference (IV, Random, 95% CI) | 0.60 [-0.63, 1.83] |

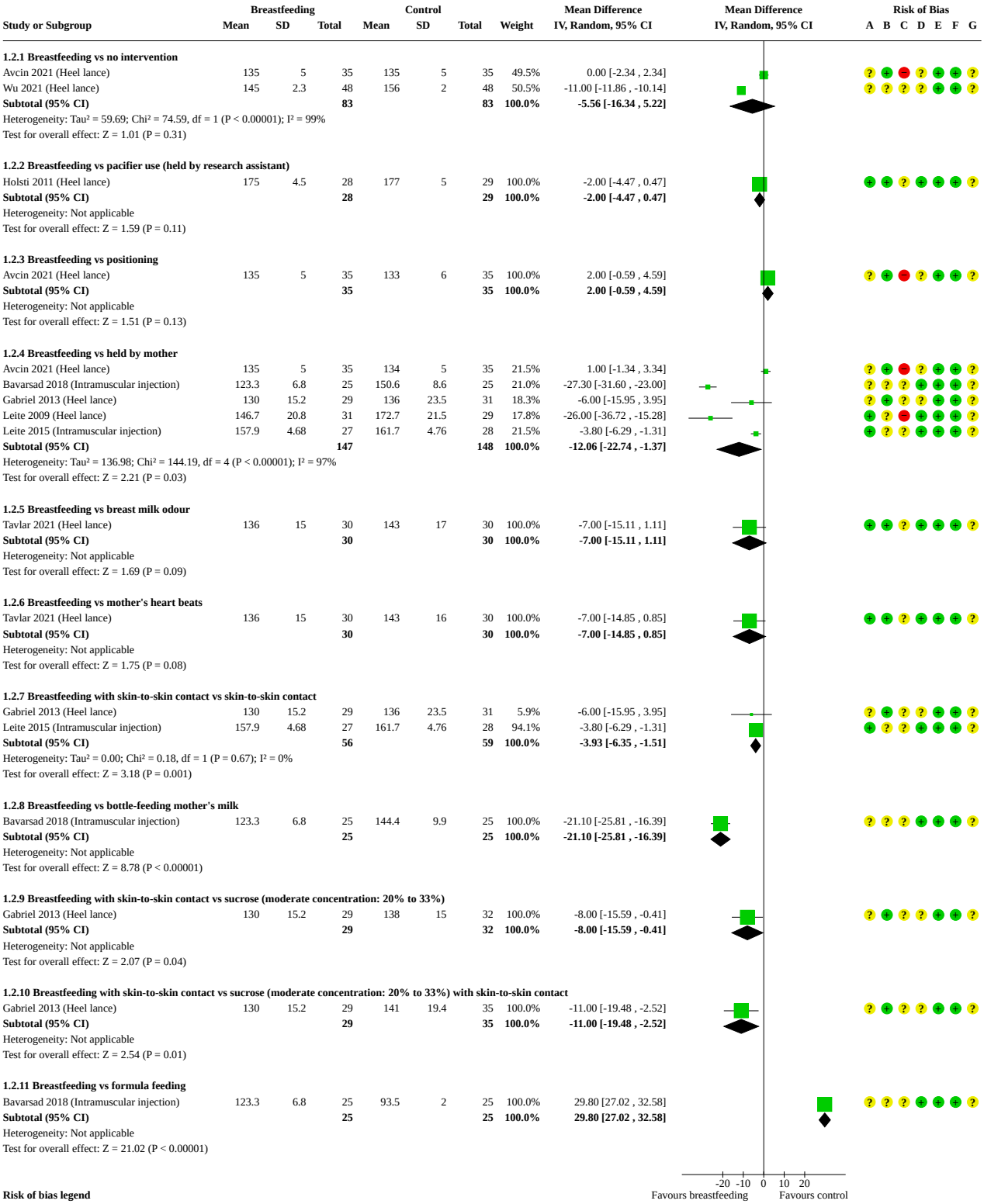
| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 1.16 Douleur Aigue Nouveau-né (DAN) Scale | 4 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 1.16.1 Breast feeding vs no intervention | 2 | 250 | Mean Difference (IV, Random, 95% CI) | -1.87 [-4.61, 0.86] |
| 1.16.2 Breastfeeding vs placebo | 1 | 89 | Mean Difference (IV, Random, 95% CI) | -6.24 [-7.38, -5.10] |
| 1.16.3 Breastfeeding vs positioning | 1 | 89 | Mean Difference (IV, Random, 95% CI) | -6.77 [-7.78, -5.76] |
| 1.16.4 Breastfeeding vs rocking | 1 | 100 | Mean Difference (IV, Random, 95% CI) | -0.04 [-0.80, 0.72] |
| 1.16.5 Breastfeeding vs pacifier use (held by research assistant) | 1 | 100 | Mean Difference (IV, Random, 95% CI) | -0.10 [-0.74, 0.54] |
| 1.16.6 Breastfeeding vs held by mother | 1 | 130 | Mean Difference (IV, Random, 95% CI) | -3.26 [-3.79, -2.73] |
| 1.16.7 Breastfeeding vs water | 1 | 100 | Mean Difference (IV, Random, 95% CI) | -0.46 [-1.20, 0.28] |
| 1.16.8 Breastfeeding vs glucose (moderate concentration: 20% to 33%) | 1 | 89 | Mean Difference (IV, Random, 95% CI) | -0.75 [-1.97, 0.47] |
| 1.16.9 Breastfeeding vs sucrose (moderate concentration: 20% to 33%) | 1 | 100 | Mean Difference (IV, Random, 95% CI) | 1.44 [0.74, 2.14] |
| 1.17 COMFORTneo scale | 1 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 1.17.1 Breastfeeding vs sucrose (moderate concentration: 20% to 33%) | 1 | 47 | Mean Difference (IV, Random, 95% CI) | 3.20 [2.71, 3.69] |
| 1.17.2 Breastfeeding vs formula feeding | 1 | 46 | Mean Difference (IV, Random, 95% CI) | 2.70 [2.21, 3.19] |
| 1.18 Composite score | 1 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 1.19 ALPS-Neo scale | 1 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 1.19.1 Breastfeeding vs breast milk odour | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -3.83 [-5.12, -2.54] |
| 1.19.2 Breastfeeding vs mother's heart beats | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -1.60 [-2.66, -0.54] |

## Analysis 1.1. Comparison 1: Breastfeeding vs control, Outcome 1: Heart rate change (beats per minute)




**Risk of bias legend**
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

## Analysis 1.2. Comparison 1: Breastfeeding vs control, Outcome 2: Heart rate (beats per minute)



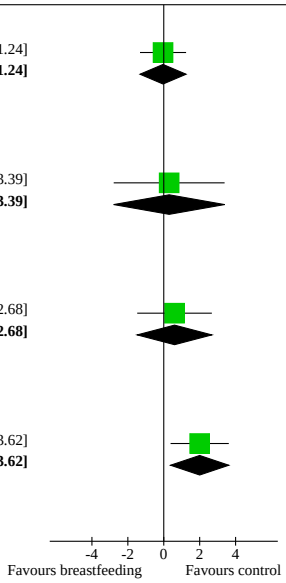

**Risk of bias legend**
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)

## Analysis 1.2. (Continued)

(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

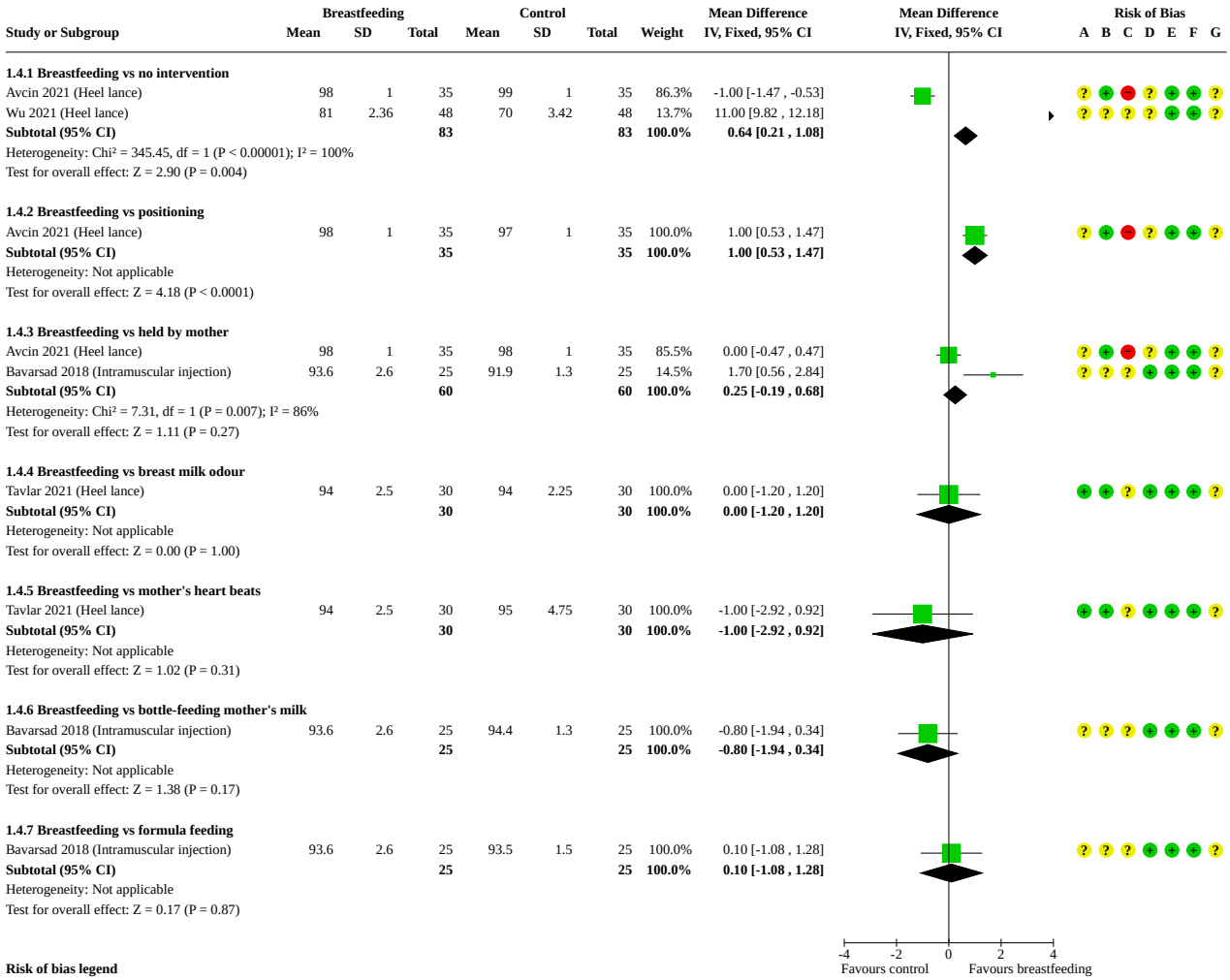
## Analysis 1.3. Comparison 1: Breastfeeding vs control, Outcome 3: Oxygen saturation change

| Study or Subgroup | Breastfeeding Mean | Breastfeeding SD | Breastfeeding Total | Control Mean | Control SD | Control Total | Weight | Mean Difference IV, Random, 95% CI | Risk of Bias A B C D E F G |
|---|---|---|---|---|---|---|---|---|---|
| **1.3.1 Breastfeeding vs no intervention** | | | | | | | | | |
| Singh 2017 (Heel lance) | 1.96 | 2.51 | 30 | 2 | 2.56 | 30 | 100.0% | -0.04 [-1.32 , 1.24] | + + ? ? + + ? |
| **Subtotal (95% CI)** | | | **30** | | | **30** | **100.0%** | **-0.04 [-1.32 , 1.24]** | |
| Heterogeneity: Not applicable | | | | | | | | | |
| Test for overall effect: Z = 0.06 (P = 0.95) | | | | | | | | | |
| **1.3.2 Breastfeeding vs pacifier use (neonate held by mother)** | | | | | | | | | |
| Phillips 2005 (Heel lance) | -1.7 | 5.6 | 28 | -2 | 7 | 36 | 100.0% | 0.30 [-2.79 , 3.39] | + + - - - - ? |
| **Subtotal (95% CI)** | | | **28** | | | **36** | **100.0%** | **0.30 [-2.79 , 3.39]** | |
| Heterogeneity: Not applicable | | | | | | | | | |
| Test for overall effect: Z = 0.19 (P = 0.85) | | | | | | | | | |
| **1.3.3 Breastfeeding vs pacifier use (neonate held by research assistant)** | | | | | | | | | |
| Phillips 2005 (Heel lance) | -1.7 | 5.6 | 28 | -2.3 | 0.3 | 25 | 100.0% | 0.60 [-1.48 , 2.68] | + + - - - - ? |
| **Subtotal (95% CI)** | | | **28** | | | **25** | **100.0%** | **0.60 [-1.48 , 2.68]** | |
| Heterogeneity: Not applicable | | | | | | | | | |
| Test for overall effect: Z = 0.57 (P = 0.57) | | | | | | | | | |
| **1.3.4 Breastfeeding vs sucrose (moderate concentration: 20% to 33%)** | | | | | | | | | |
| Codipietro 2008 (Heel lance) | -1 | 2.7 | 51 | -3 | 5.2 | 50 | 100.0% | 2.00 [0.38 , 3.62] | + + - - + + + |
| **Subtotal (95% CI)** | | | **51** | | | **50** | **100.0%** | **2.00 [0.38 , 3.62]** | |
| Heterogeneity: Not applicable | | | | | | | | | |
| Test for overall effect: Z = 2.42 (P = 0.02) | | | | | | | | | |




**Risk of bias legend**
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

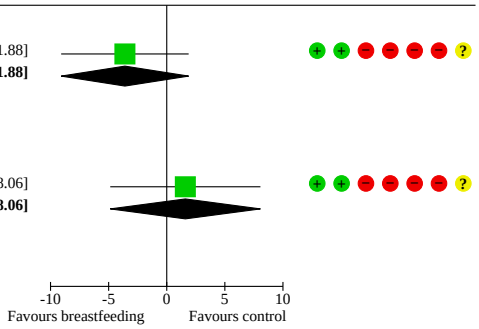
## Analysis 1.4. Comparison 1: Breastfeeding vs control, Outcome 4: Oxygen saturation




**Risk of bias legend**
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

## Analysis 1.5. Comparison 1: Breastfeeding vs control, Outcome 5: Blood pressure changes (mmHg)

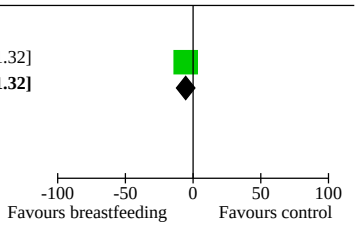
| Study or Subgroup | Breastfeeding Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| **1.5.1 Breastfeeding vs pacifier use (neonate held by mother)** | | | | | | | | |
| Phillips 2005 (Heel lance) | -5.3 | 11.2 | 25 | -1.7 | 10.2 | 37 | 100.0% | -3.60 [-9.08 , 1.88] |
| **Subtotal (95% CI)** | | | **25** | | | **37** | **100.0%** | **-3.60 [-9.08 , 1.88]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 1.29 (P = 0.20) | | | | | | | | |
| **1.5.2 Breastfeeding vs pacifier use (neonate held by research assistant)** | | | | | | | | |
| Phillips 2005 (Heel lance) | -5.3 | 11.2 | 25 | -6.9 | 11.6 | 23 | 100.0% | 1.60 [-4.86 , 8.06] |
| **Subtotal (95% CI)** | | | **25** | | | **23** | **100.0%** | **1.60 [-4.86 , 8.06]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 0.49 (P = 0.63) | | | | | | | | |




**Risk of bias legend**
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

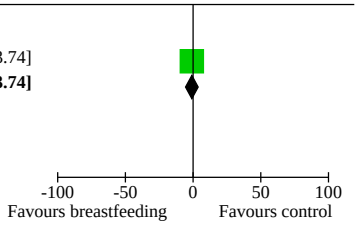
## Analysis 1.6. Comparison 1: Breastfeeding vs control, Outcome 6: Systolic blood pressure change (mmHg)

| Study or Subgroup | Breastfeeding Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| **1.6.1 Breastfeeding vs no intervention** | | | | | | | | |
| Singh 2017 (Heel lance) | 9.42 | 12.22 | 30 | 14.9 | 14.56 | 30 | 100.0% | -5.48 [-12.28 , 1.32] |
| **Subtotal (95% CI)** | | | **30** | | | **30** | **100.0%** | **-5.48 [-12.28 , 1.32]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 1.58 (P = 0.11) | | | | | | | | |



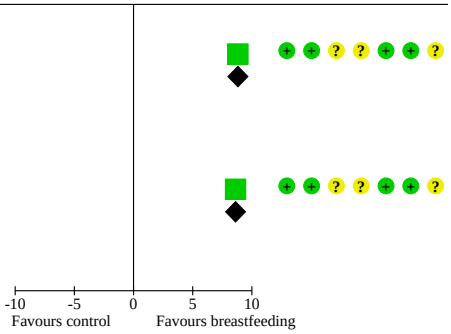

## Analysis 1.7. Comparison 1: Breastfeeding vs control, Outcome 7: Diastolic blood pressure change (mmHg)

| Study or Subgroup | Breastfeeding Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| **1.7.1 Breastfeeding vs no intervention** | | | | | | | | |
| Singh 2017 (Heel lance) | 0.94 | 8.9 | 30 | 1.86 | 9.5 | 30 | 100.0% | -0.92 [-5.58 , 3.74] |
| **Subtotal (95% CI)** | | | **30** | | | **30** | **100.0%** | **-0.92 [-5.58 , 3.74]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 0.39 (P = 0.70) | | | | | | | | |



## Analysis 1.8. Comparison 1: Breastfeeding vs control, Outcome 8: Latency to first cry

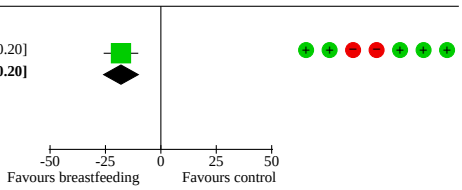
| Study or Subgroup | Breastfeeding Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| **1.8.1 Breastfeeding vs no intervention** | | | | | | | | |
| Zhu 2015 (Heel lance) | 13.65 | 2.34 | 64 | 4.83 | 2.45 | 61 | 100.0% | 8.82 [7.98 , 9.66] |
| **Subtotal (95% CI)** | | | **64** | | | **61** | **100.0%** | **8.82 [7.98 , 9.66]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 20.56 (P < 0.00001) | | | | | | | | |
| **1.8.2 Breastfeeding vs music therapy** | | | | | | | | |
| Zhu 2015 (Heel lance) | 13.65 | 2.34 | 64 | 5.03 | 2.41 | 62 | 100.0% | 8.62 [7.79 , 9.45] |
| **Subtotal (95% CI)** | | | **64** | | | **62** | **100.0%** | **8.62 [7.79 , 9.45]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 20.36 (P < 0.00001) | | | | | | | | |




**Risk of bias legend**
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

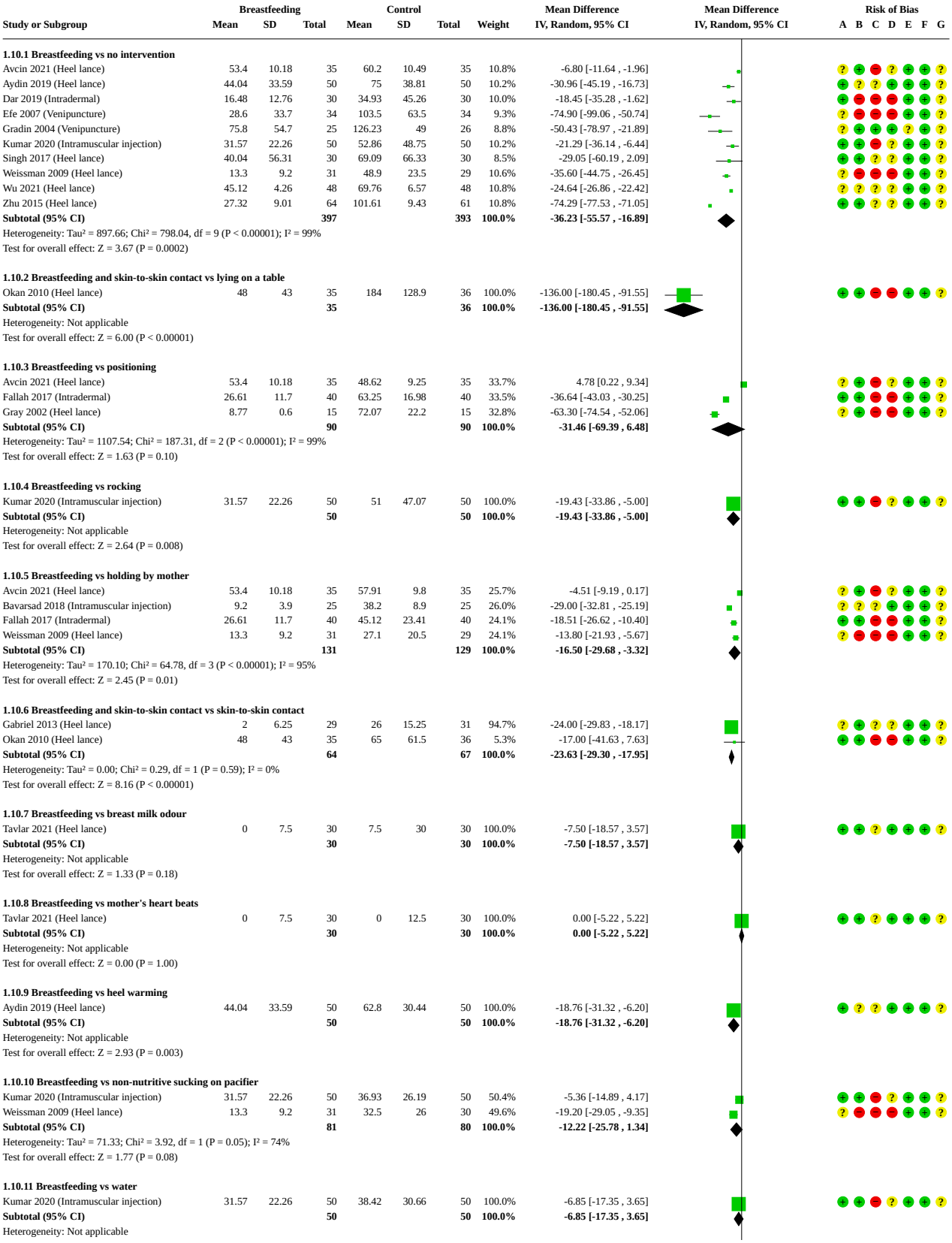
## Analysis 1.9. Comparison 1: Breastfeeding vs control, Outcome 9: Duration of first cry (seconds)

| Study or Subgroup | Breastfeeding Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| **1.9.1 Breastfeeding vs sucrose (moderate concentration: 20% to 33%)** | | | | | | | | |
| Codipietro 2008 (Heel lance) | 3 | 20 | 51 | 21 | 20 | 50 | 100.0% | -18.00 [-25.80 , -10.20] |
| **Subtotal (95% CI)** | | | **51** | | | **50** | **100.0%** | **-18.00 [-25.80 , -10.20]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 4.52 (P < 0.00001) | | | | | | | | |



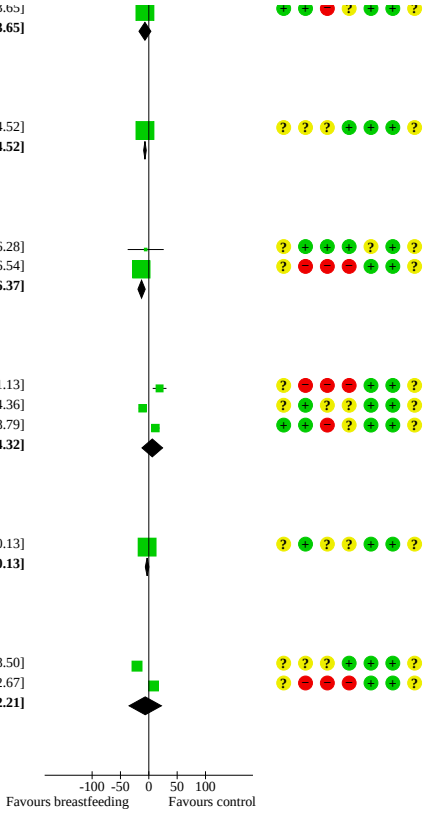

**Risk of bias legend**
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

## Analysis 1.10. Comparison 1: Breastfeeding vs control, Outcome 10: Duration of crying (seconds)



## Analysis 1.10. (Continued)

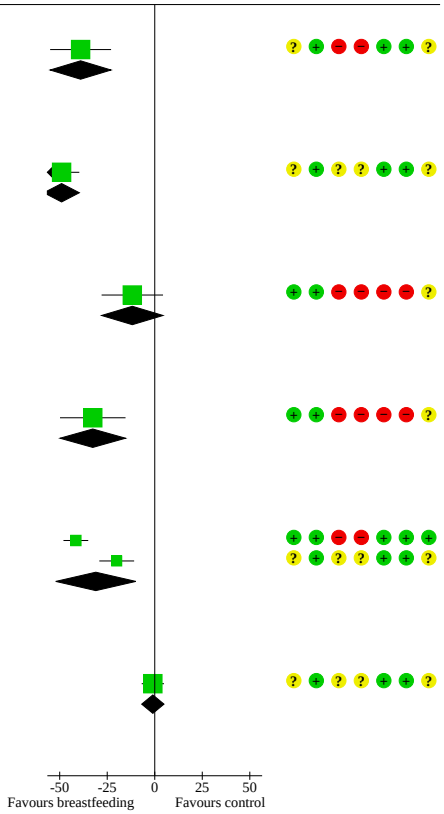
| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Kumar 2020 (Intramuscular injection) | 31.57 | 22.26 | 50 | 38.42 | 30.66 | 50 | 100.0% | -6.85 [-17.35 , 3.65] | |
| **Subtotal (95% CI)** | | | **50** | | | **50** | **100.0%** | **-6.85 [-17.35 , 3.65]** | |
| Heterogeneity: Not applicable | | | | | | | | | |
| Test for overall effect: Z = 1.28 (P = 0.20) | | | | | | | | | |
| **1.10.12 Breastfeeding vs bottle feeding mother's milk** | | | | | | | | | |
| Bavarsad 2018 (Intramuscular injection) | 9.2 | 3.9 | 25 | 16 | 4.3 | 25 | 100.0% | -6.80 [-9.08 , -4.52] | |
| **Subtotal (95% CI)** | | | **25** | | | **25** | **100.0%** | **-6.80 [-9.08 , -4.52]** | |
| Heterogeneity: Not applicable | | | | | | | | | |
| Test for overall effect: Z = 5.86 (P < 0.00001) | | | | | | | | | |
| **1.10.13 Breastfeeding vs glucose (moderate concentration: 20% to 33%)** | | | | | | | | | |
| Gradin 2004 (Venipuncture) | 75.8 | 54.7 | 25 | 81.29 | 63.29 | 28 | 4.1% | -5.49 [-37.26 , 26.28] | |
| Weissman 2009 (Heel lance) | 13.3 | 9.2 | 31 | 26.4 | 16.2 | 31 | 95.9% | -13.10 [-19.66 , -6.54] | |
| **Subtotal (95% CI)** | | | **56** | | | **59** | **100.0%** | **-12.79 [-19.21 , -6.37]** | |
| Heterogeneity: Tau² = 0.00; Chi² = 0.21, df = 1 (P = 0.65); I² = 0% | | | | | | | | | |
| Test for overall effect: Z = 3.90 (P < 0.0001) | | | | | | | | | |
| **1.10.14 Breastfeeding vs sucrose (moderate concentration: 20% to 33%)** | | | | | | | | | |
| Efe 2007 (Venipuncture) | 28.6 | 33.7 | 34 | 9.6 | 12.9 | 34 | 31.2% | 19.00 [6.87 , 31.13] | |
| Gabriel 2013 (Heel lance) | 2 | 6.25 | 29 | 13 | 18 | 32 | 34.5% | -11.00 [-17.64 , -4.36] | |
| Kumar 2020 (Intramuscular injection) | 31.57 | 22.26 | 50 | 19.9 | 12.84 | 50 | 34.3% | 11.67 [4.55 , 18.79] | |
| **Subtotal (95% CI)** | | | **113** | | | **116** | **100.0%** | **6.13 [-12.07 , 24.32]** | |
| Heterogeneity: Tau² = 238.12; Chi² = 29.35, df = 2 (P < 0.00001); I² = 93% | | | | | | | | | |
| Test for overall effect: Z = 0.66 (P = 0.51) | | | | | | | | | |
| **1.10.15 Breastfeeding and skin-to-skin contact vs sucrose (moderate concentration: 20% to 33%) and skin-to-skin contact** | | | | | | | | | |
| Gabriel 2013 (Heel lance) | 2 | 6.25 | 29 | 5 | 6.5 | 35 | 100.0% | -3.00 [-6.13 , 0.13] | |
| **Subtotal (95% CI)** | | | **29** | | | **35** | **100.0%** | **-3.00 [-6.13 , 0.13]** | |
| Heterogeneity: Not applicable | | | | | | | | | |
| Test for overall effect: Z = 1.88 (P = 0.06) | | | | | | | | | |
| **1.10.16 Breastfeeding vs formula feeding** | | | | | | | | | |
| Bavarsad 2018 (Intramuscular injection) | 9.2 | 3.9 | 25 | 30 | 4.4 | 25 | 50.2% | -20.80 [-23.10 , -18.50] | |
| Weissman 2009 (Heel lance) | 13.3 | 9.2 | 31 | 5 | 8.2 | 30 | 49.8% | 8.30 [3.93 , 12.67] | |
| **Subtotal (95% CI)** | | | **56** | | | **55** | **100.0%** | **-6.31 [-34.83 , 22.21]** | |
| Heterogeneity: Tau² = 420.23; Chi² = 133.26, df = 1 (P < 0.00001); I² = 99% | | | | | | | | | |
| Test for overall effect: Z = 0.43 (P = 0.66) | | | | | | | | | |




**Risk of bias legend**
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

## Analysis 1.11. Comparison 1: Breastfeeding vs control, Outcome 11: Percentage of time crying

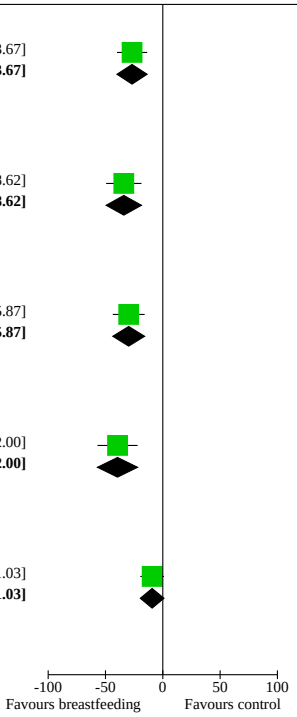
| Study or Subgroup | Breastfeeding Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| **1.11.1 Breastfeeding vs positioning** | | | | | | | | |
| Gray 2002 (Heel lance) | 4 | 7.23 | 15 | 43 | 30.83 | 15 | 100.0% | -39.00 [-55.03 , -22.97] |
| **Subtotal (95% CI)** | | | **15** | | | **15** | **100.0%** | **-39.00 [-55.03 , -22.97]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 4.77 (P < 0.00001) | | | | | | | | |
| **1.11.2 Breastfeeding with skin-to-skin contact vs skin-to-skin contact** | | | | | | | | |
| Gabriel 2013 (Heel lance) | 3 | 13.12 | 29 | 52 | 22.75 | 31 | 100.0% | -49.00 [-58.32 , -39.68] |
| **Subtotal (95% CI)** | | | **29** | | | **31** | **100.0%** | **-49.00 [-58.32 , -39.68]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 10.30 (P < 0.00001) | | | | | | | | |
| **1.11.3 Breastfeeding vs pacifier use (neonate held by mother)** | | | | | | | | |
| Phillips 2005 (Heel lance) | 33 | 32.6 | 32 | 44.8 | 36.8 | 39 | 100.0% | -11.80 [-27.95 , 4.35] |
| **Subtotal (95% CI)** | | | **32** | | | **39** | **100.0%** | **-11.80 [-27.95 , 4.35]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 1.43 (P = 0.15) | | | | | | | | |
| **1.11.4 Breastfeeding vs pacifier use (neonate held by research assistant)** | | | | | | | | |
| Phillips 2005 (Heel lance) | 33 | 32.6 | 32 | 65.6 | 33.2 | 25 | 100.0% | -32.60 [-49.83 , -15.37] |
| **Subtotal (95% CI)** | | | **32** | | | **25** | **100.0%** | **-32.60 [-49.83 , -15.37]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 3.71 (P = 0.0002) | | | | | | | | |
| **1.11.5 Breastfeeding vs sucrose (moderate concentration: 20% to 33%)** | | | | | | | | |
| Codipietro 2008 (Heel lance) | 4 | 16.7 | 51 | 45.5 | 16.7 | 50 | 51.2% | -41.50 [-48.01 , -34.99] |
| Gabriel 2013 (Heel lance) | 3 | 13.2 | 29 | 23 | 22.5 | 32 | 48.8% | -20.00 [-29.16 , -10.84] |
| **Subtotal (95% CI)** | | | **80** | | | **82** | **100.0%** | **-31.00 [-52.06 , -9.94]** |
| Heterogeneity: $Tau^2 = 214.69$; $Chi^2 = 14.06$, df = 1 (P = 0.0002); $I^2 = 93\%$ | | | | | | | | |
| Test for overall effect: Z = 2.88 (P = 0.004) | | | | | | | | |
| **1.11.6 Breastfeeding with skin-to-skin contact vs sucrose (moderate concentration: 20% to 33%) with skin-to-skin contact** | | | | | | | | |
| Gabriel 2013 (Heel lance) | 3 | 13.12 | 29 | 4 | 10.25 | 35 | 100.0% | -1.00 [-6.86 , 4.86] |
| **Subtotal (95% CI)** | | | **29** | | | **35** | **100.0%** | **-1.00 [-6.86 , 4.86]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 0.33 (P = 0.74) | | | | | | | | |




**Risk of bias legend**
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

## Analysis 1.12. Comparison 1: Breastfeeding vs control, Outcome 12: Time to first calming (seconds)

| Study or Subgroup | Breastfeeding Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI | Risk of Bias A B C D E F G |
|---|---|---|---|---|---|---|---|---|---|
| **1.12.1 Breastfeeding vs no intervention** | | | | | | | | | |
| Aydin 2019 (Heel lance) | 26.7 | 24.66 | 50 | 53.52 | 40.55 | 50 | 100.0% | -26.82 [-39.97 , -13.67] | + ? ? + + + ? |
| **Subtotal (95% CI)** | | | **50** | | | **50** | **100.0%** | **-26.82 [-39.97 , -13.67]** | |
| Heterogeneity: Not applicable | | | | | | | | | |
| Test for overall effect: Z = 4.00 (P < 0.0001) | | | | | | | | | |
| **1.12.2 Breastfeeding, swaddling and holding vs no intervention** | | | | | | | | | |
| Yilmaz 2020 (Heel lance) | 26.25 | 25.75 | 40 | 60.27 | 42.52 | 40 | 100.0% | -34.02 [-49.42 , -18.62] | + ? ? + + + ? |
| **Subtotal (95% CI)** | | | **40** | | | **40** | **100.0%** | **-34.02 [-49.42 , -18.62]** | |
| Heterogeneity: Not applicable | | | | | | | | | |
| Test for overall effect: Z = 4.33 (P < 0.0001) | | | | | | | | | |
| **1.12.3 Breastfeeding, swaddling and holding vs swaddling** | | | | | | | | | |
| Yilmaz 2020 (Heel lance) | 26.25 | 25.75 | 40 | 56 | 36.65 | 40 | 100.0% | -29.75 [-43.63 , -15.87] | + ? ? + + + ? |
| **Subtotal (95% CI)** | | | **40** | | | **40** | **100.0%** | **-29.75 [-43.63 , -15.87]** | |
| Heterogeneity: Not applicable | | | | | | | | | |
| Test for overall effect: Z = 4.20 (P < 0.0001) | | | | | | | | | |
| **1.12.4 Breastfeeding, swaddling and holding vs swaddling and holding** | | | | | | | | | |
| Yilmaz 2020 (Heel lance) | 26.25 | 25.75 | 40 | 65.75 | 50.25 | 40 | 100.0% | -39.50 [-57.00 , -22.00] | + ? ? + + + ? |
| **Subtotal (95% CI)** | | | **40** | | | **40** | **100.0%** | **-39.50 [-57.00 , -22.00]** | |
| Heterogeneity: Not applicable | | | | | | | | | |
| Test for overall effect: Z = 4.42 (P < 0.00001) | | | | | | | | | |
| **1.12.5 Breastfeeding vs heel warming** | | | | | | | | | |
| Aydin 2019 (Heel lance) | 26.7 | 24.66 | 50 | 36 | 27.92 | 50 | 100.0% | -9.30 [-19.63 , 1.03] | + ? ? + + + ? |
| **Subtotal (95% CI)** | | | **50** | | | **50** | **100.0%** | **-9.30 [-19.63 , 1.03]** | |
| Heterogeneity: Not applicable | | | | | | | | | |
| Test for overall effect: Z = 1.77 (P = 0.08) | | | | | | | | | |




**Risk of bias legend**
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

## Analysis 1.13. Comparison 1: Breastfeeding vs control, Outcome 13: Neonatal Infant Pain Scale (NIPS)

| Study or Subgroup | Breastfeeding Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| **1.13.1 Breastfeeding vs no intervention** | | | | | | | | |
| Avcin 2021 (Heel lance) | 2.88 | 0.95 | 35 | 3.45 | 0.85 | 35 | 20.5% | -0.57 [-0.99 , -0.15] |
| Aydin 2019 (Heel lance) | 4.44 | 1.21 | 50 | 6.42 | 0.91 | 50 | 20.5% | -1.98 [-2.40 , -1.56] |
| Efe 2007 (Venipuncture) | 0.9 | 1.9 | 34 | 5.6 | 2.2 | 34 | 17.3% | -4.70 [-5.68 , -3.72] |
| Wu 2021 (Heel lance) | 2.13 | 0.25 | 48 | 4.52 | 0.58 | 48 | 21.3% | -2.39 [-2.57 , -2.21] |
| Zhu 2015 (Heel lance) | 3.08 | 1.88 | 64 | 6.43 | 0.23 | 61 | 20.3% | -3.35 [-3.81 , -2.89] |
| **Subtotal (95% CI)** | | | **231** | | | **228** | **100.0%** | **-2.53 [-3.46 , -1.60]** |
| Heterogeneity: Tau² = 1.06; Chi² = 110.37, df = 4 (P < 0.00001); I² = 96% | | | | | | | | |
| Test for overall effect: Z = 5.31 (P < 0.00001) | | | | | | | | |
| **1.13.2 Breastfeeding vs positioning** | | | | | | | | |
| Avcin 2021 (Heel lance) | 2.88 | 0.95 | 35 | 2.4 | 0.84 | 35 | 50.1% | 0.48 [0.06 , 0.90] |
| Fallah 2017 (Intradermal) | 1.81 | 1.16 | 40 | 3.48 | 0.96 | 40 | 49.9% | -1.67 [-2.14 , -1.20] |
| **Subtotal (95% CI)** | | | **75** | | | **75** | **100.0%** | **-0.59 [-2.70 , 1.51]** |
| Heterogeneity: Tau² = 2.26; Chi² = 45.04, df = 1 (P < 0.00001); I² = 98% | | | | | | | | |
| Test for overall effect: Z = 0.55 (P = 0.58) | | | | | | | | |
| **1.13.3 Breastfeeding vs heel warming** | | | | | | | | |
| Aydin 2019 (Heel lance) | 4.44 | 1.21 | 50 | 6.1 | 1.07 | 50 | 100.0% | -1.66 [-2.11 , -1.21] |
| **Subtotal (95% CI)** | | | **50** | | | **50** | **100.0%** | **-1.66 [-2.11 , -1.21]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 7.27 (P < 0.00001) | | | | | | | | |
| **1.13.4 Breastfeeding vs held by mother** | | | | | | | | |
| Avcin 2021 (Heel lance) | 2.88 | 0.95 | 35 | 3.03 | 1.05 | 35 | 37.6% | -0.15 [-0.62 , 0.32] |
| Fallah 2017 (Intradermal) | 1.81 | 1.16 | 40 | 2.92 | 1.34 | 40 | 35.7% | -1.11 [-1.66 , -0.56] |
| Soltani 2018 (Heel lance) | 5.52 | 2.22 | 42 | 6.84 | 1.96 | 38 | 26.8% | -1.32 [-2.24 , -0.40] |
| **Subtotal (95% CI)** | | | **117** | | | **113** | **100.0%** | **-0.81 [-1.57 , -0.05]** |
| Heterogeneity: Tau² = 0.34; Chi² = 9.11, df = 2 (P = 0.01); I² = 78% | | | | | | | | |
| Test for overall effect: Z = 2.08 (P = 0.04) | | | | | | | | |
| **1.13.5 Breastfeeding vs non-nutritive sucking** | | | | | | | | |
| Napiorkowska-Orkisz 2022 (Heel lance) | 2.6 | 2.5 | 30 | 3.7 | 2.6 | 30 | 100.0% | -1.10 [-2.39 , 0.19] |
| **Subtotal (95% CI)** | | | **30** | | | **30** | **100.0%** | **-1.10 [-2.39 , 0.19]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 1.67 (P = 0.09) | | | | | | | | |
| **1.13.6 Breastfeeding vs music therapy** | | | | | | | | |
| Zhu 2015 (Heel lance) | 3.08 | 1.88 | 64 | 6.06 | 0.22 | 62 | 100.0% | -2.98 [-3.44 , -2.52] |
| **Subtotal (95% CI)** | | | **64** | | | **62** | **100.0%** | **-2.98 [-3.44 , -2.52]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 12.59 (P < 0.00001) | | | | | | | | |
| **1.13.7 Breastfeeding vs EMLA cream** | | | | | | | | |
| Soltani 2018 (Heel lance) | 5.52 | 2.22 | 42 | 7.37 | 1.95 | 41 | 100.0% | -1.85 [-2.75 , -0.95] |
| **Subtotal (95% CI)** | | | **42** | | | **41** | **100.0%** | **-1.85 [-2.75 , -0.95]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 4.04 (P < 0.0001) | | | | | | | | |
| **1.13.8 Breastfeeding vs glucose (moderate concentration: 20% to 33%)** | | | | | | | | |
| Bembich 2013 (Heel lance) | 3 | 1.15 | 15 | 5.1 | 1.01 | 15 | 23.9% | -2.10 [-2.87 , -1.33] |
| Chiabi 2016 (Heel lance) | 2 | 0.5 | 50 | 3 | 0.5 | 50 | 40.2% | -1.00 [-1.20 , -0.80] |
| Napiorkowska-Orkisz 2022 (Heel lance) | 2.6 | 2.5 | 30 | 3.1 | 2.2 | 30 | 15.0% | -0.50 [-1.69 , 0.69] |
| Soltani 2018 (Heel lance) | 5.52 | 2.22 | 42 | 6.45 | 1.88 | 40 | 21.0% | -0.93 [-1.82 , -0.04] |
| **Subtotal (95% CI)** | | | **137** | | | **135** | **100.0%** | **-1.17 [-1.75 , -0.60]** |
| Heterogeneity: Tau² = 0.20; Chi² = 8.20, df = 3 (P = 0.04); I² = 63% | | | | | | | | |
| Test for overall effect: Z = 4.01 (P < 0.0001) | | | | | | | | |
| **1.13.9 Breastfeeding vs sucrose (moderate concentration: 20% to 33%)** | | | | | | | | |
| Efe 2007 (Venipuncture) | 0.9 | 1.9 | 34 | 0.3 | 0.9 | 34 | 49.6% | 0.60 [-0.11 , 1.31] |
| Gabriel 2013 (Heel lance) | 1 | 0.75 | 29 | 4 | 0.75 | 32 | 50.4% | -3.00 [-3.38 , -2.62] |
| **Subtotal (95% CI)** | | | **63** | | | **66** | **100.0%** | **-1.21 [-4.74 , 2.31]** |
| Heterogeneity: Tau² = 6.40; Chi² = 77.62, df = 1 (P < 0.00001); I² = 99% | | | | | | | | |
| Test for overall effect: Z = 0.67 (P = 0.50) | | | | | | | | |
| **1.13.10 Breastfeeding and skin-to-skin contact vs sucrose (moderate concentration: 20% to 33%) and skin-to-skin contact** | | | | | | | | |
| Gabriel 2013 (Heel lance) | 1 | 0.75 | 29 | 2 | 0.5 | 35 | 100.0% | -1.00 [-1.32 , -0.68] |
| **Subtotal (95% CI)** | | | **29** | | | **35** | **100.0%** | **-1.00 [-1.32 , -0.68]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 6.14 (P < 0.00001) | | | | | | | | |
| **1.13.11 Breastfeeding, swaddling and holding vs no intervention** | | | | | | | | |
| Yilmaz 2020 (Heel lance) | 4.47 | 1.19 | 40 | 6.4 | 0.95 | 40 | 100.0% | -1.93 [-2.40 , -1.46] |
| **Subtotal (95% CI)** | | | **40** | | | **40** | **100.0%** | **-1.93 [-2.40 , -1.46]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 8.02 (P < 0.00001) | | | | | | | | |
| **1.13.12 Breastfeeding, swaddling and holding vs swaddling** | | | | | | | | |

Mean Difference IV, Random, 95% CI

Risk of Bias A B C D E F G

## Analysis 1.13. (Continued)

Test for overall effect: Z = 8.02 (P < 0.00001)

**1.13.12 Breastfeeding, swaddling and holding vs swaddling**

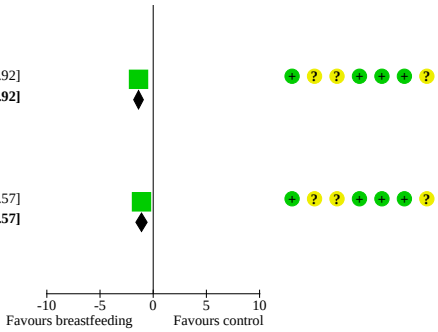
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Yilmaz 2020 (Heel lance) | 4.47 | 1.19 | 40 | 5.85 | 0.86 | 40 | 100.0% | -1.38 [-1.84 , -0.92] |
| **Subtotal (95% CI)** | | | **40** | | | **40** | **100.0%** | **-1.38 [-1.84 , -0.92]** |

Heterogeneity: Not applicable
Test for overall effect: Z = 5.94 (P < 0.00001)

**1.13.13 Breastfeeding, swaddling and holding vs swaddling and holding**

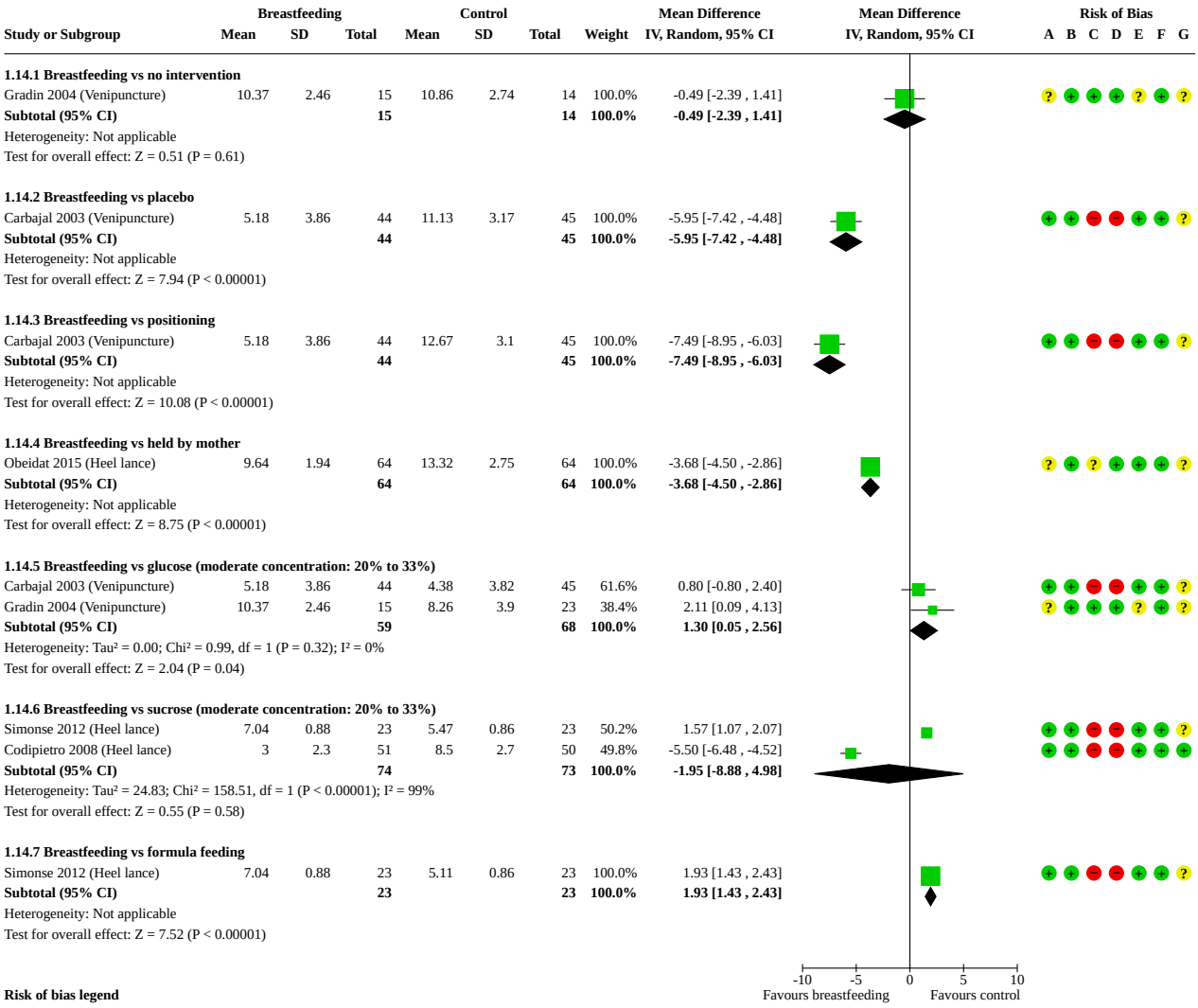
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Yilmaz 2020 (Heel lance) | 4.47 | 1.19 | 40 | 5.57 | 1.23 | 40 | 100.0% | -1.10 [-1.63 , -0.57] |
| **Subtotal (95% CI)** | | | **40** | | | **40** | **100.0%** | **-1.10 [-1.63 , -0.57]** |

Heterogeneity: Not applicable
Test for overall effect: Z = 4.07 (P < 0.0001)




**Risk of bias legend**
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

## Analysis 1.14. Comparison 1: Breastfeeding vs control, Outcome 14: Premature Infant Pain Profile Score

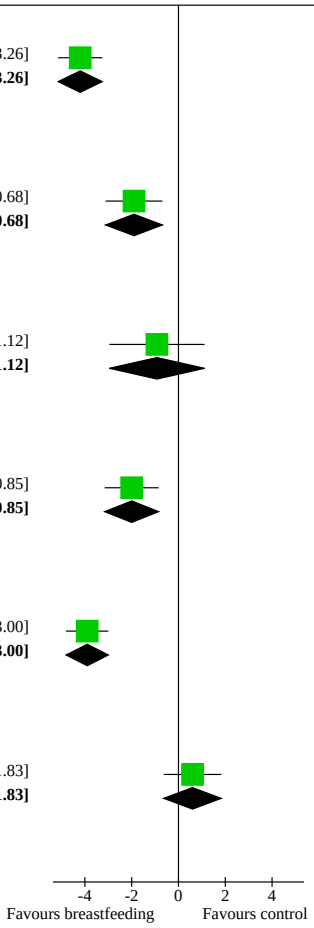



**Risk of bias legend**

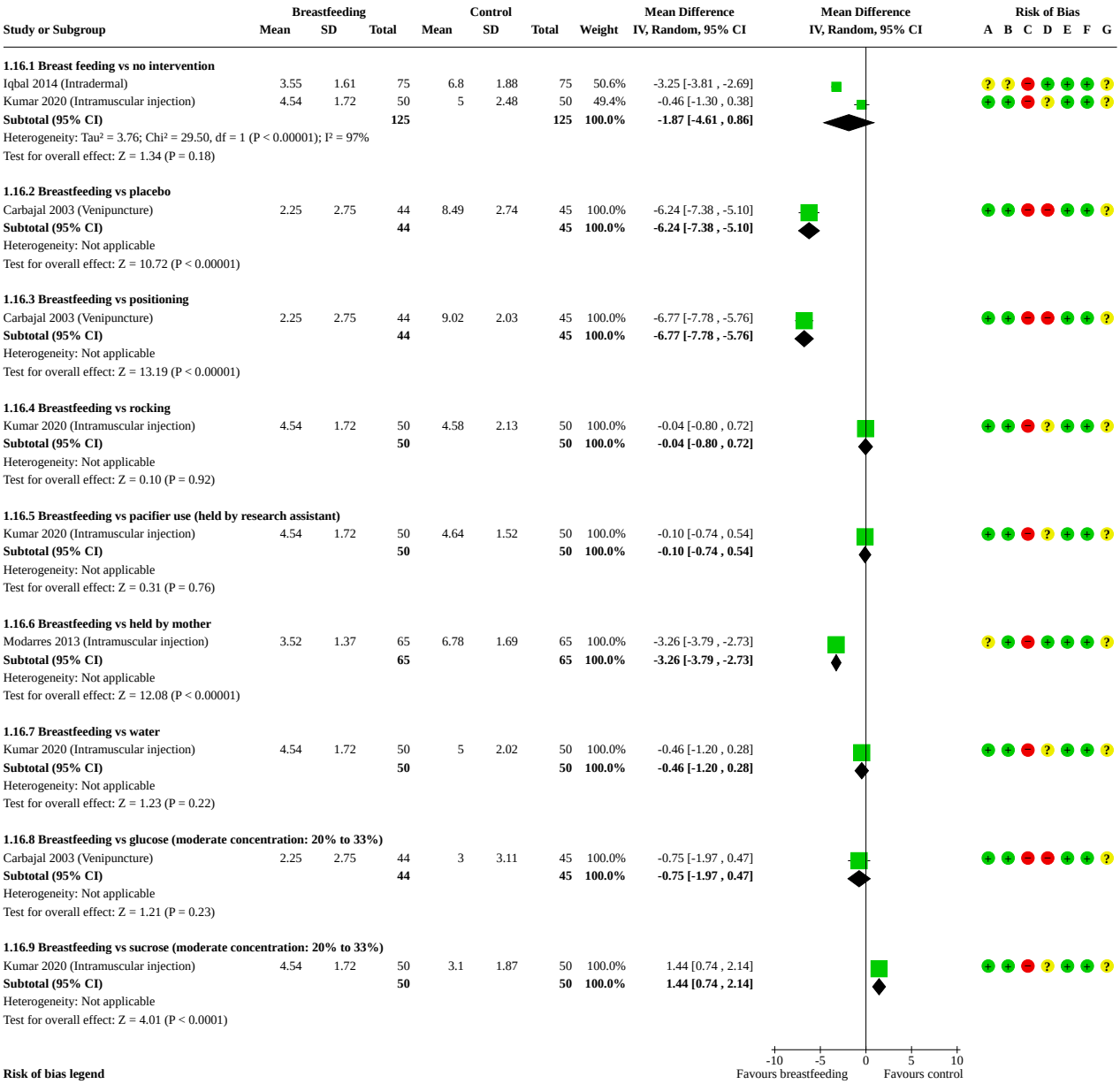
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

## Analysis 1.15. Comparison 1: Breastfeeding vs control, Outcome 15: Neonatal Facial Coding System (NFCS)

| Study or Subgroup | Breastfeeding Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| **1.15.1 Breastfeeding vs no intervention** | | | | | | | | |
| Weissman 2009 (Heel lance) | 2.9 | 2.4 | 31 | 7.1 | 1.15 | 29 | 100.0% | -4.20 [-5.14 , -3.26] |
| **Subtotal (95% CI)** | | | **31** | | | **29** | **100.0%** | **-4.20 [-5.14 , -3.26]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 8.73 (P < 0.00001) | | | | | | | | |
| **1.15.2 Breastfeeding vs holding by mother** | | | | | | | | |
| Weissman 2009 (Heel lance) | 2.9 | 2.4 | 31 | 4.8 | 2.4 | 29 | 100.0% | -1.90 [-3.12 , -0.68] |
| **Subtotal (95% CI)** | | | **31** | | | **29** | **100.0%** | **-1.90 [-3.12 , -0.68]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 3.06 (P = 0.002) | | | | | | | | |
| **1.15.3 Breastfeeding and skin-to-skin contact vs skin-to-skin contact** | | | | | | | | |
| Leite 2015 (Intramuscular injection) | 18.44 | 3.75 | 27 | 19.36 | 3.95 | 28 | 100.0% | -0.92 [-2.96 , 1.12] |
| **Subtotal (95% CI)** | | | **27** | | | **28** | **100.0%** | **-0.92 [-2.96 , 1.12]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 0.89 (P = 0.38) | | | | | | | | |
| **1.15.4 Breastfeeding vs non-nutritive sucking with pacifier** | | | | | | | | |
| Weissman 2009 (Heel lance) | 2.9 | 2.4 | 31 | 4.9 | 2.2 | 30 | 100.0% | -2.00 [-3.15 , -0.85] |
| **Subtotal (95% CI)** | | | **31** | | | **30** | **100.0%** | **-2.00 [-3.15 , -0.85]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 3.39 (P = 0.0007) | | | | | | | | |
| **1.15.5 Breastfeeding vs glucose (moderate concentration: 20% to 33%)** | | | | | | | | |
| Weissman 2009 (Heel lance) | 2.9 | 2.4 | 31 | 6.8 | 0.9 | 31 | 100.0% | -3.90 [-4.80 , -3.00] |
| **Subtotal (95% CI)** | | | **31** | | | **31** | **100.0%** | **-3.90 [-4.80 , -3.00]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 8.47 (P < 0.00001) | | | | | | | | |
| **1.15.6 Breastfeeding vs formula feeding** | | | | | | | | |
| Weissman 2009 (Heel lance) | 2.9 | 2.4 | 31 | 2.3 | 2.5 | 30 | 100.0% | 0.60 [-0.63 , 1.83] |
| **Subtotal (95% CI)** | | | **31** | | | **30** | **100.0%** | **0.60 [-0.63 , 1.83]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 0.96 (P = 0.34) | | | | | | | | |



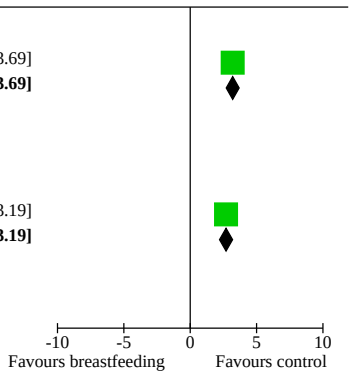
## Analysis 1.16. Comparison 1: Breastfeeding vs control, Outcome 16: Douleur Aigue Nouveau-né (DAN) Scale




**Risk of bias legend**
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

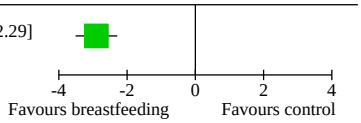
### Analysis 1.17. Comparison 1: Breastfeeding vs control, Outcome 17: COMFORTneo scale

| Study or Subgroup | Breastfeeding Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| **1.17.1 Breastfeeding vs sucrose (moderate concentration: 20% to 33%)** | | | | | | | | |
| Simonse 2012 (Heel lance) | 19 | 0.85 | 23 | 15.8 | 0.85 | 24 | 100.0% | 3.20 [2.71 , 3.69] |
| **Subtotal (95% CI)** | | | **23** | | | **24** | **100.0%** | **3.20 [2.71 , 3.69]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 12.90 (P < 0.00001) | | | | | | | | |
| **1.17.2 Breastfeeding vs formula feeding** | | | | | | | | |
| Simonse 2012 (Heel lance) | 19 | 0.85 | 23 | 16.3 | 0.85 | 23 | 100.0% | 2.70 [2.21 , 3.19] |
| **Subtotal (95% CI)** | | | **23** | | | **23** | **100.0%** | **2.70 [2.21 , 3.19]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 10.77 (P < 0.00001) | | | | | | | | |



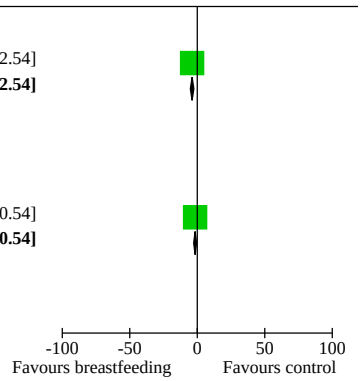

### Analysis 1.18. Comparison 1: Breastfeeding vs control, Outcome 18: Composite score

| Study or Subgroup | Breastfeeding Mean | SD | Total | Control Mean | SD | Total | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|
| Shendurnikar 2005 (Heel lance) | 5.38 | 1.77 | 50 | 8.28 | 1.31 | 50 | -2.90 [-3.51 , -2.29] |




### Analysis 1.19. Comparison 1: Breastfeeding vs control, Outcome 19: ALPS-Neo scale

| Study or Subgroup | Breastfeeding Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| **1.19.1 Breastfeeding vs breast milk odour** | | | | | | | | |
| Tavlar 2021 (Heel lance) | 0.3 | 1.05 | 30 | 4.13 | 3.44 | 30 | 100.0% | -3.83 [-5.12 , -2.54] |
| **Subtotal (95% CI)** | | | **30** | | | **30** | **100.0%** | **-3.83 [-5.12 , -2.54]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 5.83 (P < 0.00001) | | | | | | | | |
| **1.19.2 Breastfeeding vs mother's heart beats** | | | | | | | | |
| Tavlar 2021 (Heel lance) | 0.3 | 1.05 | 30 | 1.9 | 2.77 | 30 | 100.0% | -1.60 [-2.66 , -0.54] |
| **Subtotal (95% CI)** | | | **30** | | | **30** | **100.0%** | **-1.60 [-2.66 , -0.54]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 2.96 (P = 0.003) | | | | | | | | |




## Comparison 2. Supplemental breast milk vs control

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 2.1 Heart rate change (beats per minute) | 7 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 2.1.1 Supplemental breast milk vs no intervention | 2 | 122 | Mean Difference (IV, Random, 95% CI) | -4.81 [-6.28, -3.35] |
| 2.1.2 Supplemental breast milk vs placebo | 5 | 300 | Mean Difference (IV, Random, 95% CI) | -3.13 [-9.51, 3.26] |
| 2.1.3 Supplemental breast milk vs glycine | 1 | 40 | Mean Difference (IV, Random, 95% CI) | 4.00 [-2.82, 10.82] |
| 2.1.4 Supplemental breast milk vs artificial sweetener | 1 | 40 | Mean Difference (IV, Random, 95% CI) | 8.00 [-0.15, 16.15] |
| 2.1.5 Supplemental breast milk vs glucose (low concentration: < 20%) | 2 | 110 | Mean Difference (IV, Random, 95% CI) | 0.62 [-1.79, 3.03] |
| 2.1.6 Supplemental breast milk vs glucose (moderate concentration: 20% to 33%) | 3 | 172 | Mean Difference (IV, Random, 95% CI) | 3.91 [-6.05, 13.87] |
| 2.1.7 Supplemental breast milk vs glucose (high concentration: > 33%) | 1 | 50 | Mean Difference (IV, Random, 95% CI) | 10.10 [8.08, 12.12] |
| 2.1.8 Supplemental breast milk vs sucrose (moderate concentration: 20% to 33%) | 1 | 68 | Mean Difference (IV, Random, 95% CI) | 13.80 [4.23, 23.37] |
| 2.2 Heart rate (beats per minute) | 8 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 2.2.1 Supplemental breast milk vs no intervention | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -20.00 [-28.74, -11.26] |
| 2.2.2 Supplemental breast milk vs positioning | 1 | 145 | Mean Difference (IV, Random, 95% CI) | 1.00 [0.07, 1.94] |
| 2.2.3 Supplemental breast milk vs breast milk smell | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -1.00 [-9.19, 7.19] |
| 2.2.4 Supplemental breast milk vs breast milk taste + smell | 1 | 60 | Mean Difference (IV, Random, 95% CI) | 2.00 [-5.08, 9.08] |
| 2.2.5 Supplemental breast milk vs water | 4 | 276 | Mean Difference (IV, Random, 95% CI) | -7.16 [-13.91, -0.42] |
| 2.2.6 Supplemental breast milk vs glucose (moderate concentration: 20% to 33%) | 3 | 233 | Mean Difference (IV, Random, 95% CI) | 0.59 [-3.33, 4.51] |
| 2.2.7 Supplemental breast milk vs sucrose (moderate concentration: 20% to 33%) | 3 | 162 | Mean Difference (IV, Random, 95% CI) | -2.48 [-6.44, 1.48] |
| 2.2.8 Supplemental breast milk vs proparacaine eye drops | 1 | 34 | Mean Difference (IV, Random, 95% CI) | -3.10 [-16.08, 9.88] |

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 2.3 Oxygen saturation change | 3 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 2.3.1 Supplemental breast milk vs no intervention | 1 | 62 | Mean Difference (IV, Random, 95% CI) | -4.90 [-5.98, -3.82] |
| 2.3.2 Supplemental breast milk vs placebo | 2 | 131 | Mean Difference (IV, Random, 95% CI) | -0.92 [-2.47, 0.63] |
| 2.3.3 Supplemental breast milk vs glucose (low concentration: < 20%) | 1 | 50 | Mean Difference (IV, Random, 95% CI) | 0.20 [-0.88, 1.28] |
| 2.3.4 Supplemental breast milk vs glucose (moderate concentration: 20% to 33%) | 2 | 112 | Mean Difference (IV, Random, 95% CI) | -0.37 [-4.98, 4.24] |
| 2.3.5 Supplemental breast milk vs glucose (high concentration: > 33%) | 1 | 50 | Mean Difference (IV, Random, 95% CI) | 2.10 [1.22, 2.98] |
| 2.4 Oxygen saturations | 7 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 2.4.1 Supplemental breast milk vs no intervention | 1 | 60 | Mean Difference (IV, Random, 95% CI) | 6.00 [4.07, 7.93] |
| 2.4.2 Supplemental breast milk vs positioning | 1 | 155 | Mean Difference (IV, Random, 95% CI) | -0.81 [-1.26, -0.36] |
| 2.4.3 Supplemental breast milk vs breast milk smell | 1 | 60 | Mean Difference (IV, Random, 95% CI) | 2.00 [0.40, 3.60] |
| 2.4.4 Supplemental breast milk vs breast milk + smell | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -2.00 [-4.61, 0.61] |
| 2.4.5 Supplemental breast milk vs water | 4 | 276 | Mean Difference (IV, Random, 95% CI) | 0.82 [0.12, 1.52] |
| 2.4.6 Supplemental breast milk vs glucose (moderate concentration: 20% to 33%) | 3 | 233 | Mean Difference (IV, Random, 95% CI) | -0.31 [-2.76, 2.15] |
| 2.4.7 Supplemental breast milk vs sucrose (moderate concentration: 20% to 33%) | 2 | 74 | Mean Difference (IV, Random, 95% CI) | 2.01 [-0.98, 5.01] |
| 2.4.8 Supplemental breast milk vs proparacaine eye drops | 1 | 34 | Mean Difference (IV, Random, 95% CI) | -0.50 [-5.69, 4.69] |
| 2.5 Respiratory rate | 3 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 2.5.1 Supplemental breast milk vs no intervention | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -10.90 [-14.70, -7.10] |

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 2.5.2 Supplemental breast milk vs positioning | 1 | 145 | Mean Difference (IV, Random, 95% CI) | 12.09 [10.97, 13.21] |
| 2.5.3 Supplemental breast milk vs water | 1 | 84 | Mean Difference (IV, Random, 95% CI) | -3.20 [-8.93, 2.53] |
| 2.5.4 Supplemental breast milk vs EMLA cream | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -4.70 [-10.92, 1.52] |
| 2.5.5 Supplemental breast milk vs glucose (moderate concentration: 20% to 33%) | 1 | 79 | Mean Difference (IV, Random, 95% CI) | -1.50 [-6.24, 3.24] |
| 2.5.6 Supplemental breast milk vs sucrose (moderate concentration: 20% to 33%) | 1 | 60 | Mean Difference (IV, Random, 95% CI) | 0.50 [-4.61, 5.61] |
| 2.6 Systolic blood pressure | 1 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 2.6.1 Supplemental breast milk vs water | 1 | 84 | Mean Difference (IV, Random, 95% CI) | 1.10 [-2.93, 5.13] |
| 2.6.2 Supplemental breast milk vs glucose (moderate concentration: 20% to 33%) | 1 | 79 | Mean Difference (IV, Random, 95% CI) | 1.80 [-1.57, 5.17] |
| 2.7 Diastolic blood pressure | 1 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 2.7.1 Supplemental breast milk vs water | 1 | 84 | Mean Difference (IV, Random, 95% CI) | -0.50 [-5.18, 4.18] |
| 2.7.2 Supplemental breast milk vs glucose (moderate concentration: 20% to 33%) | 1 | 79 | Mean Difference (IV, Random, 95% CI) | 1.30 [-2.21, 4.81] |
| 2.8 Duration of first cry (seconds) | 2 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 2.8.1 Supplemental breast milk vs non-nutritive sucking (pacifier) | 1 | 38 | Mean Difference (IV, Random, 95% CI) | 4.00 [0.18, 7.82] |
| 2.8.2 Supplemental breast milk vs massage | 1 | 35 | Mean Difference (IV, Random, 95% CI) | -6.00 [-12.34, 0.34] |
| 2.8.3 Supplemental breast milk vs rocking | 1 | 35 | Mean Difference (IV, Random, 95% CI) | 3.00 [-1.52, 7.52] |
| 2.8.4 Supplemental breast milk vs water | 2 | 83 | Mean Difference (IV, Random, 95% CI) | -2.68 [-8.45, 3.10] |
| 2.8.5 Supplemental breast milk vs glucose (low concentration: < 20%) | 1 | 50 | Mean Difference (IV, Random, 95% CI) | 1.92 [-1.38, 5.22] |

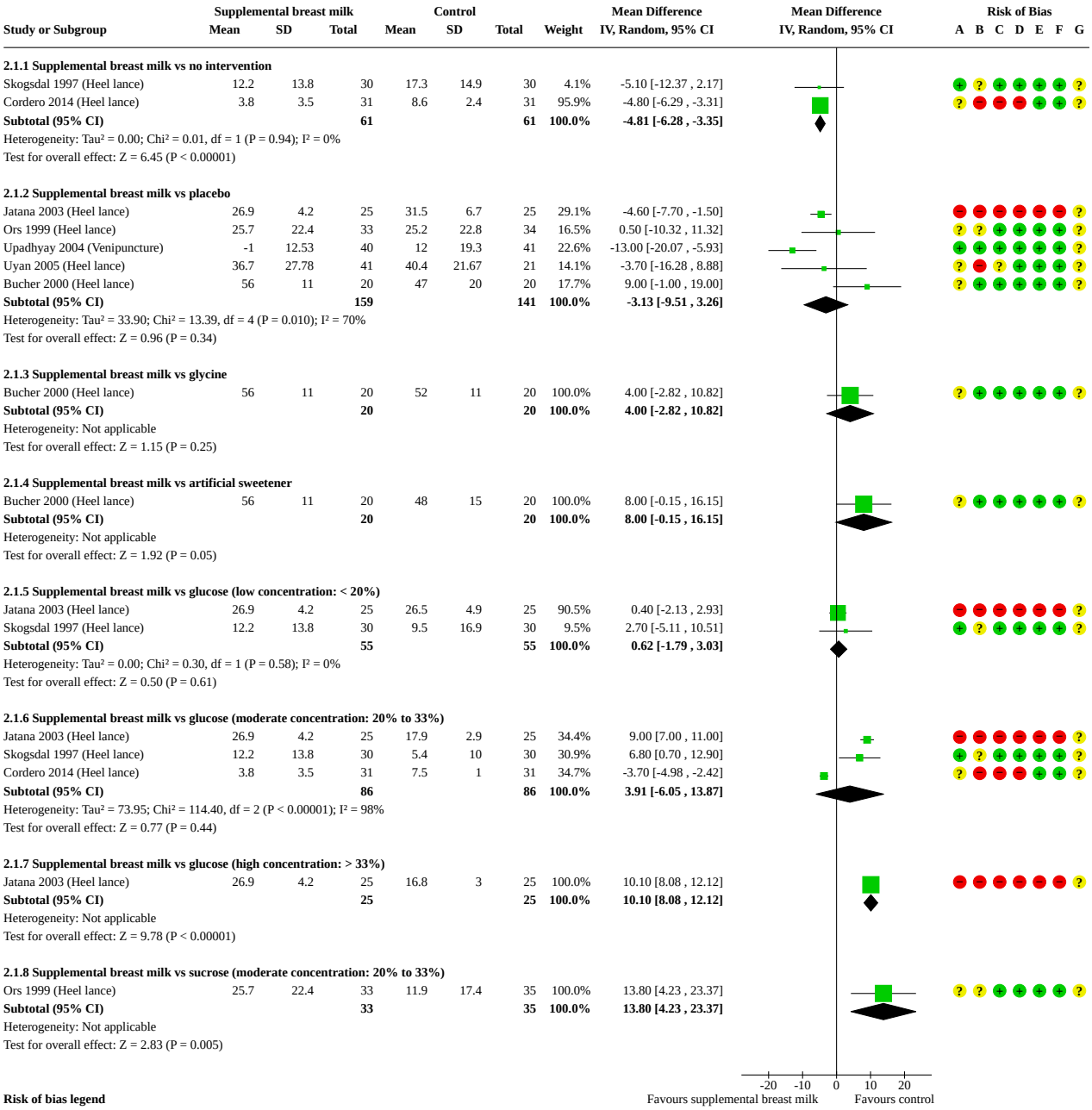
| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 2.8.6 Supplemental breast milk vs glucose (moderate concentration: 20% to 33%) | 1 | 50 | Mean Difference (IV, Random, 95% CI) | 12.78 [9.36, 16.20] |
| 2.8.7 Supplemental breast milk vs glucose (high concentration: > 33%) | 1 | 50 | Mean Difference (IV, Random, 95% CI) | 11.56 [8.54, 14.58] |
| 2.8.8 Supplemental breast milk vs sucrose (moderate concentration: 20% to 33%) | 1 | 35 | Mean Difference (IV, Random, 95% CI) | 6.00 [2.50, 9.50] |
| 2.9 Duration of crying (seconds) | 11 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 2.9.1 Supplemental breast milk vs no intervention | 1 | 60 | Mean Difference (IV, Random, 95% CI) | 36.70 [0.60, 72.80] |
| 2.9.2 Supplemental breast milk vs placebo | 7 | 357 | Mean Difference (IV, Random, 95% CI) | -8.67 [-12.32, -5.02] |
| 2.9.3 Supplemental breast milk vs positioning | 1 | 145 | Mean Difference (IV, Random, 95% CI) | -7.43 [-8.55, -6.31] |
| 2.9.4 Supplemental breast milk vs rocking | 1 | 35 | Mean Difference (IV, Random, 95% CI) | 31.00 [24.47, 37.53] |
| 2.9.5 Supplemental breast milk vs massage | 1 | 35 | Mean Difference (IV, Random, 95% CI) | -9.00 [-16.97, -1.03] |
| 2.9.6 Supplemental breast milk vs non-nutritive sucking (pacifier) | 2 | 98 | Mean Difference (IV, Random, 95% CI) | 44.23 [38.47, 49.98] |
| 2.9.7 Supplemental breast milk vs water | 3 | 238 | Mean Difference (IV, Random, 95% CI) | -54.91 [-117.56, 7.74] |
| 2.9.8 Supplemental breast milk vs glycine | 1 | 40 | Mean Difference (IV, Random, 95% CI) | 51.80 [6.33, 97.27] |
| 2.9.9 Supplemental breast milk vs artificial sweetener | 1 | 40 | Mean Difference (IV, Random, 95% CI) | 41.00 [-6.61, 88.61] |
| 2.9.10 Supplemental breast milk vs glucose (low concentration: < 20%) | 2 | 110 | Mean Difference (IV, Random, 95% CI) | 3.58 [-1.52, 8.68] |
| 2.9.11 Supplemental breast milk vs glucose (moderate concentration: 20% to 33%) | 4 | 301 | Mean Difference (IV, Random, 95% CI) | 27.41 [23.04, 31.78] |
| 2.9.12 Supplemental breast milk vs glucose (high concentration > 33%) | 1 | 50 | Mean Difference (IV, Random, 95% CI) | 27.20 [20.89, 33.51] |
| 2.9.13 Supplemental breast milk vs sucrose (low concentration < 20%) | 1 | 43 | Mean Difference (IV, Random, 95% CI) | 35.00 [29.04, 40.96] |

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 2.9.14 Supplemental breast milk vs sucrose (moderate concentration: 20% to 33%) | 3 | 163 | Mean Difference (IV, Random, 95% CI) | 34.61 [1.36, 67.86] |
| 2.9.15 Supplemental breast milk vs sucrose (low concentration: < 20%) 2 doses | 1 | 41 | Mean Difference (IV, Random, 95% CI) | 22.00 [13.09, 30.91] |
| 2.9.16 Supplemental breast milk 1 dose vs supplemental breast milk 2 doses | 1 | 41 | Mean Difference (IV, Random, 95% CI) | -11.00 [-21.22, -0.78] |
| 2.10 Percentage of time crying | 1 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 2.10.1 Supplemental breast milk vs placebo | 1 | 40 | Mean Difference (IV, Random, 95% CI) | 9.00 [-1.99, 19.99] |
| 2.10.2 Supplemental breast milk vs artificial sweetener | 1 | 40 | Mean Difference (IV, Random, 95% CI) | 15.00 [2.38, 27.62] |
| 2.10.3 Supplemental breast milk vs glycine | 1 | 40 | Mean Difference (IV, Random, 95% CI) | 1.00 [-4.61, 6.61] |
| 2.11 Neonatal Infant pain scale (NIPS) | 3 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 2.11.1 Supplemental breast milk vs no intervention | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -0.30 [-1.60, 1.00] |
| 2.11.2 Supplemental breast milk vs non-nutritive sucking (pacifier) | 1 | 60 | Mean Difference (IV, Random, 95% CI) | 1.20 [-0.14, 2.54] |
| 2.11.3 Supplemental breast milk + breast milk odour + gentle touch + verbal comfort vs gentle touch + verbal comfort | 1 | 80 | Mean Difference (IV, Random, 95% CI) | -0.19 [-0.58, 0.20] |
| 2.11.4 Supplemental breast milk + breast milk odour + gentle touch + verbal comfort vs breast milk odour + gentle touch + verbal comfort | 1 | 80 | Mean Difference (IV, Random, 95% CI) | -0.04 [-0.39, 0.31] |
| 2.11.5 Supplemental breast milk vs sucrose (moderate concentration: 20% to 33%) | 2 | 94 | Mean Difference (IV, Random, 95% CI) | 0.91 [-1.45, 3.28] |
| 2.11.6 Supplemental breast milk vs proparacaine eye drops | 1 | 34 | Mean Difference (IV, Random, 95% CI) | 0.05 [-0.29, 0.39] |
| 2.12 Premature infant pain profile (PIPP) | 9 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 2.12.1 Supplemental breast milk vs swaddling | 2 | 112 | Mean Difference (IV, Random, 95% CI) | -0.64 [-4.57, 3.29] |

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 2.12.2 Supplemental breast milk vs held by mother | 1 | 50 | Mean Difference (IV, Random, 95% CI) | 0.28 [-0.83, 1.39] |
| 2.12.3 Supplemental breast milk vs water | 4 | 250 | Mean Difference (IV, Random, 95% CI) | -1.87 [-4.59, 0.85] |
| 2.12.4 Supplemental breast milk vs glucose (moderate concentration: 20% to 33%) | 4 | 307 | Mean Difference (IV, Random, 95% CI) | 1.86 [1.02, 2.71] |
| 2.12.5 Supplemental breast milk vs sucrose (moderate concentration: 20% to 33%) | 3 | 170 | Mean Difference (IV, Random, 95% CI) | -0.02 [-0.94, 0.91] |
| 2.13 Neonatal Facial Coding System score at 3 minutes | 3 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 2.13.1 Supplemental breast milk vs placebo | 3 | 183 | Mean Difference (IV, Random, 95% CI) | -0.86 [-2.24, 0.52] |
| 2.13.2 Supplemental breast milk vs glycine | 1 | 40 | Mean Difference (IV, Random, 95% CI) | -0.47 [-0.90, -0.04] |
| 2.13.3 Supplemental breast milk vs artificial sweetener | 1 | 40 | Mean Difference (IV, Random, 95% CI) | -0.22 [-0.65, 0.21] |
| 2.14 Neonatal Facial Coding System score at 2 minutes | 1 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 2.14.1 Supplemental breast milk vs water | 1 | 45 | Mean Difference (IV, Random, 95% CI) | -0.84 [-1.09, -0.59] |
| 2.14.2 Supplemental breast milk vs water 2 doses | 1 | 44 | Mean Difference (IV, Random, 95% CI) | -0.59 [-0.83, -0.35] |
| 2.14.3 Supplemental breast milk vs sucrose (low concentration: < 20%) | 1 | 43 | Mean Difference (IV, Random, 95% CI) | 0.92 [0.64, 1.20] |
| 2.14.4 Supplemental breast milk vs sucrose (low concentration: < 20%) 2 doses | 1 | 41 | Mean Difference (IV, Random, 95% CI) | 0.16 [-0.11, 0.43] |
| 2.14.5 Supplemental breast milk 1 dose vs supplemental breast milk 2 doses | 1 | 41 | Mean Difference (IV, Random, 95% CI) | -1.14 [-1.37, -0.91] |
| 2.15 Douleur Aigue du Nouveau-né (DAN) scale | 2 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 2.15.1 Supplemental breast milk vs no intervention | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -1.00 [-2.15, 0.15] |
| 2.15.2 Supplemental breast milk vs massage | 1 | 35 | Mean Difference (IV, Random, 95% CI) | -0.50 [-0.91, -0.09] |

| Outcome or subgroup title | No. of studies | No. of participants | Statistical method | Effect size |
|---|---|---|---|---|
| 2.15.3 Supplemental breast milk vs rocking | 1 | 35 | Mean Difference (IV, Random, 95% CI) | 1.10 [0.65, 1.55] |
| 2.15.4 Supplemental breast milk vs non-nutritive sucking (pacifier) | 1 | 38 | Mean Difference (IV, Random, 95% CI) | 0.80 [0.40, 1.20] |
| 2.15.5 Supplemental breast milk vs water | 1 | 33 | Mean Difference (IV, Random, 95% CI) | -1.10 [-1.65, -0.55] |
| 2.15.6 Supplemental breast milk vs EMLA cream | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -0.64 [-1.92, 0.64] |
| 2.15.7 Supplemental breast milk vs sucrose (moderate concentration: 20% to 33%) | 2 | 95 | Mean Difference (IV, Random, 95% CI) | 0.29 [-0.34, 0.92] |
| 2.16 Body pain score | 1 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 2.16.1 Supplemental breast milk vs placebo | 1 | 40 | Mean Difference (IV, Random, 95% CI) | 0.48 [-0.38, 1.34] |
| 2.16.2 Supplemental breast milk vs glycine | 1 | 40 | Mean Difference (IV, Random, 95% CI) | 0.43 [-0.51, 1.37] |
| 2.16.3 Supplemental breast milk vs artificial sweetener | 1 | 40 | Mean Difference (IV, Random, 95% CI) | 0.16 [-0.72, 1.04] |
| 2.17 Neonatal Pain, Agitation and Sedation Scale (N-PASS) | 2 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 2.17.1 Supplemental breast milk vs no intervention | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -0.45 [-0.55, -0.35] |
| 2.17.2 Supplemental breast milk vs breast milk smell | 1 | 60 | Mean Difference (IV, Random, 95% CI) | -0.43 [-0.53, -0.33] |
| 2.17.3 Supplemental breast milk vs breast milk taste + smell | 1 | 60 | Mean Difference (IV, Random, 95% CI) | 0.60 [0.40, 0.80] |
| 2.17.4 Supplemental breast milk vs water | 1 | 84 | Mean Difference (IV, Random, 95% CI) | -2.11 [-3.51, -0.71] |
| 2.17.5 Supplemental breast milk vs glucose (moderate concentration: 20% to 33%) | 1 | 79 | Mean Difference (IV, Random, 95% CI) | -0.28 [-1.62, 1.06] |
| 2.18 Premature Infant Pain Profile - Revised scale | 1 | | Mean Difference (IV, Random, 95% CI) | Subtotals only |
| 2.18.1 Supplemental breast milk vs 24% oral sucrose | 1 | 88 | Mean Difference (IV, Random, 95% CI) | -1.10 [-1.86, -0.34] |

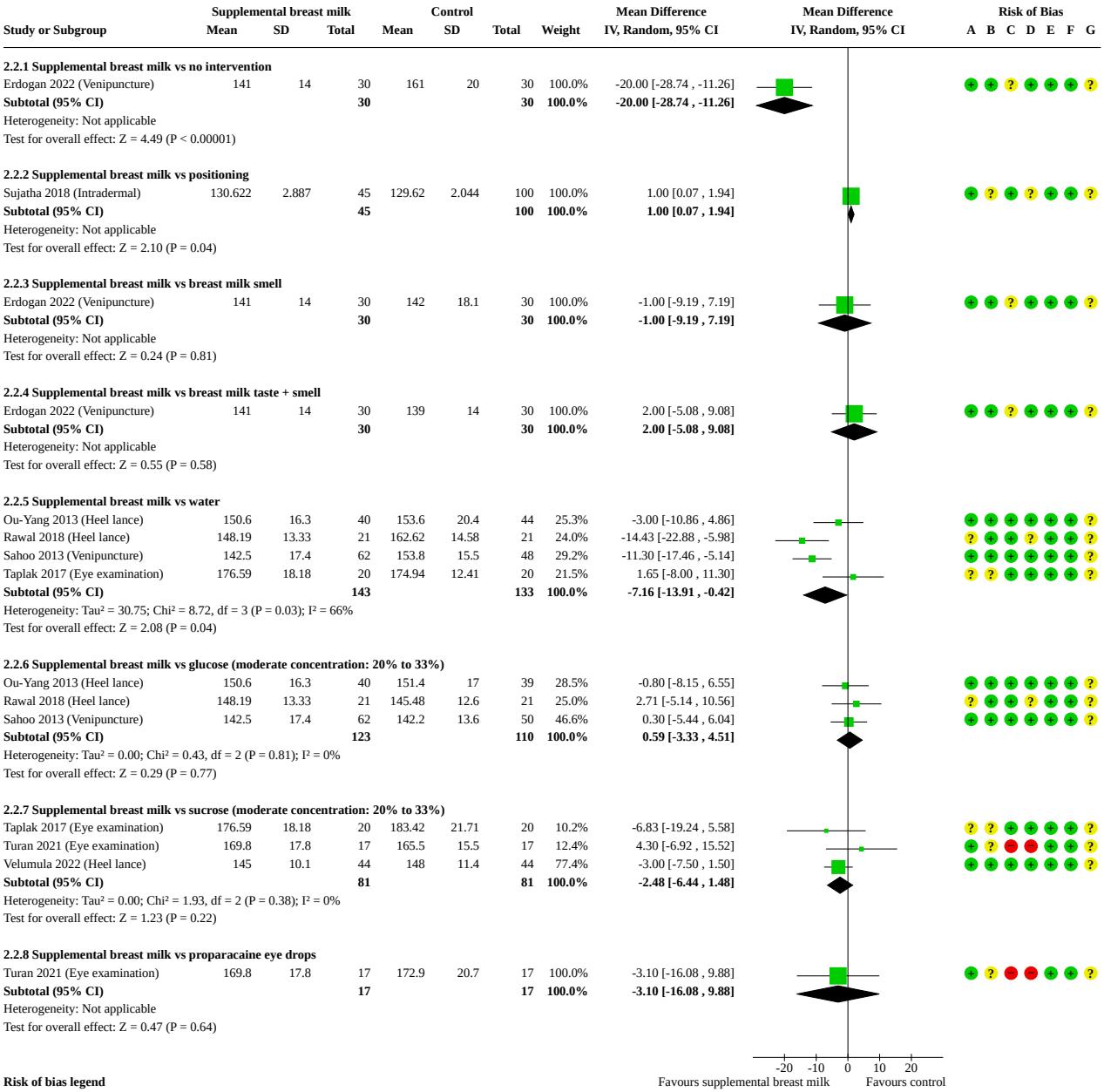
# Analysis 2.1. Comparison 2: Supplemental breast milk vs control, Outcome 1: Heart rate change (beats per minute)




**Risk of bias legend**

(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

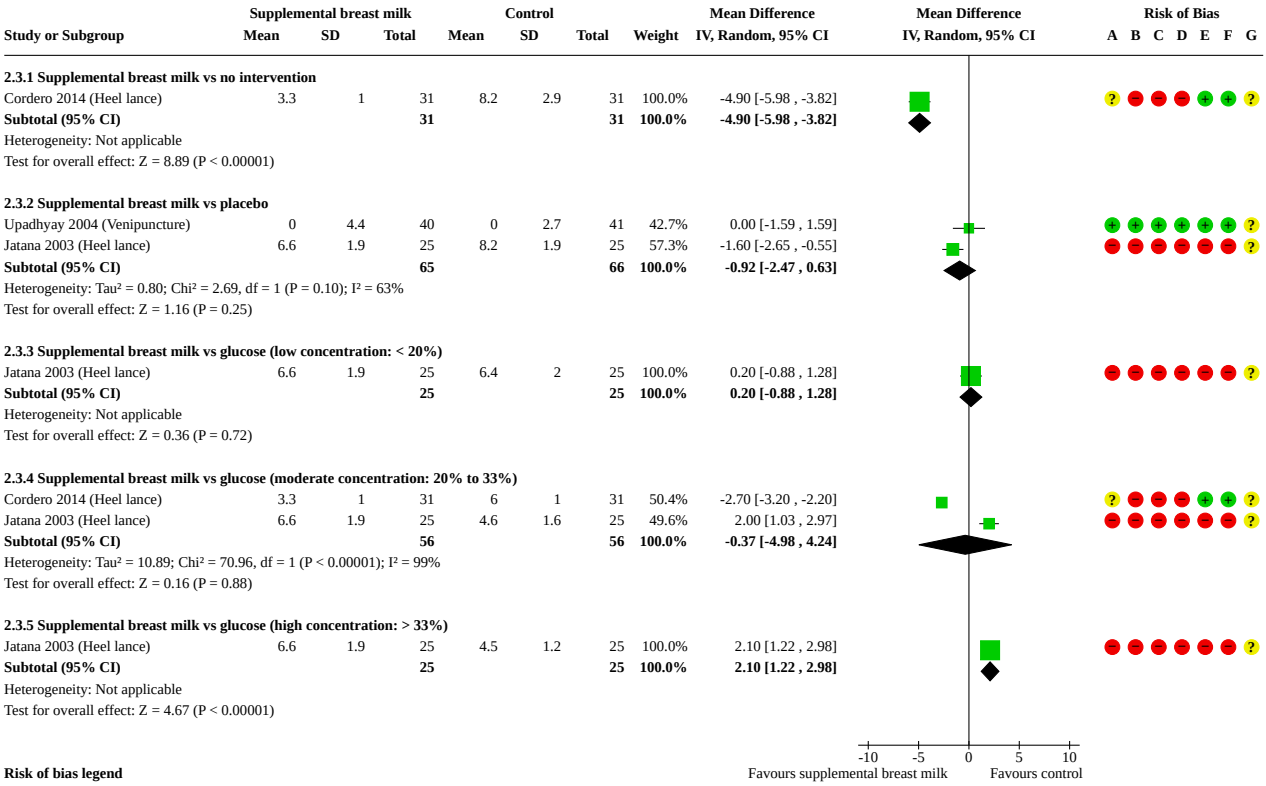
## Analysis 2.2. Comparison 2: Supplemental breast milk vs control, Outcome 2: Heart rate (beats per minute)




**Risk of bias legend**

(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

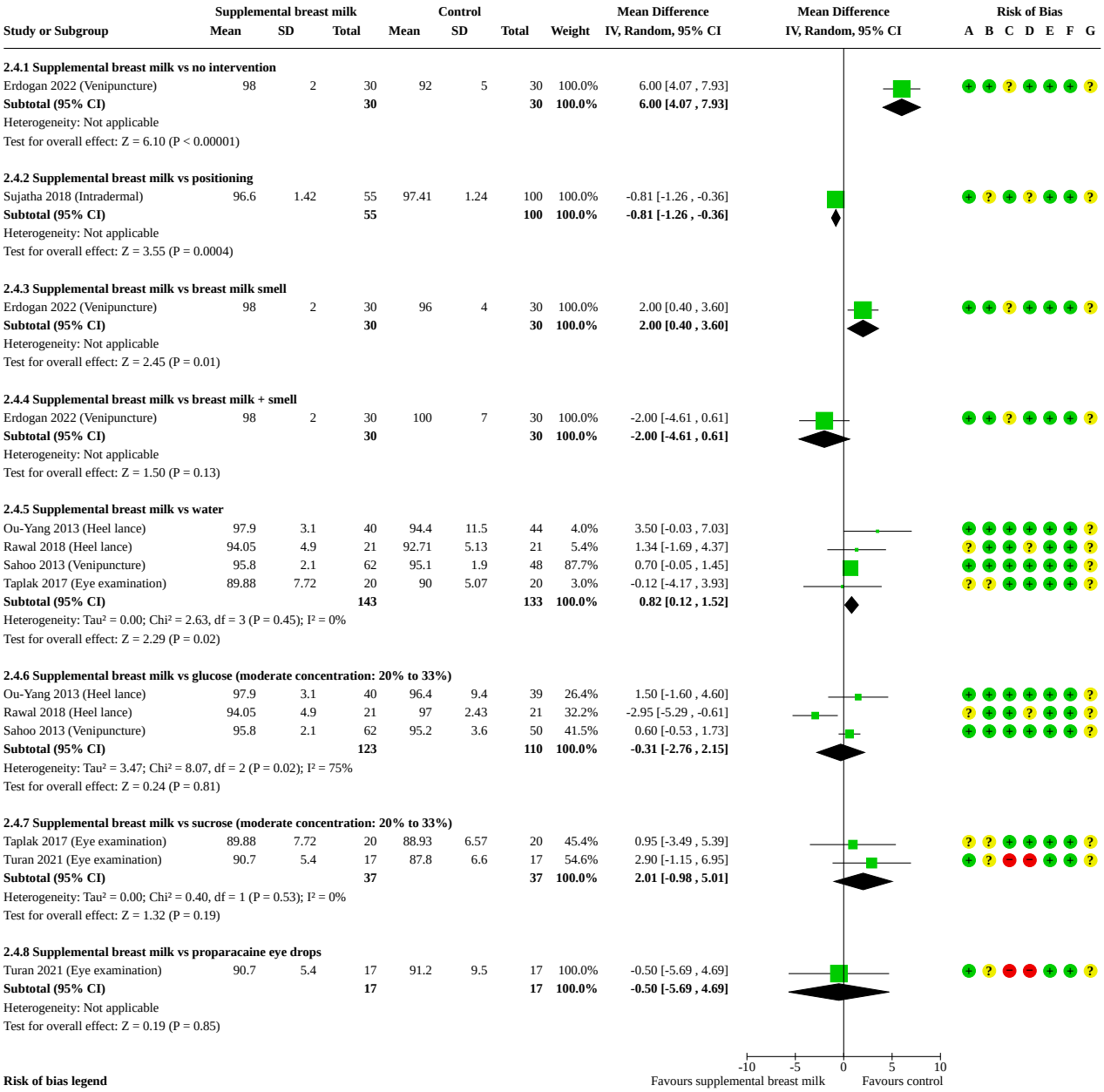
## Analysis 2.3. Comparison 2: Supplemental breast milk vs control, Outcome 3: Oxygen saturation change




**Risk of bias legend**

(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

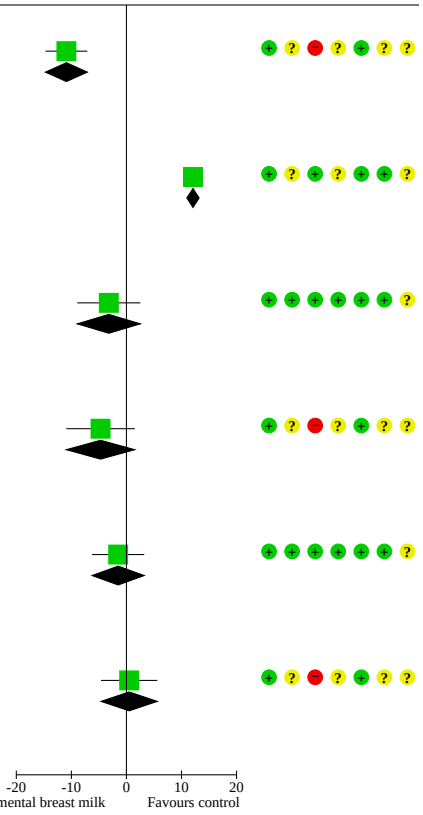
## Analysis 2.4. Comparison 2: Supplemental breast milk vs control, Outcome 4: Oxygen saturations




**Risk of bias legend**
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

## Analysis 2.5. Comparison 2: Supplemental breast milk vs control, Outcome 5: Respiratory rate

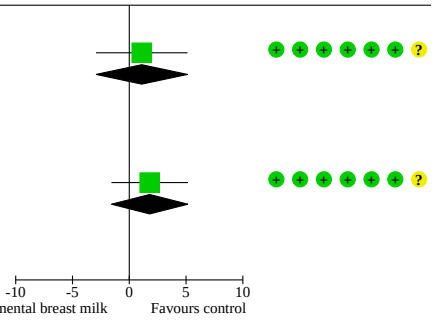
| Study or Subgroup | Supplemental breast milk Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| **2.5.1 Supplemental breast milk vs no intervention** | | | | | | | | |
| Aziznejad 2013 (Venipuncture) | 53.6 | 10.3 | 30 | 64.5 | 2.61 | 30 | 100.0% | -10.90 [-14.70 , -7.10] |
| **Subtotal (95% CI)** | | | **30** | | | **30** | **100.0%** | **-10.90 [-14.70 , -7.10]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 5.62 (P < 0.00001) | | | | | | | | |
| **2.5.2 Supplemental breast milk vs positioning** | | | | | | | | |
| Sujatha 2018 (Intradermal) | 70.73 | 3.06 | 45 | 58.64 | 3.42 | 100 | 100.0% | 12.09 [10.97 , 13.21] |
| **Subtotal (95% CI)** | | | **45** | | | **100** | **100.0%** | **12.09 [10.97 , 13.21]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 21.21 (P < 0.00001) | | | | | | | | |
| **2.5.3 Supplemental breast milk vs water** | | | | | | | | |
| Ou-Yang 2013 (Heel lance) | 42.8 | 12.9 | 40 | 46 | 13.9 | 44 | 100.0% | -3.20 [-8.93 , 2.53] |
| **Subtotal (95% CI)** | | | **40** | | | **44** | **100.0%** | **-3.20 [-8.93 , 2.53]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 1.09 (P = 0.27) | | | | | | | | |
| **2.5.4 Supplemental breast milk vs EMLA cream** | | | | | | | | |
| Aziznejad 2013 (Venipuncture) | 53.6 | 10.3 | 30 | 58.3 | 14 | 30 | 100.0% | -4.70 [-10.92 , 1.52] |
| **Subtotal (95% CI)** | | | **30** | | | **30** | **100.0%** | **-4.70 [-10.92 , 1.52]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 1.48 (P = 0.14) | | | | | | | | |
| **2.5.5 Supplemental breast milk vs glucose (moderate concentration: 20% to 33%)** | | | | | | | | |
| Ou-Yang 2013 (Heel lance) | 42.8 | 12.9 | 40 | 44.3 | 8.1 | 39 | 100.0% | -1.50 [-6.24 , 3.24] |
| **Subtotal (95% CI)** | | | **40** | | | **39** | **100.0%** | **-1.50 [-6.24 , 3.24]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 0.62 (P = 0.53) | | | | | | | | |
| **2.5.6 Supplemental breast milk vs sucrose (moderate concentration: 20% to 33%)** | | | | | | | | |
| Aziznejad 2013 (Venipuncture) | 53.6 | 10.3 | 30 | 53.1 | 9.9 | 30 | 100.0% | 0.50 [-4.61 , 5.61] |
| **Subtotal (95% CI)** | | | **30** | | | **30** | **100.0%** | **0.50 [-4.61 , 5.61]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 0.19 (P = 0.85) | | | | | | | | |




**Risk of bias legend**
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

## Analysis 2.6. Comparison 2: Supplemental breast milk vs control, Outcome 6: Systolic blood pressure

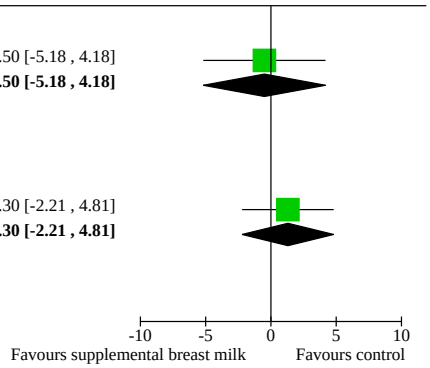
| Study or Subgroup | Supplemental breast milk Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| **2.6.1 Supplemental breast milk vs water** | | | | | | | | |
| Ou-Yang 2013 (Heel lance) | 63.5 | 8.4 | 40 | 62.4 | 10.4 | 44 | 100.0% | 1.10 [-2.93 , 5.13] |
| **Subtotal (95% CI)** | | | **40** | | | **44** | **100.0%** | **1.10 [-2.93 , 5.13]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 0.54 (P = 0.59) | | | | | | | | |
| **2.6.2 Supplemental breast milk vs glucose (moderate concentration: 20% to 33%)** | | | | | | | | |
| Ou-Yang 2013 (Heel lance) | 63.5 | 8.4 | 40 | 61.7 | 6.8 | 39 | 100.0% | 1.80 [-1.57 , 5.17] |
| **Subtotal (95% CI)** | | | **40** | | | **39** | **100.0%** | **1.80 [-1.57 , 5.17]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 1.05 (P = 0.29) | | | | | | | | |



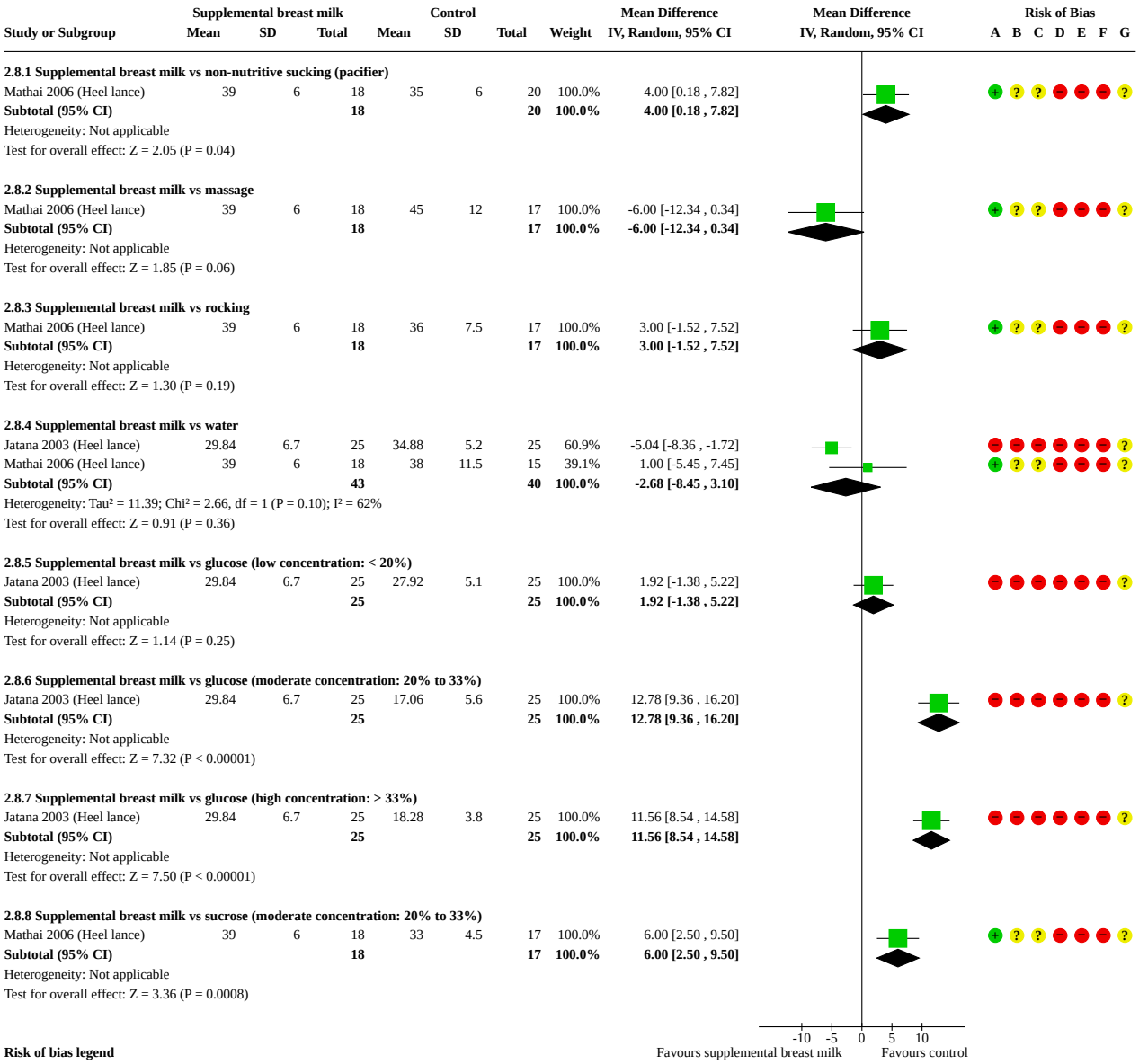

**Risk of bias legend**
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

## Analysis 2.7. Comparison 2: Supplemental breast milk vs control, Outcome 7: Diastolic blood pressure

| Study or Subgroup | Supplemental breast milk Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| **2.7.1 Supplemental breast milk vs water** | | | | | | | | |
| Ou-Yang 2013 (Heel lance) | 38.1 | 6.7 | 40 | 38.6 | 14.2 | 44 | 100.0% | -0.50 [-5.18 , 4.18] |
| **Subtotal (95% CI)** | | | **40** | | | **44** | **100.0%** | **-0.50 [-5.18 , 4.18]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 0.21 (P = 0.83) | | | | | | | | |
| **2.7.2 Supplemental breast milk vs glucose (moderate concentration: 20% to 33%)** | | | | | | | | |
| Ou-Yang 2013 (Heel lance) | 38.1 | 6.7 | 40 | 36.8 | 9 | 39 | 100.0% | 1.30 [-2.21 , 4.81] |
| **Subtotal (95% CI)** | | | **40** | | | **39** | **100.0%** | **1.30 [-2.21 , 4.81]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 0.73 (P = 0.47) | | | | | | | | |



## Analysis 2.8. Comparison 2: Supplemental breast milk vs control, Outcome 8: Duration of first cry (seconds)




**Risk of bias legend**
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

## Analysis 2.9. Comparison 2: Supplemental breast milk vs control, Outcome 9: Duration of crying (seconds)

| Study or Subgroup | Supplemental breast milk Mean [Seconds] | SD [Seconds] | Total | Control Mean [Seconds] | SD [Seconds] | Total | Weight | Mean Difference IV, Random, 95% CI [Seconds] |
|---|---|---|---|---|---|---|---|---|
| **2.9.1 Supplemental breast milk vs no intervention** | | | | | | | | |
| Yilmaz 2011 (Heel lance) | 95.5 | 76.8 | 30 | 58.8 | 65.4 | 30 | 100.0% | 36.70 [0.60 , 72.80] |
| **Subtotal (95% CI)** | | | **30** | | | **30** | **100.0%** | **36.70 [0.60 , 72.80]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 1.99 (P = 0.05) | | | | | | | | |
| **2.9.2 Supplemental breast milk vs placebo** | | | | | | | | |
| Bucher 2000 (Heel lance) | 118.6 | 94.1 | 20 | 74.4 | 72.8 | 20 | 0.5% | 44.20 [-7.94 , 96.34] |
| Jatana 2003 (Heel lance) | 104.56 | 9.2 | 25 | 113.76 | 11.8 | 25 | 38.7% | -9.20 [-15.07 , -3.33] |
| Mathai 2006 (Heel lance) | 87 | 115 | 18 | 98 | 8 | 15 | 0.5% | -11.00 [-64.28 , 42.28] |
| Ors 1999 (Heel lance) | 71.06 | 52.74 | 33 | 86.08 | 66.89 | 34 | 1.6% | -15.02 [-43.82 , 13.78] |
| Ozdogan 2010 (Heel lance) | 117 | 10.2 | 18 | 126 | 6.8 | 27 | 46.3% | -9.00 [-14.36 , -3.64] |
| Skogsdal 1997 (Heel lance) | 22.04 | 41.09 | 30 | 35.6 | 60.07 | 30 | 2.0% | -13.56 [-39.60 , 12.48] |
| Uyan 2005 (Heel lance) | 151.34 | 20.3 | 41 | 157.05 | 21.93 | 21 | 10.5% | -5.71 [-16.96 , 5.54] |
| **Subtotal (95% CI)** | | | **185** | | | **172** | **100.0%** | **-8.67 [-12.32 , -5.02]** |
| Heterogeneity: Tau² = 0.00; Chi² = 4.59, df = 6 (P = 0.60); I² = 0% | | | | | | | | |
| Test for overall effect: Z = 4.66 (P < 0.00001) | | | | | | | | |
| **2.9.3 Supplemental breast milk vs positioning** | | | | | | | | |
| Sujatha 2018 (Intradermal) | 53.51 | 3.06 | 45 | 60.94 | 3.43 | 100 | 100.0% | -7.43 [-8.55 , -6.31] |
| **Subtotal (95% CI)** | | | **45** | | | **100** | **100.0%** | **-7.43 [-8.55 , -6.31]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 13.02 (P < 0.00001) | | | | | | | | |
| **2.9.4 Supplemental breast milk vs rocking** | | | | | | | | |
| Mathai 2006 (Heel lance) | 87 | 11.5 | 18 | 56 | 8 | 17 | 100.0% | 31.00 [24.47 , 37.53] |
| **Subtotal (95% CI)** | | | **18** | | | **17** | **100.0%** | **31.00 [24.47 , 37.53]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 9.30 (P < 0.00001) | | | | | | | | |
| **2.9.5 Supplemental breast milk vs massage** | | | | | | | | |
| Mathai 2006 (Heel lance) | 87 | 11.5 | 18 | 96 | 12.5 | 17 | 100.0% | -9.00 [-16.97 , -1.03] |
| **Subtotal (95% CI)** | | | **18** | | | **17** | **100.0%** | **-9.00 [-16.97 , -1.03]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 2.21 (P = 0.03) | | | | | | | | |
| **2.9.6 Supplemental breast milk vs non-nutritive sucking (pacifier)** | | | | | | | | |
| Mathai 2006 (Heel lance) | 87 | 11.5 | 18 | 43 | 5.5 | 20 | 97.4% | 44.00 [38.17 , 49.83] |
| Yilmaz 2011 (Heel lance) | 95.5 | 76.8 | 30 | 42.8 | 63.3 | 30 | 2.6% | 52.70 [17.09 , 88.31] |
| **Subtotal (95% CI)** | | | **48** | | | **50** | **100.0%** | **44.23 [38.47 , 49.98]** |
| Heterogeneity: Tau² = 0.00; Chi² = 0.22, df = 1 (P = 0.64); I² = 0% | | | | | | | | |
| Test for overall effect: Z = 15.06 (P < 0.00001) | | | | | | | | |
| **2.9.7 Supplemental breast milk vs water** | | | | | | | | |
| Ou-Yang 2013 (Heel lance) | 29.5 | 16.25 | 40 | 70.5 | 24.75 | 44 | 33.4% | -41.00 [-49.88 , -32.12] |
| Ozdogan 2010 (Heel lance) | 117 | 10.2 | 18 | 117 | 6.8 | 26 | 33.5% | 0.00 [-5.39 , 5.39] |
| Sahoo 2013 (Venipuncture) | 37.5 | 34.25 | 62 | 162 | 35.25 | 48 | 33.1% | -124.50 [-137.62 , -111.38] |
| **Subtotal (95% CI)** | | | **120** | | | **118** | **100.0%** | **-54.91 [-117.56 , 7.74]** |
| Heterogeneity: Tau² = 3041.01; Chi² = 315.65, df = 2 (P < 0.00001); I² = 99% | | | | | | | | |
| Test for overall effect: Z = 1.72 (P = 0.09) | | | | | | | | |
| **2.9.8 Supplemental breast milk vs glycine** | | | | | | | | |
| Bucher 2000 (Heel lance) | 118.6 | 94.1 | 20 | 66.8 | 43.7 | 20 | 100.0% | 51.80 [6.33 , 97.27] |
| **Subtotal (95% CI)** | | | **20** | | | **20** | **100.0%** | **51.80 [6.33 , 97.27]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 2.23 (P = 0.03) | | | | | | | | |
| **2.9.9 Supplemental breast milk vs artificial sweetener** | | | | | | | | |
| Bucher 2000 (Heel lance) | 118.6 | 94.1 | 20 | 77.6 | 54.3 | 20 | 100.0% | 41.00 [-6.61 , 88.61] |
| **Subtotal (95% CI)** | | | **20** | | | **20** | **100.0%** | **41.00 [-6.61 , 88.61]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 1.69 (P = 0.09) | | | | | | | | |
| **2.9.10 Supplemental breast milk vs glucose (low concentration: < 20%)** | | | | | | | | |
| Jatana 2003 (Heel lance) | 104.56 | 9.2 | 25 | 101 | 9.9 | 25 | 92.8% | 3.56 [-1.74 , 8.86] |
| Skogsdal 1997 (Heel lance) | 22.04 | 41.09 | 30 | 18.2 | 33.79 | 30 | 7.2% | 3.84 [-15.20 , 22.88] |
| **Subtotal (95% CI)** | | | **55** | | | **55** | **100.0%** | **3.58 [-1.52 , 8.68]** |
| Heterogeneity: Tau² = 0.00; Chi² = 0.00, df = 1 (P = 0.98); I² = 0% | | | | | | | | |
| Test for overall effect: Z = 1.37 (P = 0.17) | | | | | | | | |
| **2.9.11 Supplemental breast milk vs glucose (moderate concentration: 20% to 33%)** | | | | | | | | |
| Jatana 2003 (Heel lance) | 104.56 | 9.2 | 25 | 74.8 | 11 | 25 | 40.4% | 29.76 [24.14 , 35.38] |
| Ou-Yang 2013 (Heel lance) | 29.5 | 16.25 | 40 | 2 | 11.25 | 39 | 35.7% | 27.50 [21.35 , 33.65] |
| Sahoo 2013 (Venipuncture) | 37.5 | 34.25 | 62 | 10 | 17.5 | 50 | 17.1% | 27.50 [17.69 , 37.31] |
| Skogsdal 1997 (Heel lance) | 22.04 | 41.09 | 30 | 9.13 | 18.8 | 30 | 6.9% | 12.91 [-3.26 , 29.08] |
| **Subtotal (95% CI)** | | | **157** | | | **144** | **100.0%** | **27.41 [23.04 , 31.78]** |
| Heterogeneity: Tau² = 4.07; Chi² = 3.74, df = 3 (P = 0.29); I² = 20% | | | | | | | | |
| Test for overall effect: Z = 12.30 (P < 0.00001) | | | | | | | | |
| **2.9.12 Supplemental breast milk vs glucose (high concentration > 33%)** | | | | | | | | |

## Analysis 2.9. (Continued)

Test for overall effect: Z = 12.30 (P < 0.00001)

**2.9.12 Supplemental breast milk vs glucose (high concentration > 33%)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Jatana 2003 (Heel lance) | 104.56 | 9.2 | 25 | 77.36 | 13.2 | 25 | 100.0% | 27.20 [20.89 , 33.51] |
| **Subtotal (95% CI)** | | | **25** | | | **25** | **100.0%** | **27.20 [20.89 , 33.51]** |

Heterogeneity: Not applicable
Test for overall effect: Z = 8.45 (P < 0.00001)

**2.9.13 Supplemental breast milk vs sucrose (low concentration < 20%)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Ozdogan 2010 (Heel lance) | 117 | 10.2 | 18 | 82 | 9.3 | 25 | 100.0% | 35.00 [29.04 , 40.96] |
| **Subtotal (95% CI)** | | | **18** | | | **25** | **100.0%** | **35.00 [29.04 , 40.96]** |

Heterogeneity: Not applicable
Test for overall effect: Z = 11.51 (P < 0.00001)

**2.9.14 Supplemental breast milk vs sucrose (moderate concentration: 20% to 33%)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mathai 2006 (Heel lance) | 87 | 11.5 | 18 | 79 | 8 | 17 | 38.2% | 8.00 [1.47 , 14.53] |
| Ors 1999 (Heel lance) | 71.06 | 52.74 | 33 | 37.89 | 33.21 | 35 | 33.5% | 33.17 [12.08 , 54.26] |
| Yilmaz 2011 (Heel lance) | 95.5 | 76.8 | 30 | 23.3 | 48.2 | 30 | 28.3% | 72.20 [39.75 , 104.65] |
| **Subtotal (95% CI)** | | | **81** | | | **82** | **100.0%** | **34.61 [1.36 , 67.86]** |

Heterogeneity: Tau² = 742.84; Chi² = 18.52, df = 2 (P < 0.0001); I² = 89%
Test for overall effect: Z = 2.04 (P = 0.04)

**2.9.15 Supplemental breast milk vs sucrose (low concentration: < 20%) 2 doses**

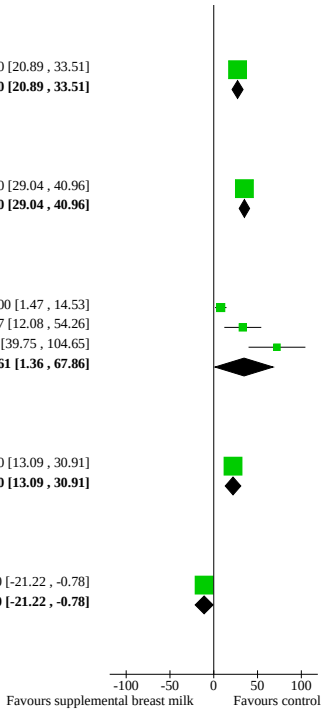
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Ozdogan 2010 (Heel lance) | 117 | 10.2 | 18 | 95 | 18.5 | 23 | 100.0% | 22.00 [13.09 , 30.91] |
| **Subtotal (95% CI)** | | | **18** | | | **23** | **100.0%** | **22.00 [13.09 , 30.91]** |

Heterogeneity: Not applicable
Test for overall effect: Z = 4.84 (P < 0.00001)

**2.9.16 Supplemental breast milk 1 dose vs supplemental breast milk 2 doses**

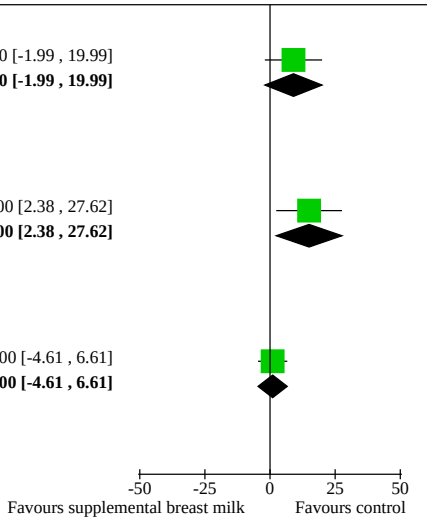
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Ozdogan 2010 (Heel lance) | 117 | 10.2 | 18 | 128 | 22.2 | 23 | 100.0% | -11.00 [-21.22 , -0.78] |
| **Subtotal (95% CI)** | | | **18** | | | **23** | **100.0%** | **-11.00 [-21.22 , -0.78]** |

Heterogeneity: Not applicable
Test for overall effect: Z = 2.11 (P = 0.03)



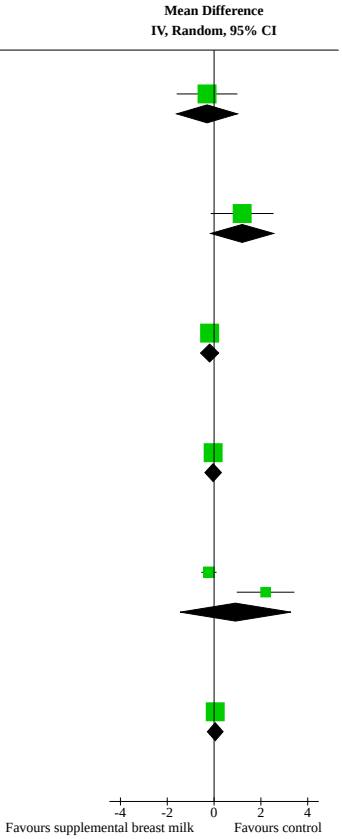

## Analysis 2.10. Comparison 2: Supplemental breast milk vs control, Outcome 10: Percentage of time crying

| Study or Subgroup | Supplemental breast milk Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| **2.10.1 Supplemental breast milk vs placebo** | | | | | | | | |
| Bucher 2000 (Heel lance) | 91 | 10 | 20 | 82 | 23 | 20 | 100.0% | 9.00 [-1.99 , 19.99] |
| **Subtotal (95% CI)** | | | **20** | | | **20** | **100.0%** | **9.00 [-1.99 , 19.99]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 1.60 (P = 0.11) | | | | | | | | |
| **2.10.2 Supplemental breast milk vs artificial sweetener** | | | | | | | | |
| Bucher 2000 (Heel lance) | 91 | 10 | 20 | 76 | 27 | 20 | 100.0% | 15.00 [2.38 , 27.62] |
| **Subtotal (95% CI)** | | | **20** | | | **20** | **100.0%** | **15.00 [2.38 , 27.62]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 2.33 (P = 0.02) | | | | | | | | |
| **2.10.3 Supplemental breast milk vs glycine** | | | | | | | | |
| Bucher 2000 (Heel lance) | 91 | 10 | 20 | 90 | 8 | 20 | 100.0% | 1.00 [-4.61 , 6.61] |
| **Subtotal (95% CI)** | | | **20** | | | **20** | **100.0%** | **1.00 [-4.61 , 6.61]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 0.35 (P = 0.73) | | | | | | | | |



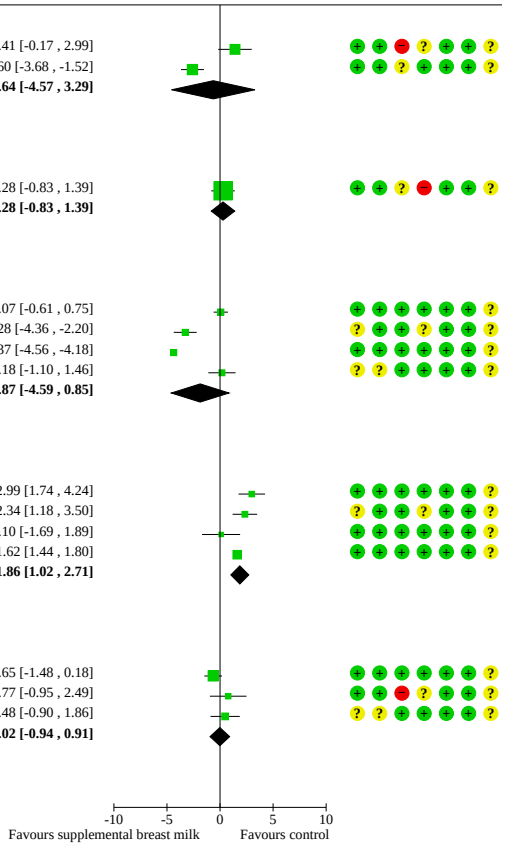
## Analysis 2.11. Comparison 2: Supplemental breast milk vs control, Outcome 11: Neonatal Infant pain scale (NIPS)

| Study or Subgroup | Supplemental breast milk Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| **2.11.1 Supplemental breast milk vs no intervention** | | | | | | | | |
| Yilmaz 2011 (Heel lance) | 4.8 | 2.8 | 30 | 5.1 | 2.3 | 30 | 100.0% | -0.30 [-1.60 , 1.00] |
| **Subtotal (95% CI)** | | | **30** | | | **30** | **100.0%** | **-0.30 [-1.60 , 1.00]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 0.45 (P = 0.65) | | | | | | | | |
| **2.11.2 Supplemental breast milk vs non-nutritive sucking (pacifier)** | | | | | | | | |
| Yilmaz 2011 (Heel lance) | 4.8 | 2.8 | 30 | 3.6 | 2.5 | 30 | 100.0% | 1.20 [-0.14 , 2.54] |
| **Subtotal (95% CI)** | | | **30** | | | **30** | **100.0%** | **1.20 [-0.14 , 2.54]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 1.75 (P = 0.08) | | | | | | | | |
| **2.11.3 Supplemental breast milk + breast milk odour + gentle touch + verbal comfort vs gentle touch + verbal comfort** | | | | | | | | |
| Lan 2021 (Heel lance) | 0.42 | 0.82 | 40 | 0.61 | 0.95 | 40 | 100.0% | -0.19 [-0.58 , 0.20] |
| **Subtotal (95% CI)** | | | **40** | | | **40** | **100.0%** | **-0.19 [-0.58 , 0.20]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 0.96 (P = 0.34) | | | | | | | | |
| **2.11.4 Supplemental breast milk + breast milk odour + gentle touch + verbal comfort vs breast milk odour + gentle touch + verbal comfort** | | | | | | | | |
| Lan 2021 (Heel lance) | 0.42 | 0.82 | 40 | 0.46 | 0.79 | 40 | 100.0% | -0.04 [-0.39 , 0.31] |
| **Subtotal (95% CI)** | | | **40** | | | **40** | **100.0%** | **-0.04 [-0.39 , 0.31]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 0.22 (P = 0.82) | | | | | | | | |
| **2.11.5 Supplemental breast milk vs sucrose (moderate concentration: 20% to 33%)** | | | | | | | | |
| Turan 2021 (Eye examination) | 6.29 | 0.5 | 17 | 6.51 | 0.47 | 17 | 53.1% | -0.22 [-0.55 , 0.11] |
| Yilmaz 2011 (Heel lance) | 4.8 | 2.8 | 30 | 2.6 | 2 | 30 | 46.9% | 2.20 [0.97 , 3.43] |
| **Subtotal (95% CI)** | | | **47** | | | **47** | **100.0%** | **0.91 [-1.45 , 3.28]** |
| Heterogeneity: Tau² = 2.72; Chi² = 13.87, df = 1 (P = 0.0002); I² = 93% | | | | | | | | |
| Test for overall effect: Z = 0.76 (P = 0.45) | | | | | | | | |
| **2.11.6 Supplemental breast milk vs proparacaine eye drops** | | | | | | | | |
| Turan 2021 (Eye examination) | 6.29 | 0.5 | 17 | 6.24 | 0.5 | 17 | 100.0% | 0.05 [-0.29 , 0.39] |
| **Subtotal (95% CI)** | | | **17** | | | **17** | **100.0%** | **0.05 [-0.29 , 0.39]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 0.29 (P = 0.77) | | | | | | | | |



# Analysis 2.12. Comparison 2: Supplemental breast milk vs control, Outcome 12: Premature infant pain profile (PIPP)

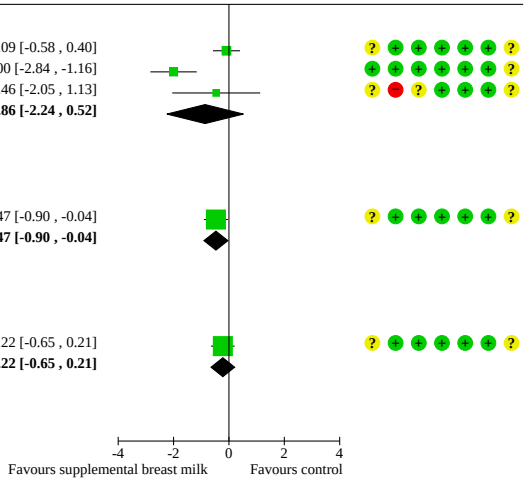
| Study or Subgroup | Supplemental breast milk Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI | Risk of Bias A B C D E F G |
|---|---|---|---|---|---|---|---|---|---|
| **2.12.1 Supplemental breast milk vs swaddling** | | | | | | | | | |
| Desai 2017 (Suctioning) | 12.88 | 3.59 | 36 | 11.47 | 3.23 | 36 | 48.9% | 1.41 [-0.17 , 2.99] | + + − ? + + ? |
| Rosali 2015 (Eye examination) | 12.7 | 1.7 | 20 | 15.3 | 1.78 | 20 | 51.1% | -2.60 [-3.68 , -1.52] | + + ? + + + ? |
| **Subtotal (95% CI)** | | | **56** | | | **56** | **100.0%** | **-0.64 [-4.57 , 3.29]** | |
| Heterogeneity: Tau² = 7.56; Chi² = 16.91, df = 1 (P < 0.0001); I² = 94% | | | | | | | | | |
| Test for overall effect: Z = 0.32 (P = 0.75) | | | | | | | | | |
| **2.12.2 Supplemental breast milk vs held by mother** | | | | | | | | | |
| Nanavati 2013 (Adhesive tape removal) | 6.2 | 2.1 | 25 | 5.92 | 1.89 | 25 | 100.0% | 0.28 [-0.83 , 1.39] | + + ? − + + ? |
| **Subtotal (95% CI)** | | | **25** | | | **25** | **100.0%** | **0.28 [-0.83 , 1.39]** | |
| Heterogeneity: Not applicable | | | | | | | | | |
| Test for overall effect: Z = 0.50 (P = 0.62) | | | | | | | | | |
| **2.12.3 Supplemental breast milk vs water** | | | | | | | | | |
| Bozlak 2017 (Eye examination) | 8.45 | 1.38 | 29 | 8.38 | 1.24 | 29 | 25.3% | 0.07 [-0.61 , 0.75] | + + + + + + ? |
| Rawal 2018 (Heel lance) | 6.86 | 2.01 | 21 | 10.14 | 1.53 | 21 | 24.7% | -3.28 [-4.36 , -2.20] | ? + + ? + + ? |
| Sahoo 2013 (Venipuncture) | 6.84 | 0.5 | 62 | 11.21 | 0.52 | 48 | 25.7% | -4.37 [-4.56 , -4.18] | + + + + + + ? |
| Taplak 2017 (Eye examination) | 16.06 | 2.41 | 20 | 15.88 | 1.66 | 20 | 24.3% | 0.18 [-1.10 , 1.46] | ? ? + + + + ? |
| **Subtotal (95% CI)** | | | **132** | | | **118** | **100.0%** | **-1.87 [-4.59 , 0.85]** | |
| Heterogeneity: Tau² = 7.50; Chi² = 195.68, df = 3 (P < 0.00001); I² = 98% | | | | | | | | | |
| Test for overall effect: Z = 1.35 (P = 0.18) | | | | | | | | | |
| **2.12.4 Supplemental breast milk vs glucose (moderate concentration: 20% to 33%)** | | | | | | | | | |
| Bueno 2012 (Heel lance) | 7.54 | 3.61 | 56 | 4.55 | 3.17 | 57 | 21.7% | 2.99 [1.74 , 4.24] | + + + + + + ? |
| Rawal 2018 (Heel lance) | 6.86 | 2.01 | 21 | 4.52 | 1.81 | 21 | 23.3% | 2.34 [1.18 , 3.50] | ? + + ? + + ? |
| Rodrigues 2017 (Suctioning) | 11.35 | 3.05 | 20 | 11.25 | 2.73 | 20 | 14.5% | 0.10 [-1.69 , 1.89] | + + + + + + ? |
| Sahoo 2013 (Venipuncture) | 6.84 | 0.5 | 62 | 5.22 | 0.45 | 50 | 40.4% | 1.62 [1.44 , 1.80] | + + + + + + ? |
| **Subtotal (95% CI)** | | | **159** | | | **148** | **100.0%** | **1.86 [1.02 , 2.71]** | |
| Heterogeneity: Tau² = 0.46; Chi² = 8.74, df = 3 (P = 0.03); I² = 66% | | | | | | | | | |
| Test for overall effect: Z = 4.30 (P < 0.0001) | | | | | | | | | |
| **2.12.5 Supplemental breast milk vs sucrose (moderate concentration: 20% to 33%)** | | | | | | | | | |
| Bozlak 2017 (Eye examination) | 8.45 | 1.38 | 29 | 9.1 | 1.82 | 29 | 49.5% | -0.65 [-1.48 , 0.18] | + + + + + + ? |
| Desai 2017 (Suctioning) | 12.88 | 3.59 | 36 | 12.11 | 3.87 | 36 | 21.3% | 0.77 [-0.95 , 2.49] | + + − ? + + ? |
| Taplak 2017 (Eye examination) | 16.06 | 2.41 | 20 | 15.58 | 2.02 | 20 | 29.1% | 0.48 [-0.90 , 1.86] | ? ? + + + + ? |
| **Subtotal (95% CI)** | | | **85** | | | **85** | **100.0%** | **-0.02 [-0.94 , 0.91]** | |
| Heterogeneity: Tau² = 0.27; Chi² = 3.27, df = 2 (P = 0.19); I² = 39% | | | | | | | | | |
| Test for overall effect: Z = 0.04 (P = 0.97) | | | | | | | | | |




**Risk of bias legend**
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

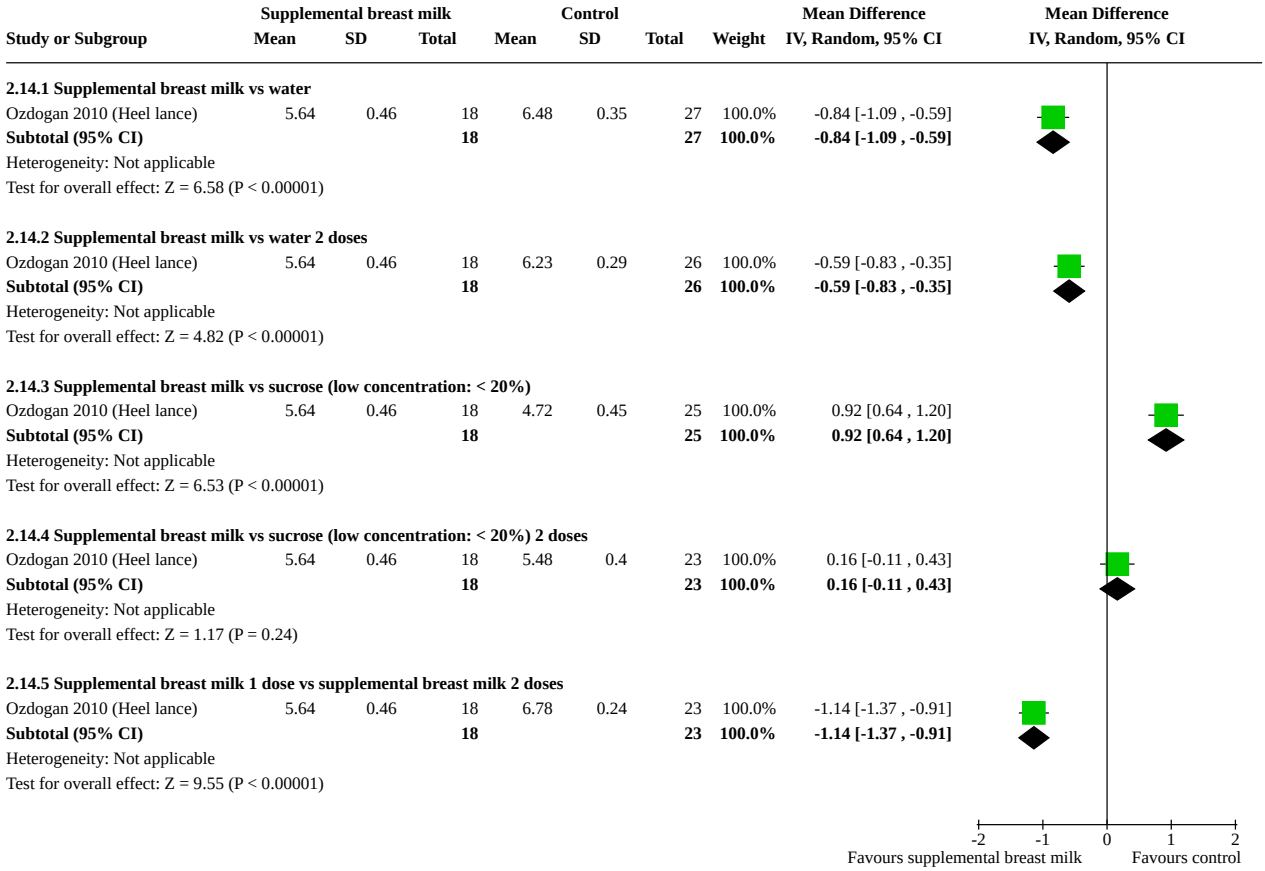
## Analysis 2.13. Comparison 2: Supplemental breast milk vs control, Outcome 13: Neonatal Facial Coding System score at 3 minutes

| Study or Subgroup | Supplemental breast milk Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| **2.13.1 Supplemental breast milk vs placebo** | | | | | | | | |
| Bucher 2000 (Heel lance) | 2.86 | 0.84 | 20 | 2.95 | 0.73 | 20 | 38.5% | -0.09 [-0.58 , 0.40] |
| Upadhyay 2004 (Venipuncture) | 0.6 | 1.3 | 40 | 2.6 | 2.4 | 41 | 35.2% | -2.00 [-2.84 , -1.16] |
| Uyan 2005 (Heel lance) | 3.08 | 2.77 | 41 | 3.54 | 3.14 | 21 | 26.4% | -0.46 [-2.05 , 1.13] |
| **Subtotal (95% CI)** | | | **101** | | | **82** | **100.0%** | **-0.86 [-2.24 , 0.52]** |
| Heterogeneity: Tau² = 1.23; Chi² = 14.93, df = 2 (P = 0.0006); I² = 87% | | | | | | | | |
| Test for overall effect: Z = 1.22 (P = 0.22) | | | | | | | | |
| **2.13.2 Supplemental breast milk vs glycine** | | | | | | | | |
| Bucher 2000 (Heel lance) | 2.86 | 0.84 | 20 | 3.33 | 0.52 | 20 | 100.0% | -0.47 [-0.90 , -0.04] |
| **Subtotal (95% CI)** | | | **20** | | | **20** | **100.0%** | **-0.47 [-0.90 , -0.04]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 2.13 (P = 0.03) | | | | | | | | |
| **2.13.3 Supplemental breast milk vs artificial sweetener** | | | | | | | | |
| Bucher 2000 (Heel lance) | 2.86 | 0.84 | 20 | 3.08 | 0.49 | 20 | 100.0% | -0.22 [-0.65 , 0.21] |
| **Subtotal (95% CI)** | | | **20** | | | **20** | **100.0%** | **-0.22 [-0.65 , 0.21]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 1.01 (P = 0.31) | | | | | | | | |

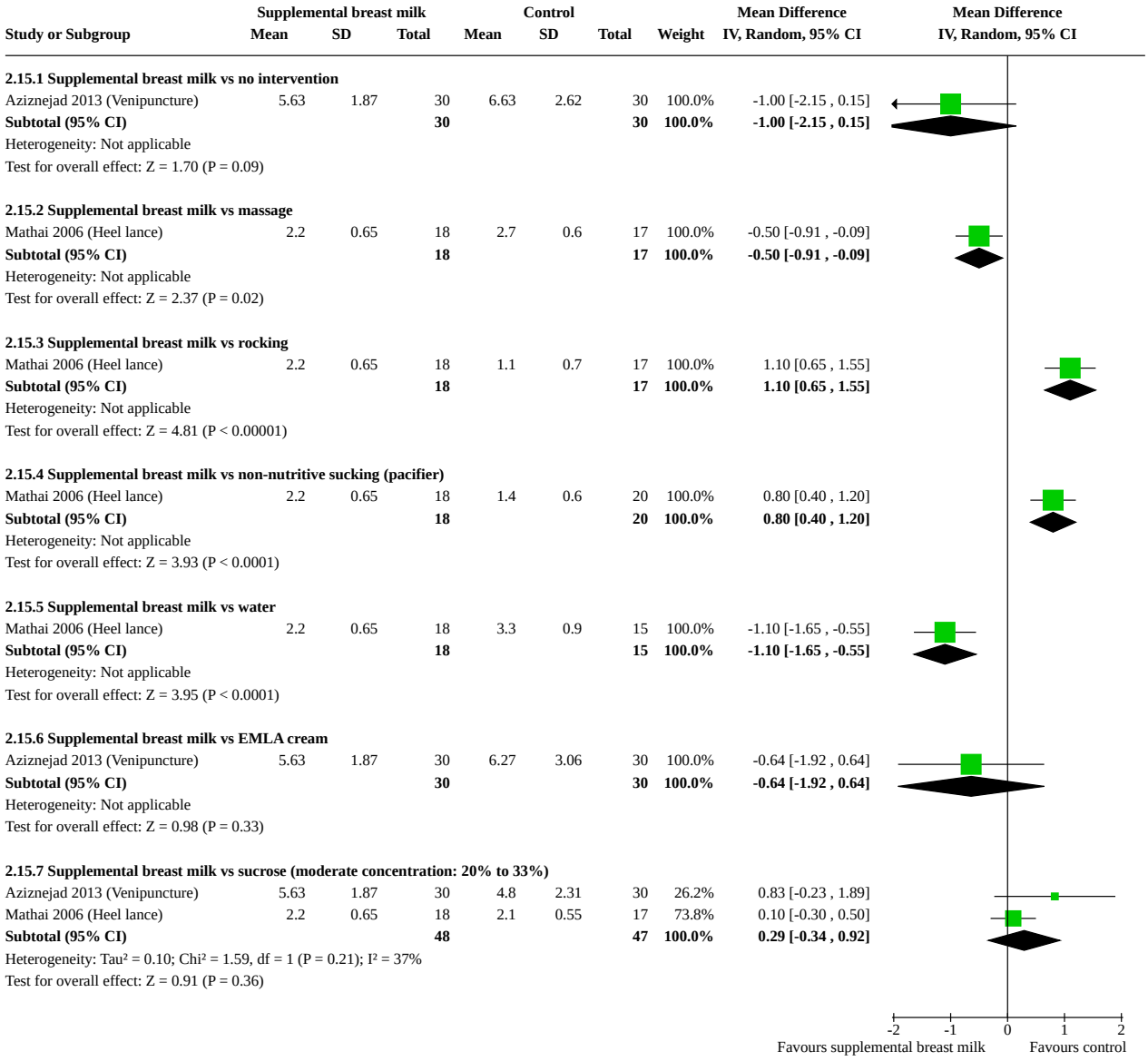



**Risk of bias legend**
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding (performance bias and detection bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

## Analysis 2.14. Comparison 2: Supplemental breast milk vs control, Outcome 14: Neonatal Facial Coding System score at 2 minutes

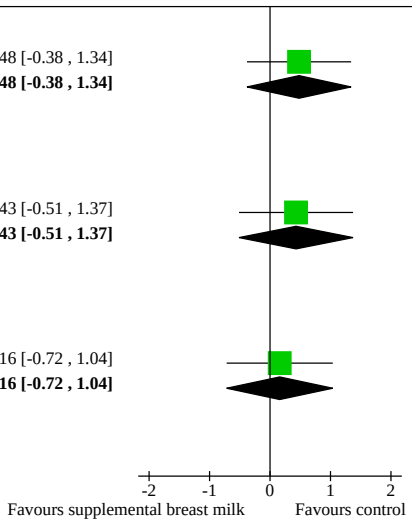


## Analysis 2.15. Comparison 2: Supplemental breast milk vs control, Outcome 15: Douleur Aigue du Nouveau-né (DAN) scale



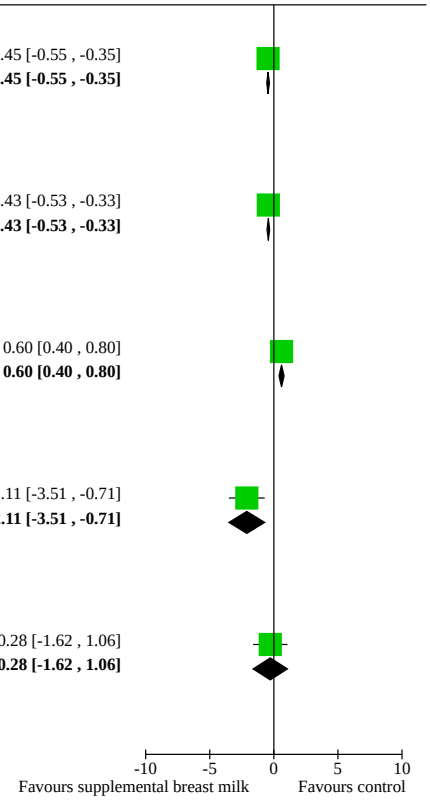
## Analysis 2.16. Comparison 2: Supplemental breast milk vs control, Outcome 16: Body pain score

| Study or Subgroup | Supplemental breast milk Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| **2.16.1 Supplemental breast milk vs placebo** | | | | | | | | |
| Bucher 2000 (Heel lance) | 4.43 | 1.28 | 20 | 3.95 | 1.49 | 20 | 100.0% | 0.48 [-0.38 , 1.34] |
| **Subtotal (95% CI)** | | | **20** | | | **20** | **100.0%** | **0.48 [-0.38 , 1.34]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 1.09 (P = 0.27) | | | | | | | | |
| **2.16.2 Supplemental breast milk vs glycine** | | | | | | | | |
| Bucher 2000 (Heel lance) | 4.43 | 1.28 | 20 | 4 | 1.73 | 20 | 100.0% | 0.43 [-0.51 , 1.37] |
| **Subtotal (95% CI)** | | | **20** | | | **20** | **100.0%** | **0.43 [-0.51 , 1.37]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 0.89 (P = 0.37) | | | | | | | | |
| **2.16.3 Supplemental breast milk vs artificial sweetener** | | | | | | | | |
| Bucher 2000 (Heel lance) | 4.43 | 1.28 | 20 | 4.27 | 1.54 | 20 | 100.0% | 0.16 [-0.72 , 1.04] |
| **Subtotal (95% CI)** | | | **20** | | | **20** | **100.0%** | **0.16 [-0.72 , 1.04]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 0.36 (P = 0.72) | | | | | | | | |

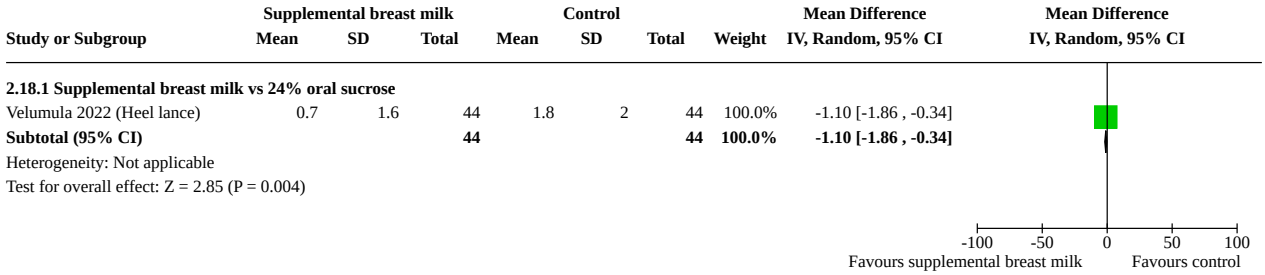



## Analysis 2.17. Comparison 2: Supplemental breast milk vs control, Outcome 17: Neonatal Pain, Agitation and Sedation Scale (N-PASS)

| Study or Subgroup | Supplemental breast milk Mean | SD | Total | Control Mean | SD | Total | Weight | Mean Difference IV, Random, 95% CI |
|---|---|---|---|---|---|---|---|---|
| **2.17.1 Supplemental breast milk vs no intervention** | | | | | | | | |
| Erdogan 2022 (Venipuncture) | 0.9 | 0.16 | 30 | 1.35 | 0.24 | 30 | 100.0% | -0.45 [-0.55 , -0.35] |
| **Subtotal (95% CI)** | | | **30** | | | **30** | **100.0%** | **-0.45 [-0.55 , -0.35]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 8.54 (P < 0.00001) | | | | | | | | |
| **2.17.2 Supplemental breast milk vs breast milk smell** | | | | | | | | |
| Erdogan 2022 (Venipuncture) | 0.9 | 0.16 | 30 | 1.33 | 0.24 | 30 | 100.0% | -0.43 [-0.53 , -0.33] |
| **Subtotal (95% CI)** | | | **30** | | | **30** | **100.0%** | **-0.43 [-0.53 , -0.33]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 8.17 (P < 0.00001) | | | | | | | | |
| **2.17.3 Supplemental breast milk vs breast milk taste + smell** | | | | | | | | |
| Erdogan 2022 (Venipuncture) | 0.9 | 0.16 | 30 | 0.3 | 0.53 | 30 | 100.0% | 0.60 [0.40 , 0.80] |
| **Subtotal (95% CI)** | | | **30** | | | **30** | **100.0%** | **0.60 [0.40 , 0.80]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 5.94 (P < 0.00001) | | | | | | | | |
| **2.17.4 Supplemental breast milk vs water** | | | | | | | | |
| Ou-Yang 2013 (Heel lance) | 5.55 | 3.29 | 40 | 7.66 | 3.24 | 44 | 100.0% | -2.11 [-3.51 , -0.71] |
| **Subtotal (95% CI)** | | | **40** | | | **44** | **100.0%** | **-2.11 [-3.51 , -0.71]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 2.96 (P = 0.003) | | | | | | | | |
| **2.17.5 Supplemental breast milk vs glucose (moderate concentration: 20% to 33%)** | | | | | | | | |
| Ou-Yang 2013 (Heel lance) | 5.55 | 3.29 | 40 | 5.83 | 2.77 | 39 | 100.0% | -0.28 [-1.62 , 1.06] |
| **Subtotal (95% CI)** | | | **40** | | | **39** | **100.0%** | **-0.28 [-1.62 , 1.06]** |
| Heterogeneity: Not applicable | | | | | | | | |
| Test for overall effect: Z = 0.41 (P = 0.68) | | | | | | | | |



**Analysis 2.18. Comparison 2: Supplemental breast milk vs control, Outcome 18: Premature Infant Pain Profile - Revised scale**




## APPENDICES

### Appendix 1. Cochrane CENTRAL search strategy

Searched via CRS

Search date: 2 August 2022

| | | |
|---|---|---|
| 1 | MESH DESCRIPTOR Breast Feeding EXPLODE ALL AND CENTRAL:TARGET | 2207 |
| 2 | MESH DESCRIPTOR Colostrum EXPLODE ALL AND CENTRAL:TARGET | 136 |
| 3 | MESH DESCRIPTOR Milk, Human EXPLODE ALL AND CENTRAL:TARGET | 1174 |
| 4 | (breastfeed* or breast feed* or breast fed or breastfed or breast milk or breast-milk* or colostrum or expressed breast milk or EBM or foremilk or hindmilk or ((human or breast* or mother* or expressed or maternal or donor*) ADJ2 milk*)) AND CENTRAL:TARGET | 13850 |
| 5 | #1 OR #2 OR #3 OR #4 | 13850 |
| 6 | MESH DESCRIPTOR Analgesia EXPLODE ALL AND CENTRAL:TARGET | 8658 |
| 7 | MESH DESCRIPTOR Analgesics EXPLODE ALL AND CENTRAL:TARGET | 54106 |
| 8 | MESH DESCRIPTOR Anxiety EXPLODE ALL AND CENTRAL:TARGET | 9443 |
| 9 | MESH DESCRIPTOR Behavior EXPLODE ALL AND CENTRAL:TARGET | 101051 |
| 10 | MESH DESCRIPTOR Crying EXPLODE ALL AND CENTRAL:TARGET | 336 |
| 11 | MESH DESCRIPTOR Facial Expression EXPLODE ALL AND CENTRAL:TARGET | 719 |
| 12 | MESH DESCRIPTOR Fear EXPLODE ALL AND CENTRAL:TARGET | 1731 |
| 13 | MESH DESCRIPTOR Gestures EXPLODE ALL AND CENTRAL:TARGET | 71 |
| 14 | MESH DESCRIPTOR Heart Rate EXPLODE ALL AND CENTRAL:TARGET | 20713 |
| 15 | MESH DESCRIPTOR Infant Behavior EXPLODE ALL AND CENTRAL:TARGET | 356 |

*(Continued)*

| | | |
|---|---|---|
| 16 | MESH DESCRIPTOR Oxygen Consumption EXPLODE ALL AND CENTRAL:TARGET | 7083 |
| 17 | MESH DESCRIPTOR Pain EXPLODE ALL AND CENTRAL:TARGET | 56453 |
| 18 | MESH DESCRIPTOR Pain Management EXPLODE ALL AND CENTRAL:TARGET | 4387 |
| 19 | MESH DESCRIPTOR Pain Measurement EXPLODE ALL AND CENTRAL:TARGET | 23183 |
| 20 | MESH DESCRIPTOR Pain Threshold EXPLODE ALL AND CENTRAL:TARGET | 1838 |
| 21 | MESH DESCRIPTOR Panic EXPLODE ALL AND CENTRAL:TARGET | 268 |
| 22 | MESH DESCRIPTOR Wakefulness EXPLODE ALL AND CENTRAL:TARGET | 1077 |
| 23 | (analgesi* or anxiet* or anxious or behavior* or behaviour* or cry* or discomfort* or distress* or Douleur Aigue du Nouveau ne or DAN or facial expression* or fear* or fright* or gesture* or grimac* or heart rate* or Median Premature Infant Pain Profile score* or Neonatal Facial Action* or Neonatal Facial Activity Coding System or Neonatal Facial Coding Score* or NFCS or neonatal facial coding system or nociceptive reaction* or orosensorial antinociceptive effect* or oxygen consumption or oxygen saturation* or pain* or panic* or sleep wake state* or wakefulness) AND CENTRAL:TARGET | 511893 |
| 24 | #23 OR #22 OR #21 OR #20 OR #19 OR #18 OR #17 OR #16 OR #15 OR #14 OR #13 OR #12 OR #11 OR #10 OR #9 OR #8 OR #7 OR #6 | 578244 |
| 25 | MESH DESCRIPTOR Biolistics EXPLODE ALL AND CENTRAL:TARGET | 2 |
| 26 | MESH DESCRIPTOR Blood Specimen Collection EXPLODE ALL AND CENTRAL:TARGET | 747 |
| 27 | MESH DESCRIPTOR Cannula EXPLODE ALL AND CENTRAL:TARGET | 192 |
| 28 | MESH DESCRIPTOR Catheterization EXPLODE ALL AND CENTRAL:TARGET | 9960 |
| 29 | MESH DESCRIPTOR Catheterization, Peripheral EXPLODE ALL AND CENTRAL:TARGET | 1080 |
| 30 | MESH DESCRIPTOR Catheterization, Central Venous EXPLODE ALL AND CENTRAL:TARGET | 885 |
| 31 | MESH DESCRIPTOR Diagnostic Techniques and Procedures EXPLODE ALL AND CENTRAL:TARGET | 251795 |
| 32 | MESH DESCRIPTOR Heel EXPLODE ALL AND CENTRAL:TARGET | 261 |
| 33 | MESH DESCRIPTOR Infusions, Parenteral EXPLODE ALL AND CENTRAL:TARGET | 12886 |
| 34 | MESH DESCRIPTOR Infusions, Intravenous EXPLODE ALL AND CENTRAL:TARGET | 10678 |
| 35 | MESH DESCRIPTOR Injections, Intravenous EXPLODE ALL AND CENTRAL:TARGET | 7757 |
| 36 | MESH DESCRIPTOR Injections EXPLODE ALL AND CENTRAL:TARGET | 23669 |

(Continued)

| | | |
|---|---|---|
| 37 | MESH DESCRIPTOR Injections, Intradermal EXPLODE ALL AND CENTRAL:TARGET | 741 |
| 38 | MESH DESCRIPTOR Injections, Jet EXPLODE ALL AND CENTRAL:TARGET | 103 |
| 39 | MESH DESCRIPTOR Injections, Subcutaneous EXPLODE ALL AND CENTRAL:TARGET | 4792 |
| 40 | MESH DESCRIPTOR Injections, Intramuscular EXPLODE ALL AND CENTRAL:TARGET | 4179 |
| 41 | MESH DESCRIPTOR Needles EXPLODE ALL AND CENTRAL:TARGET | 1306 |
| 42 | MESH DESCRIPTOR Needlestick Injuries EXPLODE ALL AND CENTRAL:TARGET | 90 |
| 43 | MESH DESCRIPTOR Phlebotomy EXPLODE ALL AND CENTRAL:TARGET | 494 |
| 44 | MESH DESCRIPTOR Punctures EXPLODE ALL AND CENTRAL:TARGET | 3097 |
| 45 | MESH DESCRIPTOR Vaccination EXPLODE ALL AND CENTRAL:TARGET | 2983 |
| 46 | (biolistics or Blood Specimen Collection* or blood collection* or blood sampl* or cannula* or catheterization* or (Diagnostic Techniques and Procedure*) or (heel* ADJ2 (lance* or lancing or prick* or stick*)) or immunization* or infusion* or injection* or ((intravenous or intravascular) ADJ2 access*) or needle* or (painf* ADJ2 procedur*) or phlebotom* or puncture* or vaccinat* or venepuncture* or venipuncture*) AND CENTRAL:TARGET | 243465 |
| 47 | #46 OR #45 OR #44 OR #43 OR #42 OR #41 OR #40 OR #39 OR #38 OR #37 OR #36 OR #35 OR #34 OR #33 OR #32 OR #31 OR #30 OR #29 OR #28 OR #27 OR #26 OR #25 | 452150 |
| 48 | MESH DESCRIPTOR Infant, Newborn EXPLODE ALL AND CENTRAL:TARGET | 17913 |
| 49 | infant or infants or infant's or "infant s" or infantile or infancy or newborn* or "new born" or "new borns" or "newly born" or neonat* or baby* or babies or premature or prematures or prematurity or preterm or preterms or "pre term" or premies or "low birth weight" or "low birthweight" or VLBW or LBW or ELBW or NICU AND CENTRAL:TARGET | 97638 |
| 50 | #48 OR #49 | 97638 |
| 51 | #5 AND #24 AND #47 AND #50 | 1132 |
| 52 | 2011 TO 2022:YR AND CENTRAL TARGET | 1050031 |
| 53 | #51 AND #52 | 691 |

## Appendix 2. MEDLINE strategy

| | |
|---|---|
| | Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations, Daily and Versions 1946 to 1 August 2022 |

*(Continued)*

| # | Searches | Results |
|---|---|---|
| 1 | exp Breast Feeding/ | 42470 |
| 2 | exp Colostrum/ | 6688 |
| 3 | exp Milk, Human/ | 21799 |
| 4 | (breastfeed* or breast feed* or breast fed or breastfed or breast milk or breastmilk* or colostrum or expressed breast milk or EBM or foremilk or hindmilk or ((human or breast* or mother* or expressed or maternal or donor*) adj2 milk*)).mp. | 97610 |
| 5 | or/1-4 [Breastfeeding/Breast Milk] | 97610 |
| 6 | exp Analgesia/ | 47926 |
| 7 | exp Analgesics/ | 574200 |
| 8 | exp Anxiety/ | 104573 |
| 9 | exp Behavior/ | 2021826 |
| 10 | exp Crying/ | 2824 |
| 11 | exp Facial Expression/ | 16610 |
| 12 | exp Fear/ | 38061 |
| 13 | exp Gestures/ | 3195 |
| 14 | exp Heart Rate/ | 178919 |
| 15 | exp Infant Behavior/ | 3770 |
| 16 | exp Oxygen Consumption/ | 109242 |
| 17 | exp Pain/ | 440387 |
| 18 | exp Pain Management/ | 39582 |
| 19 | exp Pain Measurement/ | 93497 |
| 20 | exp Pain Threshold/ | 13990 |
| 21 | exp Panic/ | 2685 |
| 22 | exp Wakefulness/ | 19740 |
| 23 | (analgesi* or anxiet* or anxious or behavior* or behaviour* or cry* or discomfort* or distress* or Douleur Aigue du Nouveau ne or DAN or facial expression* or fear* or fright* or gesture* or grimac* or heart rate* or Median Premature Infant Pain Profile score* or Neonatal Facial Action* or Neonatal Facial Activity Coding System or Neonatal Facial Coding Score* or NFCS or neonatal facial coding system or nociceptive reaction* or orosensorial antinociceptive effect* | 4298943 |

(Continued)

| | | |
|---|---|---|
| | or oxygen consumption or oxygen saturation* or pain* or panic* or sleep wake state* or wakefulness).mp. | |
| 24 | or/6-23 [Analgesia/Pain/Signs of Pain in Neonate] | 5726393 |
| 25 | exp Biolistics/ | 1124 |
| 26 | exp Blood Specimen Collection/ | 16256 |
| 27 | exp Cannula/ | 1589 |
| 28 | exp Catheterization/ or exp Catheterization, Peripheral/ or exp Catheterization, Central Venous/ | 205228 |
| 29 | exp "Diagnostic Techniques and Procedures"/ | 7724337 |
| 30 | exp Heel/ | 3609 |
| 31 | exp Infusions, Parenteral/ | 94950 |
| 32 | exp infusions, intravenous/ | 56750 |
| 33 | exp Injections, Intravenous/ or exp Injections/ or exp Injections, Intradermal/ or exp Injections, Jet/ or exp Injections, Subcutaneous/ or exp Injections, Intramuscular/ | 295067 |
| 34 | exp Needles/ | 16949 |
| 35 | exp Needlestick Injuries/ | 3683 |
| 36 | exp Phlebotomy/ | 5984 |
| 37 | exp Punctures/ | 117705 |
| 38 | exp Vaccination/ | 102201 |
| 39 | (biolistics or Blood Specimen Collection* or blood collection* or blood sampl* or cannula* or catheterization* or (Diagnostic Techniques and Procedure*) or (heel* adj2 (lance* or lancing or prick* or stick*)) or immunization* or infusion* or injection* or ((intravenous or intravascular) adj2 access*) or needle* or (painf* adj2 procedur*) or phlebotom* or puncture* or vaccinat* or venepuncture* or venipuncture*).mp. | 1922715 |
| 40 | or/25-39 [Painful procedures] | 8974522 |
| 41 | exp infant, newborn/ | 657614 |
| 42 | (newborn* or new born or new borns or newly born or baby* or babies or premature or prematurity or preterm or pre term or low birth weight or low birthweight or VLBW or LBW or infant or infants or 'infant s' or infant's or infantile or infancy or neonat*).ti,ab. | 929475 |
| 43 | or/41-42 [Neonatal Terms] | 1221817 |
| 44 | randomized controlled trial.pt. | 574311 |
| 45 | controlled clinical trial.pt. | 94967 |

(Continued)

| | | |
|---|---|---|
| 46 | randomized.ab. | 570933 |
| 47 | placebo.ab. | 230652 |
| 48 | drug therapy.fs. | 2517339 |
| 49 | randomly.ab. | 388372 |
| 50 | trial.ab. | 611164 |
| 51 | groups.ab. | 2389178 |
| 52 | or/44-51 | 5425910 |
| 53 | exp animals/ not humans.sh. | 5033970 |
| 54 | 52 not 53 [RCT Filter--Cochrane] | 4726055 |
| 55 | 5 and 24 and 40 and 43 and 54 | 2085 |
| 56 | limit 55 to yr="2011 -Current" | 1012 |

## Appendix 3. Embase strategy

| | |
|---|---|
| | Embase 1974 to 1 August 2022 |

| # | Searches | Results |
|---|---|---|
| 1 | breast feeding/ | 60904 |
| 2 | colostrum/ | 8176 |
| 3 | exp breast milk/ | 31564 |
| 4 | (breastfeed* or breast feed* or breast fed or breastfed or breast milk or breast-milk* or colostrum or expressed breast milk or EBM or foremilk or hindmilk or ((human or breast* or mother* or expressed or maternal or donor*) adj2 milk*)).mp. | 119494 |
| 5 | or/1-4 [Breastfeeding/Breast Milk] | 119494 |
| 6 | exp *analgesic agent/ | 400630 |
| 7 | exp *analgesia/ | 69478 |
| 8 | *anxiety/ or *fear/ | 76621 |
| 9 | *behavior/ or behavioral stress/ or coping behavior/ or defensive behavior/ or emotion/ or feeding behavior/ or illness behavior/ or motor activity/ | 376404 |
| 10 | crying/ or nonverbal communication/ | 16094 |

*(Continued)*

| | | |
|---|---|---|
| 11 | facial expression/ | 24241 |
| 12 | *fear/ | 18396 |
| 13 | *gesture/ or *psychomotor activity/ | 3910 |
| 14 | exp *heart rate/ | 65092 |
| 15 | *child behavior/ | 19282 |
| 16 | oxygen consumption/ or exp *metabolism/ | 1543515 |
| 17 | *pain/ or *injection pain/ or *injection site pain/ | 96669 |
| 18 | exp *pain/ | 447738 |
| 19 | exp *pain measurement/ or exp *pain assessment/ | 12510 |
| 20 | exp *pain threshold/ | 4095 |
| 21 | *panic/ | 10066 |
| 22 | *wakefulness/ | 6851 |
| 23 | (analgesi* or anxiet* or anxious or behavior* or behaviour* or cry* or discomfort* or distress* or Douleur Aigue du Nouveau ne or DAN or facial expression* or fear* or fright* or gesture* or grimac* or heart rate* or Median Premature Infant Pain Profile score* or Neonatal Facial Action* or Neonatal Facial Activity Coding System or Neonatal Facial Coding Score* or NFCS or neonatal facial coding system or nociceptive reaction* or orosensorial antinociceptive effect* or oxygen consumption or oxygen saturation* or pain* or panic* or sleep wake state* or wakefulness).mp. | 5664078 |
| 24 | or/6-23 [Analgesia/Pain/Signs of Pain in Neonate] | 7361074 |
| 25 | biostatic activity/ | 11 |
| 26 | *blood sampling/ or hematological procedure/ | 16348 |
| 27 | exp *cannula/ | 3216 |
| 28 | *catheterization/ or blood vessel catheterization/ | 11980 |
| 29 | exp *diagnostic procedure/ | 4533106 |
| 30 | exp *heel/ | 1724 |
| 31 | exp *parenteral drug administration/ | 25121 |
| 32 | exp *intravenous drug administration/ | 5361 |
| 33 | exp *injection/ | 16360 |
| 34 | exp *jet injection/ | 465 |
| 35 | exp *intradermal drug administration/ | 251 |

*(Continued)*

| | | |
|---|---|---|
| 36 | exp *subcutaneous drug administration/ | 1010 |
| 37 | exp *intramuscular drug administration/ | 1052 |
| 38 | exp *needle/ | 11942 |
| 39 | exp *needlestick injury/ | 2370 |
| 40 | *blood sampling/ | 15374 |
| 41 | exp *puncture/ | 3876 |
| 42 | exp *vaccination/ | 87430 |
| 43 | (biolistics or Blood Specimen Collection* or blood collection* or blood sampl* or cannula* or catheterization* or (Diagnostic Techniques and Procedure*) or (heel* adj2 (lance* or lancing or prick* or stick*)) or immunization* or infusion* or injection* or ((intravenous or intravascular) adj2 access*) or needle* or (painf* adj2 procedur*) or phlebotom* or puncture* or vaccinat* or venepuncture* or venipuncture*).mp. | 2522550 |
| 44 | or/25-43 [Painful procedures] | 6707512 |
| 45 | exp newborn/ | 575507 |
| 46 | (newborn* or new born or new borns or newly born or baby* or babies or premature or prematurity or preterm or pre term or low birth weight or low birthweight or VLBW or LBW or infant or infants or 'infant s' or infant's or infantile or infancy or neonat*).ti,ab. | 1133961 |
| 47 | or/45-46 [Neonate] | 1345500 |
| 48 | Randomized controlled trial/ or Controlled clinical study/ | 910963 |
| 49 | random$.ti,ab,kw. | 1822081 |
| 50 | Randomization/ | 94537 |
| 51 | placebo.ti,ab,kw. | 344651 |
| 52 | ((double or single or doubly or singly) adj (blind or blinded or blindly)).ti,ab,kw. | 259006 |
| 53 | double blind procedure/ | 197258 |
| 54 | (controlled adj7 (study or design or trial)).ti,ab,kw. | 414358 |
| 55 | parallel group$1.ti,ab. | 29780 |
| 56 | (crossover or cross over).ti,ab. | 117393 |
| 57 | ((assign$ or match or matched or allocation) adj5 (alternate or group$1 or intervention$1 or patient$1 or subject$1 or participant$1)).ti,ab. | 384820 |
| 58 | (open adj label).ti,ab. | 98528 |
| 59 | (quasirandom* or quasi-random* or randomi* or randomly).ti,ab,kw,kf. | 1486117 |

*(Continued)*

| | | |
|---|---|---|
| 60 | (control* adj2 (group? or random*)).ti,ab,kw,kf. | 1209908 |
| 61 | or/48-60 [ Terms based on Cochrane Central strategy- How Central is Created] | 3115654 |
| 62 | (exp animals/ or exp invertebrate/ or animal experiment/ or animal model/ or animal tissue/ or animal cell/ or nonhuman/) and (human/ or normal human/ or human cell/) | 23918834 |
| 63 | exp animals/ or exp invertebrate/ or animal experiment/ or animal model/ or animal tissue/ or animal cell/ or nonhuman/ | 30867992 |
| 64 | 63 not 62 [Animal Exclusion-https://community-cochrane-org.ezproxy.uvm.edu/sites/default/files/uploads/inline-files/Embase%20animal%20filter.pdf] | 6949158 |
| 65 | 61 not 64 [Filter: RCT-EMBASE] | 2678272 |
| 66 | 5 and 24 and 44 and 47 and 65 | 420 |
| 67 | 66 and ("2011" or "2012" or "2013" or "2014" or "2015" or "2016" or "2017" or "2018" or "2019" or 202*).yr. | 316 |

## Appendix 4. CINAHL strategy

| | | |
|---|---|---|
| | CINAHL Complete (Ebsco) | |
| | 02-Aug-22 | |
| | Advanced search screen | Results |
| S1 | ( (breastfeed* or breast feed* or breast fed or breast milk or breastmilk* or colostrum or expressed breast milk or EBM or foremilk or hindmilk or ((human or breast* or mother* or expressed or maternal or donor*) N2 milk*)) ) AND ( (analgesi* or anxiet* or anxious or behavior* or behaviour* or cry* or discomfort* or distress* or Douleur Aigue du Nouveau ne or DAN or facial expression* or fear* or fright* or gesture* or grimac* or heart rate* or Median Premature Infant Pain Profile score* or Neonatal Facial Action* or Neonatal Facial Activity Coding System or Neonatal Facial Coding Score* or NFCS or neonatal facial coding system or nociceptive reaction* or orosensorial antinociceptive effect* or oxygen consumption or oxygen saturation* or pain* or panic* or sleep wake state* or wakefulness) ) AND ( (biolistics or Blood Specimen Collection* or blood collection* or blood sampl* or cannula* or catheterization* or (Diagnostic Techniques and Procedure*) or (heel* N2 (lance* or lancing or prick* or stick*)) or immunization* or infusion* or injection* or ((intravenous or intravascular) N2 access*) or needle* or (painf* N2 procedur*) or phlebotom* or puncture* or vaccinat* or venepuncture* or venipuncture*) ) AND ( (infant or infants or infant's or infantile or infancy or newborn* or "new born" or "new borns" or "newly born" or neonat* or baby* or babies or premature or prematures or prematurity or preterm or preterms or "pre term" or premies or "low birth weight" or "low birthweight" or VLBW or LBW) ) AND ( (randomized controlled trial OR controlled clinical trial OR randomized OR randomised OR placebo OR clinical trials as topic OR randomly OR trial OR PT clinical trial) ) [Published Date: 20110101-20221231 ] | 96 |

## Appendix 5. Trial registry strategies

| Date | Source | Terms | Results |
|---|---|---|---|
| 2 August 2022 | ICTRP | Breastfeeding [Title] AND Pain [Condition] | 2 |
| 2 August 2022 | ICTRP | Breast milk [Title] AND Pain [Condition] | 3 |
| 2 August 2022 | ICTRP | Breastfeeding [Title] AND Procedure [Condition] | 0 |
| 2 August 2022 | ICTRP | Breastfeeding [Title] AND Prick [Condition] | 0 |
| 2 August 2022 | ICTRP | Breastfeeding [Title] AND Needle [Condition] | 0 |
| 2 August 2022 | ICTRP | Breastfeeding [Intervention] AND pain [Title] | 3 |
| 2 August 2022 | ICTRP | Breastfeeding [Intervention] AND needle [Title] | 0 |
| 2 August 2022 | ICTRP | Breast milk [Intervention] AND pain [Title] | 8 |
| 2 August 2022 | ICTRP | Breast milk [Intervention] AND procedure [Title] | 2 |
| 2 August 2022 | ICTRP | Breast milk [Intervention] AND needle [Title] | 1 |
| 2 August 2022 | ICTRP | Breast milk [Intervention] AND neonatal [Condition] | 0 |
| 2 August 2022 | ICTRP | Breastfeeding [Intervention] AND neonate [Condition] | 0 |
| 2 August 2022 | ICTRP | Breastfeeding [Intervention] AND catheter [Title] | 0 |
| 2 August 2022 | ICTRP | Breastfeeding [Intervention] AND catheter [Condition] | 0 |
| 2 August 2022 | ISRCTN | Breastfeeding [Intervention] AND Neonatal [condition] | 0 |
| 2 August 2022 | ISRCTN | Breastfeeding [Intervention] AND Pain [condition] | 0 |
| 2 August 2022 | ISRCTN | Breastfeeding [Intervention] AND Pain [Text] | 0 |
| 2 August 2022 | clinicaltrials-gov | Breastfeeding [Other term] AND neonatal [Condition] AND Child: Birth-17 | 30 |
| 2 August 2022 | clinicaltrials-gov | procedure neonatal [conditon] AND Breastfeeding | 0 |
| 2 August 2022 | clinicaltrials-gov | neonatal pain [condition] AND breast milk [Other term] AND Child age group | 14 |
| 2 August 2022 | clinicaltrials-gov | Neonatal pain [Condition] AND breastfeed | 0 |
| 2 August 2022 | clinicaltrials-gov | neonatal pain [condition] AND breast milk [Other term] | 14 |
| Total | | | 77 |

## Appendix 6. 2011 Search methods and strategies

We ran searches using the OVID search platforms in the following databases: MEDLINE, EMBASE, CINAHL and CCTR. We retrieved a total of **2203** references from all 3 databases.

The following tables and text record the search strategies and terms used.

**MEDLINE:**

The search strategy for MEDLINE (1948 to **16 September 2011**) retrieved **699** references. We used a combination of MeSH and free text terms for

| Set | History | Results | Comments |
|---|---|---|---|
| 1 | Infant, newborn/ or infant, low birth weight/ or infant, small for gestational age/ or infant, very low birth weight/ or infant, premature/ or exp Infant, Newborn, Diseases/ or pregnancy, high-risk/ or quadruplets/ or quintuplets/ or superfetation/ or triplets/ or twins/ or twins, dizygotic/ or twins, monozygotic/ or (infan: or neonat: or newborn: or prematur: or iugr or sga or vlbw or lbw or elbw).ti,ab. or ((intrauterine adj2 growth adj2 restrict:) or (intrauterine adj2 growth adj2 retard:)).ti,ab. | 809582 | Infant age group Terms |
| 2 | Breast Feeding/ or Milk, Human/ or (breastfeed* or (breast adj2 milk) or breastmilk or breastfed or (breast adj2 feed*) or (breast adj2 fed)).mp. | 41519 | Breast Feeding terms |
| 3 | Pain Measurement/ or exp pain/ or Antibody Formation/ or Crying/ or anxiety/ or fear/ or panic/ or (adverse adj2 effect:).ti,ab. or (side adj2 effect:).ti,ab. or (skin adj2 reaction:).ti,ab. or (distress* or discomfort* or fright* or anxious).ti,ab. | 698718 | Pain terms |
| 4 | 1 and 2 and 3 | 1283 | Base clinical set 1 |
| 5 | needles/ or (needle: adj2 (gauge: or length: or thick: or angle: or size:)).ti,ab. or injections/ or injections, intramuscular/ or injections, subcutaneous/ or injections, intradermal/ or injections, jet/ or biolistics/ or ((needle: or inject: or vaccinat:) adj2 (technique: or techinc: or aspirat: or angle: or speed: or slow: or fast: or order:)).ti,ab. or punctures/ or blood specimen collection/ or phlebotomy/ or infusions, parenteral/ or infusions, intravenous/ or injections, intravenous/ or catheterization/ or catheterization, central venous/ or catheterization, peripheral/ or cannula*.mp. or ((intravenous or intravascular) adj2 access).ti,ab. or venipuncture*.mp. or (painf* adj2 procedur*).ti,ab. | 338692 | Procedure terms |
| 6 | 2 and 5 | 359 | Base clinical set 2 |
| 7 | 4 or 6 | 1579 | Base Results |
| 8 | ("clinical trial, all" or clinical trial).pt. or clinical trials as topic/ or clinical trial, phase i.pt. or clinical trials, phase i as topic/ or clinical trial, phase iii.pt. or clinical trials, phase iii as topic/ or clinical trial, phase iv.pt. or clinical trials, phase iv as topic/ or controlled clinical trial.pt. or controlled clinical trials as topic/ or meta-analysis.pt. or meta-analysis as topic/ or multicenter study.pt. or multicenter studies as topic/ or randomized controlled trial.pt. or randomized controlled trials as topic/ or eval- | 4754353 | Study Design/Methodology terms |

(Continued)

| | | | |
|---|---|---|---|
| | uation studies as topic/ or validation studies as topic/ or evaluation study.pt. or validation study.pt. or double-blind method/ or random allocation/ or single-blind method/ or (guideline* or cochrane or medline or cinahl or embase or CCTR or scopus or "web of science" or lilacs or (systematic* adj2 review*)).mp. or comparative study/ or (random* or (doubl* adj2 dummy) or ((singl* or doubl* or tripl* or trebl*) adj25 (mask* or blind*)) or rct or rcts or (control adj25 trial*) or multicent* or placebo* or metaanalys* or (meta adj5 analys*) or sham or effectiveness or efficacy or compar*).ti,ab. or (guideline* or cochrane or medline or cinahl or embase or CCTR or scopus or "web of science" or lilacs or (systematic* adj2 review*)).mp. or comparative study/ | | |
| 9 | 7 and 8 | 699 | Final results |

**EMBASE**

The search strategy for EMBASE (1980 to **2011 Week 36**) retrieved **1212** references. We used a combination of EMBASE and free text terms for

| Set | History | Results | Comments |
|---|---|---|---|
| 1 | newborn/ or newborn period/ or low birth weight/ or extremely low birth weight/ or small for date infant/ or very low birth weight/ or Prematurity/ or exp newborn disease/ or multiple pregnancy/ or twin pregnancy/ or twins/ or dizygotic twins/ or monozygotic twins/ or human triplets/ or intrauterine growth retardation/ or small for date infant/ or (infan: or neonat: or newborn: or prematur: or iugr or sga or vlbw or lbw or elbw or (intrautrine adj2 growth adj2 restrict:) or (intrauterine adj2 growth adj2 retard:)).ti,ab. | 1397165 | Infant age group Terms |
| 2 | breast feeding/ or breast milk/ or (breastfeed* or (breast adj2 milk) or breastmilk or breastfed or (breast adj2 feed*) or (breast adj2 fed)).mp. | 48144 | Breast Feeding terms |
| 3 | Pain Assessment/ or pain/ or injection pain/ or vaccination reaction/ or exp application site reaction/ or exp injection site reaction/ or antibody production/ or crying/ or facial expression/ or gesture/ or fear/ or anticipatory anxiety/ or anxiety/ or (adverse adj2 effect:).ti,ab. or (side adj2 effect:).ti,ab. or (skin adj2 reaction:).ti,ab. or (distress* or discomfort* or fright* or anxious).ti,ab. | 726581 | Pain terms |
| 4 | 1 and 2 and 3 | 1466 | Base clinical set 1 |
| 5 | needle/ or exp Injection/ or intradermal drug administration/ or intramuscular drug administration/ or intraosseous drug administration/ or subcutaneous drug administration/ or transdermal drug administration/ or (needle: adj2 (gauge: or length: or thick: or angle: or size:)).ti,ab. or injections/ or injections, intramuscular/ or injections, subcutaneous/ or injections, intradermal/ or injections, jet/ or biolistics/ or ((needle: or inject: or vaccinat:) adj2 (technique: or techinc: or aspirat: or angle: or speed: or slow: or fast: or order:)).ti,ab. or cannula*.mp. or ((intravenous or intravascular) adj2 access).ti,ab. or | 693172 | Procedure terms |

(Continued)

| | | | |
|---|---|---|---|
| | venipuncture*.mp. or (painf* adj2 procedur*).ti,ab. or puncture/ or blood sampling/ or vein puncture/ or phlebotomy/ or intravenous drug administration/ or parenteral drug administration/ or intravenous drug administration/ or catheterization/ or blood vessel catheterization/ or central venous catheter/ or "catheters and tubes"/ or cannula/ or ((intravenous or intravascular) adj2 access).ti,ab. or venipuncture*.mp. | | |
| 6 | 2 and 5 | 1163 | Base clinical set 2 |
| 7 | 4 or 6 | 2514 | Base Results |
| 8 | ct.fs. or clinical trial/ or controlled clinical trial/ or multicenter study/ or phase 1 clinical trial/ or phase 2 clinical trial/ or phase 3 clinical trial/ or phase 4 clinical trial/ or cohort analysis/ or double blind procedure/ or single blind procedure/ or triple blind procedure/ or meta analysis/ or randomized controlled trial/ or "systematic review"/ or case control study/ or longitudinal study/ or prospective study/ or retrospective study/ or multicenter study/ or validation study/ or (((evaluation or validation) adj2 study) or ((evaluation or validation) adj2 studies)).ti,ab. or double-blind method/ or random allocation/ or single-blind method/ or (guideline* or cochrane or medline or cinahl or embase or CCTR or scopus or "web of science" or lilacs or (systematic* adj2 review*)).mp. or comparative study/ or (random* or (doubl* adj2 dummy) or ((singl* or doubl* or tripl* or trebl*) adj25 (mask* or blind*)) or rct or rcts or (control adj25 trial*) or multicent* or placebo* or metaanalys* or (meta adj5 analys*) or sham or effectiveness or efficacy or compar*).ti,ab. | 5294788 | Study Design/Methodology terms |
| 9 | 7 or 8 | 1212 | Final results |

**EBM Reviews - Cochrane Central Register of Controlled Trials**

The search strategy for CCTR (3rd **Quarter 2011**) retrieved **174** references. This database consists exclusively of RCTs, no study design terms were used. We used a combination of primarily MeSH and free text terms for

| Set | History | Results | Comments |
|---|---|---|---|
| 1 | Infant, newborn/ or infant, low birth weight/ or infant, small for gestational age/ or infant, very low birth weight/ or infant, premature/ or exp Infant, Newborn, Diseases/ or pregnancy, high-risk/ or quadruplets/ or quintuplets/ or superfetation/ or triplets/ or twins/ or twins, dizygotic/ or twins, monozygotic/ or newborn/ or newborn period/ or low birth weight/ or extremely low birth weight/ or small for date infant/ or very low birth weight/ or Prematurity/ or exp newborn disease/ or multiple pregnancy/ or twin pregnancy/ or dizygotic twins/ or monozygotic twins/ or human triplets/ or intrauterine growth retardation/ or small for date infant/ or (infan: or neonat: or newborn: or prematur: or iugr or sga or vlbw or lbw or elbw).ti,ab. or ((intrauterine adj2 growth adj2 restrict:) or (intrauterine adj2 growth adj2 retard:)).ti,ab. | 25542 | Infant age group Terms |

*(Continued)*

| | | | |
|---|---|---|---|
| 2 | Breast Feeding/ or Milk, Human/ or breast milk/ or (breastfeed* or (breast adj2 milk) or breastmilk or breastfed or (breast adj2 feed*) or (breast adj2 fed)).mp. | 2349 | Breast Feeding terms |
| 3 | Pain Measurement/ or exp pain/ or Antibody Formation/ or Crying/ or anxiety/ or fear/ or panic/ or Pain Assessment/ or injection pain/ or vaccination reaction/ or exp application site reaction/ or exp injection site reaction/ or antibody production/ or facial expression/ or gesture/ or anticipatory anxiety/ or anxiety/ or (adverse adj2 effect:).ti,ab. or (side adj2 effect:).ti,ab. or (skin adj2 reaction:).ti,ab. or (distress* or discomfort* or fright* or anxious).ti,ab. | 80214 | Pain terms |
| 4 | 1 and 2 and 3 | 146 | Base clinical set 1 |
| 5 | needle/ or exp Injection/ or intradermal drug administration/ or intramuscular drug administration/ or intraosseous drug administration/ or subcutaneous drug administration/ or transdermal drug administration/ or injections/ or injections, intramuscular/ or injections, subcutaneous/ or injections, intradermal/ or injections, jet/ or biolistics/ or puncture/ or blood sampling/ or vein puncture/ or phlebotomy/ or intravenous drug administration/ or parenteral drug administration/ or intravenous drug administration/ or catheterization/ or blood vessel catheterization/ or central venous catheter/ or "catheters and tubes"/ or cannula/ or needles/ or (needle: adj2 (gauge: or length: or thick: or angle: or size:)).ti,ab. or injections/ or ((needle: or inject: or vaccinat:) adj2 (technique: or techinc: or aspirat: or angle: or speed: or slow: or fast: or order:)).ti,ab. or punctures/ or blood specimen collection/ or phlebotomy/ or infusions, parenteral/ or infusions, intravenous/ or injections, intravenous/ or catheterization, central venous/ or catheterization, peripheral/ or cannula*.mp. or ((intravenous or intravascular) adj2 access).ti,ab. or venipuncture*.mp. or (painf* adj2 procedur*).ti,ab. | 28461 | Procedure terms |
| 6 | 2 and 5 | 49 | Base clinical set 2 |
| 7 | 4 or 6 | 174 | Final results |

**CINAHL**

CINAHL as of 4 October 2011

S1 (MH "Infant, Newborn") OR (MH "Infant, Low Birth Weight") OR (MH "Infant, Small for Gestational Age") OR (MH "Infant, Very Low Birth Weight") OR (MH "Infant, Premature") OR (MH "Infant, Newborn, Diseases+") OR (MH "Pregnancy, High Risk") OR (MH "Pregnancy, Multiple") OR (MH "Twins") OR (MH "Childbirth, Premature") OR (MH "Multiple Offspring") OR (MH "Fetal Growth Retardation") OR (TX quadruplet* OR quintuplet* OR superfetation OR triplet* OR twin* OR infan* OR neonat* OR newborn* OR prematur* OR iugr OR sga OR vlbw OR lbw OR elbw) OR (TX (intrauterine N2 growth N2 restrict*)) OR (TX (intrauterine N2 growth N2 retard*))

S2 (MH "Breast Feeding") OR (MH "Breast Feeding Positions") OR (MH "Latching, Breastfeeding") OR (MH "Milk, Human") OR (TX breastfeed* OR breastmilk or breastfed) OR (TX (breast N2 milk) OR (TX breast N2 feed*) OR (TX breast N2 fed))

S3 (MH "Pain+") OR (MH "Treatment Related Pain") OR (MH "Antibody Formation") OR (MH "Crying") OR (MH "Facial Expression") OR (MH "Anxiety") OR (MH "Anticipatory Anxiety") OR (MH "Fear") OR (MH "Suffering") OR (TX panic OR distress* OR discomfort* OR fright* OR anxious) OR (TX (adverse N2 effect*)) OR (TX (side N2 effect*)) OR (TX (skin N2 reaction*))

S4 S1 and S2 and S3

S5 (MH "Needles") OR (MH "Injections") OR (MH "Injection Sites") OR (MH "Injections, Intradermal") OR (MH "Injections, Intramuscular+") OR (MH "Injections, Intravenous") OR (MH "Injections, Subcutaneous+") OR (MH "Administration, Intravenous+") OR (MH "Infusions, Parenteral+") OR (MH "Punctures") OR (MH "Arterial Puncture") OR (MH "Venipuncture") OR (MH "Phlebotomy") OR (MH "Blood Specimen Collection") OR (MH "Catheterization") OR (MH "Catheterization, Central Venous+") OR (MH "Catheterization, Peripheral+") OR (MH "Catheterization, Umbilical Vessels") OR (MH "Venous Cutdown")OR (TX (needle* N2 gauge*)) OR (TX (needle* N2 length*)) OR (TX (needle* N2 thick*)) OR (TX (needle* N2 angle*)) OR (TX (needle* N2 size*)) OR (TX (needle* N2 techni*)) or (TX (Needle* N2 aspirat*)) OR (TX (needle* N2 angle*)) OR (TX (needle* N2 speed)) OR (TX (needle N2 slow*)) OR (TX (needle* N2 fast*)) OR (TX (needle N2 order*)) OR (TX (inject* N2 techni*)) or (TX (inject* N2 aspirat*)) OR (TX (inject* N2 angle*)) OR (TX (inject* N2 speed)) OR (TX (inject* N2 slow*)) OR (TX (inject* N2 fast*)) OR (TX (inject* N2 order*)) OR (TX (vaccinat* N2 techni*)) or (TX (vaccinat* N2 aspirat*)) OR (TX (vaccinat* N2 angle*)) OR (TX (vaccinat* N2 speed)) OR (TX (vaccinat* N2 slow*)) OR (TX (vaccinat* N2 fast*)) OR (TX (vaccinat*t N2 order*)) OR (TX cannula*) OR (TX venipunctur*) OR (TX (pain* N2 procedure*))

S6 S2 and S5

S7 S4 or S6

S8 (MH "Triple-Blind Studies") OR (MH "Single-Blind Studies") OR (MH "Randomized Controlled Trials") OR (MH "Double-Blind Studies") OR (TX (cochrane OR medline OR cinahl OR embase OR CCTR OR scopus OR lilacs)) OR (TX (systematic* N2 review*)) OR (TX (web N2 science))
**Results 118 references**

## Appendix 7. Risk of bias tool

**Allocation concealment (checking for possible selection bias). Was allocation adequately concealed?**

For each included study, we categorised the method used to conceal the allocation sequence as:

- low risk (e.g. telephone or central randomisation; consecutively numbered sealed opaque envelopes);
- high risk (open random allocation; unsealed or non-opaque envelopes, alternation; date of birth); or
- unclear risk.

**Blinding of personnel (checking for possible performance bias). Was knowledge of the allocated intervention adequately prevented during the study?**

For each included study, we categorised the methods used to blind study participants and personnel from knowledge of which intervention a participant received. Blinding was assessed separately for different outcomes or class of outcomes. We categorised the methods as:

- low risk, high risk or unclear risk for participants; and
- low risk, high risk or unclear risk for personnel.

**Blinding of outcome assessment (checking for possible detection bias). Was knowledge of the allocated intervention adequately prevented at the time of outcome assessment?**

For each included study, we categorised the methods used to blind outcome assessment. Blinding was assessed separately for different outcomes or class of outcomes. We categorised the methods as:

- low risk for outcome assessors;
- high risk for outcome assessors; or
- unclear risk for outcome assessors.

**Incomplete outcome data (checking for possible attrition bias through withdrawals, dropouts, protocol deviations). Were incomplete outcome data adequately addressed?**

For each included study and for each outcome, we described the completeness of data including attrition and exclusions from the analysis. We noted whether attrition and exclusions were reported, the numbers included in the analysis at each stage (compared with the total randomised participants), reasons for attrition or exclusion where reported, and whether missing data were balanced across groups or were related to outcomes. Where sufficient information was reported or supplied by the trial authors, we re-included missing data in the analyses. We categorised the methods as:

- low risk (< 20% missing data);
- high risk (≥ 20% missing data); or
- unclear risk.

**Selective reporting bias. Are reports of the study free of suggestion of selective outcome reporting?**

For each included study, we described how we investigated the possibility of selective outcome reporting bias and what we found. For studies in which study protocols were published in advance, we compared prespecified outcomes versus outcomes eventually reported in

the published results. If the study protocol was not published in advance, we contacted study authors to gain access to the study protocol. We assessed the methods as:

- low risk (where it is clear that all of the study's prespecified outcomes and all expected outcomes of interest to the review have been reported);
- high risk (where not all the study's prespecified outcomes have been reported; one or more reported primary outcomes were not prespecified outcomes of interest and are reported incompletely and so cannot be used; study fails to include results of a key outcome that would have been expected to have been reported); or
- unclear risk.

**Other sources of bias. Was the study apparently free of other problems that could put it at a high risk of bias?**

For each included study, we described any important concerns we had about other possible sources of bias (for example, whether there was a potential source of bias related to the specific study design or whether the trial was stopped early due to some data-dependent process). We assessed whether each study was free of other problems that could put it at risk of bias as:

- low risk;
- high risk;
- unclear risk

If needed, we explored the impact of the level of bias through undertaking sensitivity analyses.

## WHAT'S NEW

| Date | Event | Description |
|---|---|---|
| 29 August 2023 | New search has been performed | We reran the searches on 1 August 2022. We included 46 new studies. We updated the methods to include evaluation of the evidence using GRADE methodology and we used 'certainty of evidence' according to the current standards of Cochrane methodology. We updated the results with separate evaluation for different interventions and comparisons.<br><br>The conclusions have changed: "Moderate-/low-certainty evidence suggests that breastfeeding or supplemental breast milk likely reduce pain in neonates undergoing painful procedures compared to no intervention/positioning/holding or placebo or non-pharmacological interventions. Low-certainty evidence suggests that moderate concentration (20% to 33%) glucose/sucrose may lead to little or no difference in reducing pain compared to breastfeeding." |
| 29 August 2023 | New citation required and conclusions have changed | A new search was conducted and 46 new studies were included in this version. The results were also evaluated using GRADE criteria and certainty of evidence statements were incorporated with the conclusions. |

## HISTORY

Protocol first published: Issue 4, 2004
Review first published: Issue 3, 2006

| Date | Event | Description |
|---|---|---|
| 11 October 2011 | New citation required but conclusions have not changed | This review has been updated to include new studies.<br><br>No change to conclusions (Shah 2012). |

| Date | Event | Description |
|---|---|---|
| 11 October 2011 | New search has been performed | This updates the review 'Breastfeeding or breast milk for procedural pain in neonates' (Shah 2006). |
| 11 September 2008 | Amended | Converted to new review format (Shah 2006). |

## CONTRIBUTIONS OF AUTHORS

Praes Shah (PS): protocol development; editing the protocol; identification of trials; writing the review; editing the review; collecting and entering data in RevMan; and revision of the review.

Ranjit Torgalkar (RT): review updating and writing; identification of new studies; entering data in RevMan; revision of the review; determining risk of bias.

Vibhuti Shah (VS): protocol editing; review editing; checking the search for trials; identification of studies; and checking the data in RevMan.

## DECLARATIONS OF INTEREST

PSS is an Associate Editor for Cochrane Neonatal; however, he was not involved in the editorial process for this review.

RT declares there is no conflict of interest.

VS is an Associate Editor for Cochrane Neonatal; however, she was not involved in the editorial process for this review.

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### Internal sources

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- Mount Sinai Hospital, University of Toronto, Canada

  Drs P Shah, V Shah and R Torglakar are affiliated to Department of Paediatrics, University of Toronto.

### External sources

- The Gerber Foundation, USA

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- Vermont Oxford Network, USA

  Cochrane Neonatal Reviews are produced with support from Vermont Oxford Network, a worldwide collaboration of health professionals dedicated to providing evidence-based care of the highest quality for newborn infants and their families.

## DIFFERENCES BETWEEN PROTOCOL AND REVIEW

We made the following changes to the protocol (Aliwalas 2004).

- Lucia Aliwalas and Cecilia Herbozo, who contributed to previous versions of review, were removed from authorship, and Ranjit Torgalkar was added as an author who contributed to this version of the review.
- Types of participants: we have defined the terms 'term' and 'preterm' infants clearly.
- We have now included the painful intervention assessed in each included study (along with the names of the study authors) in the analyses section so that readers have a clear understanding of the different procedures for which different interventions were studied.
- Outcomes are now clearly identified with respect to change as well as absolute values. We also identified several pain scales used in the individual studies, which are now defined and reported in the results.
- In this version, we also included summary of findings tables and certainty of the evidence for each comparison.

## INDEX TERMS

### Medical Subject Headings (MeSH)

Acetaminophen [therapeutic use]; Breast Feeding; *Milk, Human; Pain [etiology] [prevention & control]; *Pain, Procedural [prevention & control]

### MeSH check words

Female; Humans; Infant; Infant, Newborn